Nima Rezaei
Editor

Cancer Immunology

Cancer Immunotherapy for Organ-Specific Tumors

Springer

Cancer Immunology

Nima Rezaei
Editor

Cancer Immunology

Cancer Immunotherapy for Organ-Specific Tumors

Springer

Editor
Nima Rezaei, MD, PhD
Research Center for Immunodeficiencies
Children's Medical Center
Pediatrics Center of Excellence
Tehran University of Medical Sciences
Tehran
Iran

Department of Immunology
Molecular Immunology Research Center
School of Medicine
Tehran University of Medical Sciences
Tehran
Iran

Universal Scientific Education and
Research, Network (USERN)
Tehran
Iran

ISBN 978-3-662-46409-0 ISBN 978-3-662-46410-6 (eBook)
DOI 10.1007/978-3-662-46410-6

Library of Congress Control Number: 2015936588

Springer Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer-Verlag GmbH Berlin Heidelberg is part of Springer Science+Business Media (www.springer.com)

This book would not have been possible without the continuous encouragement by my parents and my wife Maryam.
I wish to dedicate it to my daughters, Ariana and Arnika, with the hope that progress in diagnosis and treatment of these diseases may result in improved survival and quality of life for the next generations and at the same time that international collaboration in research will happen without barriers.
Whatever I have learned comes from my mentors. This book is therefore dedicated also to all of them but most importantly to the patients and their families, whose continuous support has guided me during the years.

Foreword

Several empirical observations suggested a long time ago that established human tumors could melt away in response to perturbations of the immune system, such as during acute infection. Such regressions of tumors occurred most often but not exclusively when infection occurred at the tumor site and sparked the interest of investigators in identifying the mechanism leading to such occurrences based on the assumption that infection acted as an adjuvant to boost existing but insufficient immune surveillance against neoplasms. These anecdotal observations are not only reflected in the scientific literature such as the classic reports of William Cooley in the late 1800s but even discussed by classic authors such as the doctor–writer Anton Chekhov.

It took time, however, to elevate these concepts derived from empirical observations to a science of molecular precision. Skepticism dominated the scene for a long time, including during the late 1980s, when the introduction of systemic IL-2 therapy for the treatment of advanced melanoma and renal cell carcinoma provided consistent and reproducible evidence that some advanced

cancers could regress and remain in long-term remission with a treatment that had for sure no direct effect on cancer cells. Retrospectively, as too often occurs in science, this skepticism was unwarranted, and the detractors of cancer immunotherapy made a disservice by slowing the progression of this budding discipline. Common criticisms were not directed against the observation that cancers could regress but rather focused on denial about the overall effectiveness of treatment, the sporadic nature of the regressions, and the relatively high toxicity. In other words, the skeptics confused the clinical effectiveness of a treatment with the value of a promising scientific observation.

I am emphasizing this because it is important to remember those difficult moments now that books as sophisticated and comprehensive are presented on a topic that was not even considered true science by most just a few decades ago. Fortunately, several investigators did not give up but, focusing on the value of an uncommon but reproducible observation, carried the field forward.

Thus, this book! An achievement difficult to predict only two decades ago!

It is a book that encompasses more than 75 chapters spanning from biological aspects of innate and adaptive immune responses to systems biology approaches to biomarker discovery to portrayals of clinical successes and discussion of regulatory processes that are about to revolutionize the development and licensing of new investigational agents.

The big change occurred after the identification and molecular characterization of antigens recognized by antibodies and/or T cells. Moreover, the characterization of molecular mechanisms controlling the cross talks between cancer and non-neoplastic somatic cells expanded the field and the understanding of the mechanistic bases of immune-mediated tumor rejection. These unarguable observations gave molecular precision to what was previously perceived as voodoo practice. However, the true revolution came with the clinical demonstration that some of the novel biological agents could significantly improve the survival of patients, receiving, therefore, acceptance and recognition as standard therapies through regulatory licensing.

Yet, challenges remain, and it is not the time to relax. Still, the benefits, though reproducible, are marginal both in terms of number of patients benefiting from the treatment and length of survival for those who benefit. Most importantly, the outcomes are capricious and unpredictable. Predictive and surrogate biomarkers are missing in spite of novel technologies and strategies that could help in the identification and stratification of patients. Still, most clinical trials are designed to look at outcomes rather than comprehensively learn in case of failures. Still, a gap exists between the potentials for what we could do to better understand the biology of immune responsiveness and what we actually do.

This book is written for those who want to move the field forward at both the clinical and the scientific levels. Such a compendium can provide a contemporary overlook at what has happened lately, which is remarkably logarithmic from a time perspective. Yet, we wonder how elemental this edition may seem just within a few years if the field will continue to evolve at the current pace. We hope that a second edition will follow soon. Perhaps the editors should have asked for a clairvoyant's chapter. Hopefully, one of the young readers of this edition may step forward and help define the new frontiers of cancer immunotherapy.

Francesco M. Marincola, MD

Preface

The rapid flow of studies in the field of cancer immunology during the last decade has increased our understanding of the interactions between the immune system and cancerous cells. In particular, it is now well known that such interactions result in the induction of epigenetic changes in cancerous cells and the selection of less immunogenic clones as well as alterations in immune responses. Understanding the cross talk between nascent transformed cells and cells of the immune system has led to the development of combinatorial immunotherapeutic strategies to combat cancer.

Cancer Immunology, a three-volume book series, is intended as an up-to-date, clinically relevant review of cancer immunology and immunotherapy. *Cancer Immunology*: *A Translational Medicine Context* is focused on the immunopathology of cancers; *Cancer Immunology*: *Bench to Bedside Immunotherapy of Cancers* is a translation text explaining novel approaches in the immunotherapy of cancers; and finally, *Cancer Immunology*: *Cancer*

Immunotherapy for Organ-Specific Tumors thoroughly addresses the immunopathology and immunotherapy of organ-specific cancers.

In *Cancer Immunology*: *Cancer Immunotherapy for Organ-Specific Tumors*, the immunopathology and immunotherapy of various cancers categorized on an organ-specific basis are discussed in detail. Notably, the principal focus is to put the basic knowledge gained on tumor immunology and immunotherapy in the other two volumes into clinical perspective with the aim to educate clinicians on the most recent approaches used in the immunotherapy of various tumors.

Twenty-four chapters are allocated to meet this purpose. At the very beginning, an overview of the beneficial effects of immunotherapy are outlined in Chap. 1; then, in Chaps. 2 and 3, various aspects of the immunotherapy of solid tumors are discussed, including vaccination against solid tumors and immunotherapy for pediatric solid tumors. Thereafter, five chapters are devoted to hematological malignancies, specifically their immune microenvironment as well as the immunotherapeutic approaches; multiple myeloma, myeloid and lymphoid leukemias, as well as Hodgkin and non-Hodgkin lymphomas are discussed in Chaps. 3, 4, 5, 6, 7, and 8.

Due to the global prevalence of gastrointestinal tumors, precise discussions are brought up in Chaps. 9, 10, 11, 12, and 13; esophageal, gastric, liver, colon, and pancreatic cancers are tackled down one by one, respectively. Skin cancers, including melanoma and squamous-cell carcinoma as well as head, neck, and oral tumors, are illustrated in Chaps. 14, 15, and 16.

A chapter is allocated to the immunopathology and immunotherapy of bone and connective tissue tumors, followed by descriptions of progress made on the immunotherapy of central nervous system and lung tumors, in Chaps. 17 and 18, respectively.

Chapters 19, 20, 21, and 22 aim to educate the reader on the immunopathology and immunotherapy of genitourinary tract tumors. Chapter 23 provides the reader with the most important detail on the application of immunotherapy in breast cancers.

To put an end to this volume and actually to the whole book series, immunology and immunotherapy of graft-versus-host disease as a common complication of organ transplantation would be highlighted.

I hope that this translational book will be comprehensible, cogent, and of special value for researchers and clinicians who wish to extend their knowledge on cancer immunology.

Nima Rezaei, MD, PhD

Acknowledgment

I would like to express my gratitude to the technical editor of this book, Maryam Ebadi, MD. With no doubt, the book would not have been completed without her contribution.

Nima Rezaei, MD, PhD

Contents

Contributors

Faouzia Ajili, PhD Laboratory of Human and Experimental Pathology, Pasteur Institute of Tunis, Tunis, Tunisia

Yoshito Akagi Department of Surgery, Kurume University School of Medicine, Fukuoka, Fukuoka, Japan

Andreas E. Albers, MD, PhD Department of Otolaryngology and Head and Neck Surgery, Campus Benjamin Franklin, Charité-Universitätsmedizin Berlin, Berlin, Germany

Julie A. Alosi, MD Department of Surgical Oncology, Roswell Park Cancer Institute, Buffalo, NY, USA

Kenneth C. Anderson, MD Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA

Richard C.E. Anderson, MD The Gabriele Bartoli Brain Tumor Research Laboratory, Department of Neurological Surgery, The Neurological Institute, Columbia University College of Physicians and Surgeons, New York, NY, USA

Department of Neurological Surgery, Morgan Stanley Children's Hospital of New York Presbyterian, New York, NY, USA

Zahra Aryan, MD, MPH Research Center for Immunodeficiencies, Children's Medical Center, Pediatrics Center of Excellence, Tehran, Iran

Endocrinology and Metabolism Research Center, Molecular Immunology Research Center, Tehran University of Medical Sciences, Tehran, Iran

David E. Avigan, MD Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

Scott W. Baldwin, MD Department of Surgical Oncology, Roswell Park Cancer Institute, Buffalo, NY, USA

Michal Bar-Natan, MD Department of Hematology/Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

Shahe Boghossian, MD, PhD Department of Surgery, University Hospital of Worcester, Worcester, UK

Department of Surgery and Vascular Surgery, University Hospital of North Staffordshire, West Midlands Deanery, Stoke on Trent, West Midlands, UK

Maurizio Chiriva-Internati, PhD Division of Oncology and Hematology, Department of Internal Medicine, Texas Tech University Health Sciences Center, Lubbock, TX, USA

Kiromic LLC, Lubbock, TX, USA

Laura W. Bush Institute for Women's Health and Center for Women's Health and Gender-Based Medicine, Amarillo, TX, USA

Carolina Cimminiello, MD Unit of Immuno-biotherapy of Melanoma and Solid Tumors, San Raffaele Foundation Scientific Institute, Milan, Italy

Department of Medical Oncology, San Raffaele Scientific Institute, Milan, Italy

Everardo Cobos, MD Department of Oncology and Hematology, Texas Tech University Health Sciences Center, Lubbock, TX, USA

Kiromic LLC, Lubbock, TX, USA

Oscar J. Cordero, PhD Department of Biochemistry and Molecular Biology, University of Santiago de Compostela, Santiago de Compostela, Galicia, Spain

Scott W. Dahlbeck, MD Department of Oncology and Hematology, Texas Tech University Health Sciences Center, Lubbock, TX, USA

Kiromic LLC, Lubbock, TX, USA

Zhen-Yu Ding, MD State Key Laboratory of Biotherapy, Cancer Center, West China Hospital, West China Medical School, Sichuan University, Chengdu, China

Martin J. D'Souza, MD Department of Pharmaceutical Sciences, Mercer University, College of Pharmacy, Atlanta, GA, USA

Maryam Ebadi, MD Department of Immunology, Molecular Immunology Research Center, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran

Jose A. Figueroa, MD Department of Oncology and Hematology, Texas Tech University Health Sciences Center, Lubbock, TX, USA

Kiromic LLC, Lubbock, TX, USA

Maria Gkotzamanidou, MD, PhD Department of Medical Oncology, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA, USA

Martin Goodman, MD Department of Hematology/Oncology, Tufts University School of Medicine, Boston, MA, USA

Fabio Grizzi, PhD Department of Immunology and Inflammation, Humanitas Research and Clinical Center, Milan, Rozzano, Italy

Doreen Haase, PhD (SIgN) Singapore Immunology Network, (A*STAR) Agency for Science, Technology and Research, Singapore, Singapore

Peter Hyckel, PhD em, MD, DMD Department of Oral Maxillary and Facial Surgery/Plastic Surgery, Jena University, Jena, Thuringia, Germany

Kyogo Itoh, MD, PhD Department of Immunology and Immunotherapy, Kurume University School of Medicine, Fukuoka, Fukuoka, Japan

Naveena B. Janakiram, PhD Hematology Oncology Section, Department of Medicine, Center for Cancer Prevention and Drug Development, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

Department of Medicine, PCS Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

Marjorie R. Jenkins, MD Laura W. Bush Institute for Women's Health and Center for Women's Health and Gender-Based Medicine, Amarillo, TX, USA

Emmanuel Katsanis, MD Department of Pediatrics, University of Arizona, Tucson, AZ, USA

Andreas M. Kaufmann, PhD Department of Gynecology, Campus Benjamin Franklin and Campus Mitte, Charité-Universitätsmedizin Berlin, Berlin, Germany

Tessa Kerre, MD, PhD Department of Clinical Chemistry, Microbiology and Immunology, Ghent University, Ghent University Hospital, Ghent, Belgium

Department of Hematology – Immunology, Ghent University Hospital, Ghent, Belgium

Lisa M. Kopp, DO Department of Pediatrics, University of Arizona, Tucson, AZ, USA

Nicolaus Kröger, MD Department of Stem Cell Transplantation, University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Germany

Mark L. Lang, PhD Department of Microbiology and Immunology, PCS Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

Sherene Loi, MBBS (Hons), FRACP, PhD Division of Cancer Medicine, Group Leader, Translational Breast Cancer Genomics Lab, Cancer Therapeutics Program, Division of Research, Peter MacCallum Cancer Centre, East Melbourne, VIC, Australia

Cristina Maccalli, PhD Unit of Immuno-biotherapy of Melanoma and Solid Tumors, San Raffaele Foundation Scientific Institute, Milan, Italy

Department of Medical Oncology, San Raffaele Scientific Institute, Milan, Italy

Kai Masur, PhD ZIKplasmatis, Leibniz Institute for Plasma Science and Technology, INP Greifswald e.V., Greifswald, Germany

Håkan Mellstedt, MD, PhD Department of Oncology, Cancer Centre Karolinska, Karolinska University Hospital Solna, Stockholm, Sweden

Hans-Robert Metelmann, MD, DDS, PhD Department of Oral and Maxillo-Facial Surgery/Plastic Surgery, Greifswald University, Greifswald, Germany

Leonardo Mirandola, PhD Department of Oncology and Hematology, Texas Tech University Health Sciences Center, Lubbock, TX, USA

Altaf Mohammed, PhD Hematology Oncology Section, Department of Medicine, Center for Cancer Prevention and Drug Development, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

Department of Medicine, PCS Cancer Center, University of Oklahoma Health Science Center, Oklahoma City, OK, USA

Diane D. Nguyen, MD Department of Oncology and Hematology, Texas Tech University Health Sciences Center, Lubbock, TX, USA

Kiromic LLC, Lubbock, TX, USA

Marek Nowak, MD, PhD Gynecology Department with Gynecologic Oncology Unit, Polish Mother's Health Center Research Institute, Lodz, Poland

Jacqueline Oxenberg, DO Department of Surgical Oncology, Roswell Park Cancer Institute, Buffalo, NY, USA

Department of Surgical Oncology, Geisinger Wyoming Valley Hospital, Wilkes-Barre, PA, USA

Giorgio Parmiani, MD Unit of Immuno-biotherapy of Melanoma and Solid Tumors, San Raffaele Foundation Scientific Institute, Milan, Italy

Department of Medical Oncology, San Raffaele Scientific Institute, Milan, Italy

Fred Podmelle, MD, DDS Department of Oral and Maxillo-Facial Surgery/Plastic Surgery, Greifswald University, Greifswald, Germany

Xu Qian, MD Department of Otolaryngology and Head and Neck Surgery, Campus Benjamin Franklin, Charité-Universitätsmedizin Berlin, Berlin, Germany

Chinthalapally V. Rao, PhD Hematology Oncology Section, Department of Medicine, Center for Cancer Prevention and Drug Development, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

Department of Medicine, PCS Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

Nishitha M. Reddy, MBBS, MSCI Hematology and Stem Cell Transplantation Section, Division of Hematology/Oncology, Department of Medicine, Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN, USA

Nima Rezaei, MD, PhD Research Center for Immunodeficiencies, Children's Medical Center, Pediatrics Center of Excellence, Tehran University of Medical Sciences, Tehran, Iran

Department of Immunology, Molecular Immunology Research Center, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Universal Scientific Education and Research Network (USERN), Tehran, Iran

Vincenzo Russo, MD Unit of Cancer Gene Therapy, San Raffaele Foundation Scientific Institute, Milan, Italy

Department of Medical Oncology, San Raffaele Scientific Institute, Milan, Italy

Amene Saghazadeh, MD Department of Immunology, Molecular Immunology Research Center, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Mahdia Saghazadeh, MD Department of Immunology, Molecular Immunology Research Center, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Muhammad Wasif Saif, MD, MBBS Department of GI Oncology, Tufts University School of Medicine, Boston, MA, USA

Tetsuro Sasada, MD, PhD Department of Immunology and Immunotherapy, Kurume University School of Medicine, Kurume, Fukuoka, Japan

Joseph J. Skitzki, MD Department of Surgical Oncology, Geisinger Wyoming Valley Hospital, Wilkes-Barre, PA, USA

Department of Immunology, Roswell Park Cancer Institute, Buffalo, NY, USA

Christopher R. Showers, BS The Gabriele Bartoli Brain Tumor Research Laboratory, Department of Neurological Surgery, The Neurological Institute, Columbia University College of Physicians and Surgeons, New York, NY, USA

Department of Neurological Surgery, Columbia University Medical Center, New York, NY, USA

Sylvia Snauwaert, MD, PhD Department of Clinical Chemistry, Microbiology and Immunology, Ghent University, Ghent University Hospital, Ghent, Belgium

Adam M. Sonabend, MD The Gabriele Bartoli Brain Tumor Research Laboratory, Department of Neurological Surgery, The Neurological Institute, Columbia University College of Physicians and Surgeons, New York, NY, USA

John Stagg, PhD Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Montreal, QC, Canada

Ryan Stevenson, MD Department of Hematology/Oncology, Tufts University School of Medicine, Boston, MA, USA

Thomas Stübig, MD Department of Stem Cell Transplantation, University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Germany

Ryuji Takahashi, MD, PhD Department of Surgery, Kurume University School of Medicine, Kurume, Fukuoka, Japan

Gianluigi Taverna, MD Department of Immunology and Inflammation, Humanitas Research and Clinical Center, Milan, Rozzano, Italy

Uhi Toh, MD, PhD Department of Surgery, Kurume University School of Medicine, Kurume, Fukuoka, Japan

Bart Vandekerckhove, MD, PhD Department of Clinical Chemistry, Microbiology and Immunology, Ghent University, Ghent University Hospital, Ghent, Belgium

Rubén Varela-Calviño, PhD Department of Biochemistry and Molecular Biology, University of Santiago de Compostela, Santiago de Compostela, A Coruña, Spain

Thomas von Woedtke, PhD Department of Plasma Medicine, Leibniz Institute for Plasma Science and Technology, INP Greifswald e.V., Greifswald, Germany

Yu-Quan Wei, MD, PhD State Key Laboratory of Biotherapy, Cancer Center, West China Hospital, West China Medical School, Sichuan University, Chengdu, China

Jacek R. Wilczyński, MD, PhD Gynecology Department with Gynecologic Oncology Unit, Polish Mother's Health Center Research Institute, Lodz, Poland

Yi Zeng, MD, PhD Department of Pediatrics, Steele Children's Research Center, University of Arizona, Tucson, AZ, USA

Abbreviations

5-ASA	5-Aminosalicylic acid
5-FU	5-Fluorouracil
AA	Anaplastic astrocytoma
AA	Arachidonic acid
ACT	Adoptive cell therapy
ADCC	Antibody-dependent cell-mediated cytotoxicity
ADCP	Ag-dependent cellular phagocytosis
ADCs	Antibody-drug conjugates
AFP	Alpha-fetoprotein
Ag	Antigens
AIDS	Acquired immunodeficiency syndrome
AIM	Antigen isolated from immunoselected melanoma
AJCC	The American Joint Committee on Cancer
AKAP4	A-kinase anchor protein 4
ALCL	Anaplastic large cell lymphoma
ALDH1	Aldehyde dehydrogenase-1
ALK	Anaplastic lymphoma kinase
ALL	Acute lymphatic leukaemia
Allo SCT	Allogeneic stem cell transplantation
AML	Acute myeloid leukemia
AMP	Adenosine monophosphate
AO	Anaplastic oligodendroglioma
AOA	Anaplastic oligoastrocytoma
AOM	Azoxymethane
AP-1	Activating protein-1
APC	Adenomatosis polyposis coli
APC	Antigen-presenting cells
APLs	Aspirin-triggered lipoxins
APM	Antigen-processing machinery
AS04	Adjuvant system 04
ASCT	Autologous stem cell transplantation
ATCs	Autologous tumor cells
ATF	Activating transcription factor
ATG	Anti-thymocyte globulin
ATR	Antitumor responses
ATRTs	Atypical teratoid-rhabdoid tumors

BAFF	B-cell-activating factor
BBB	Blood-brain barrier
BCC	Basal cell carcinoma
BCG	Bacillus Calmette-Guérin
BCMA	B-cell maturation antigen
bFGF	Basic fibroblast growth factor
BID	Bowel inflammatory disease
BM	Bone marrow
BMI	Body mass index
BMSCs	BM stromal cells
BMT	Bone marrow transplantation
B-NHLs	B-cell non-Hodgkin's lymphomas
BTLA	B- and T-lymphocyte attenuator
C	Chemotherapy
CAC	Colitis-associated cancer
CAFs	Cancer-associated fibroblasts
CAK	Cytokine activated cells
CAR	Chimeric antigen receptor
CD	Cytosine deaminase
CDC	Complement-dependent cytotoxicity
CDR	Complementary-determining region
CEA	Carcinoembryogenic antigen
cHL	Classical HL
CHP	Cholesterol-bearing hydrophobized pullulan
CI	Confidence interval
CIK	Cytokine-induced killer
cILCs	Colonic innate lymphoid cells
CIN	Cervical intraepithelial neoplasia
CIS	Carcinoma in situ
CLL	Chronic lymphocytic leukemia
CLP	Common lymphoid progenitor
CMC	Complement-mediated cytotoxicity
CML	Chronic myeloid leukaemia
CMP	Common myeloid progenitor cells
CMV	Cytomegalovirus
CNS	Central nervous system
COG	Children's Oncology Group
COX	Cyclooxygenase
COX-2	Cyclooxygenase-2
CPG ODN	CpG oligodeoxynucleotides
CR	Complete remission
CR	Complete response
CRC	Colorectal cancer
CRI	Cancer-related inflammation
CRP	C-reactive protein
CRPC	Castration-resistant prostatic carcinoma
CSCs	Cancer stem cells
CSF-1	Colony-stimulating factor

CTAs	Cancer/testis antigens
CTL	Cytotoxic T lymphocyte
CTL4	Cytotoxic T lymphocyte antigen-4
CTLA	Cytotoxic T-lymphocyte-associated
CTLA-4	Cytotoxic T lymphocyte-associated antigen 4
CTLs	Cytotoxic T lymphocytes
CTTNB1	Beta-catenin gene
DALY	Disability-adjusted life year
DAMPs	Damage-associated molecular patterns
DAPK	Death-associated protein kinase
DC	Dendritic cell
DFI	Disease-free interval
DFS	Disease-free survival
DHA	Docosahexaenoic acid
DHFR	Dihydrofolate reductase
DKK1	Dickkopf-related protein 1
DLBCL	Diffuse large B-cell lymphoma
DLI	Donor lymphocyte infusion
DMFI	Distant-metastasis-free interval
DMH	Dimethylhydrazine
DR5	Death receptor 5
DSS	Dextran sulfate sodium
DTH	Delayed-type hypersensitivity
EAU	European Association of Urology
EBV	Epstein-Barr virus
ECAD	E-cadherin
ECM	Extracellular matrix
ECP	Extracorporeal photochemotherapy
EFS	Event-free survival
EGCs	Esophageal and gastric cancers
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EMEA	European Medicines Agency
EMT	Epithelial-mesenchymal transition
EOC	Epithelial ovarian cancer
EORTC	European Organisation for Research and Treatment of Cancer
EP1	Prostaglandin E receptor-1
EPA	Eicosapentaenoic acid
Eph	Ephrin
ER	Estrogen receptor
ERK1/2	Extracellular signal-regulated kinase 1/2
ESCC	Esophageal squamous cell carcinoma
ESHAP	Etoposide, doxorubicin, methylprednisolone, cytarabine, and cisplatin
ET-1	Endothelin-1
ET_AR	Endothelin A receptor
EWSR1	Ewing's sarcoma breakpoint region 1
FAP	Familial adenomatous polyposis

FasL	Fas ligand
FcR	Fc receptor
FDA	Federal Drug Administration
FFS	Failure-free survival
FGF	Fibroblast growth factor
FGF2	Fibroblast growth factor 2
FGFR 4	Fibroblastic growth factor receptor 4
FIGO	International Federation of Gynecology and Obstetrics
FL	Follicular lymphoma
FLI1	Friend leukemia virus integration 1
FOLFIRI	5-Fluorouracil, leucovorin, irinotecan
FOLFOX	5-Fluorouracil, leucovorin, oxaliplatin
FOXp3	Forkhead box P3
FRα	Folate receptor α
GAA	Glioblastoma-associated antigen
GBM	Glioblastoma multiforme
GC	Gemcitabine and carboplatin
G-CSF	Granulocyte-CSF
GCT	Germ cell tumors
GI	Gastrointestinal
GISTs	Gastrointestinal stromal tumors
GITR	Glucocorticoid-induced tumor necrosis factor receptor
GLSG	German low-grade lymphoma study group
Gly	Glycine
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GMP	Good manufacturing practice
GPC3	Glypican-3
GPI	Glycosylphosphatidylinositol
GPR9	G protein-coupled receptor 9
GSC	Glioma stem cells
GSTP1	Glutathione S-transferase P1
GSTP1	Glutathione S-transferase p1 gene
GVH	Graft-versus-host
GVHD	Graft versus host disease
GVL	Graft-versus-leukaemia
GVT	Graft-versus-tumor
HAART	Highly active antiretroviral treatment
HAMA	Human anti-mouse antibody
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HDI	Human development index
HDL	High-density lipoprotein
HER2	Human epidermal growth factor receptor 2
HETE	Hydroperoxyeicosatetraenoic acid
HGG	High-grade glioma
HHV8	Human herpesvirus 8
HIF	Hypoxia-inducible factor
HIV	Human immunodeficiency virus

HL	Hodgkin's lymphoma
HLA	Human leukocyte antigen
HLA-G	Human leukocyte antigen G
HMG	High-mobility group
HMGB1	High-mobility group box 1
HNSCC	Squamous cell carcinoma of the head and neck
HOX	Homeobox
HPCs	Hematopoietic progenitor cells
HPV	Human papillomavirus
HRS	Hodgkin and Reed-Sternberg
HRT	Hormone replacement therapy
HSC	Hematopoietic stem cells
HSCT	Hematopoietic stem cell transplantation
HSP	Heat shock protein
HSPPCs	Heat shock protein peptide complexes
HSPs	Heat shock proteins
HTLV-I	Human T-lymphotropic virus-I
HVG	Host-versus-graft
IAP	Inhibitor of apoptosis protein
IBD	Inflammatory bowel disease
ICE	Ifosfamide, carboplatin, and etoposide
IDH	Isocitrate dehydrogenase
IDO	Indoleamine 2,3-dioxygenase
IEDB	Immune Epitope Database and Analysis Resources
IFN	Interferon
IFN-α	Interferon-α
IFNγ	Interferon gamma
IGF-1	Insulin-like growth factor-1
IGF-1R	Insulin-like growth factor 1 receptor
IGF-BPs	Insulin-like growth factors binding proteins
IGFs	Insulin-like growth factors
IGKC	Immunoglobulin κ C
IHC	Immunohistochemistry
IL	Interleukin
IL-10	Interleukin-10
IL-18	Interleukin-18
IL-18R	IL-18 receptor
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-8	Interleukin-8
IMSCs	Immature myeloid suppressor cells
IMTs	Inflammatory myofibroblastic tumors
INF-γ	Gamma interferon
INGR	International Neuroblastoma Risk Group
iNOS	Inducible nitric oxide synthase
INSS	International Neuroblastoma Staging System
Ipb	Ipilimumab

IPI	International prognostic index
IRC	Immune-related criteria
irPFS	Immune-related progression-free survival
irRC	Immune-related response criteria
IRS	Intergroup Rhabdomyosarcoma Study
I-TAC	Interferon-inducible T-cell α-chemoattractant
ITK	Inducible T cell kinase
IV	Intravenous
IVIG	Intravenous immunoglobulin
kg	Kilogram
KHL	Keyhole limpet hemocyanin
KIF	Kinesin superfamily protein
KIR	Killer-cell immunoglobulin-like receptor
KLH	Keyhole limpet hemocyanin
KO	Knockout
KRAS	Kristin rat sarcoma
KS	Kaposi's sarcoma
KSHV	Kaposi's sarcoma herpesvirus
LAA	Leukemia-associated antigen
LAK	Lymphokine-activated killer
LCMC	Lung Cancer Mutation Consortium
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
LMP1	Latent membrane protein 1
LOH	Loss of heterozygosity
LOX	Lipoxygenase
LPA	Lysophosphatidic acid
LPS	Lipopolysaccharide
LSA	Leukemia-specific antigen
LSC	Leukemic stem cell
LT	Lymphotoxins
LTs	Leukotrienes
M	Months
mAb	Monoclonal antibody
MALP-2	Macrophage-activating lipopeptide
MAPKs	Mitogen-activated protein kinases
MCA	Methylcholanthrene
MCL	Mantle cell lymphoma
MCP-1	Monocyte chemotactic protein 1
MCP-3	Monocyte chemoattractant protein-3
MCPs	Macrophage chemotactic proteins
M-CSF	Monocyte colony-stimulating factor
MDS	Myelodysplasia
MDSCs	Myeloid-derived suppressor cells
MFH	Malignant fibrous histiocytoma
mg	Milligram
MGMT	Methylguanine-DNA-methyltransferase
MGMT	O(6-methylguanine-DNA methyltransferase

MGUS	Monoclonal gammopathy of undetermined significance
MHC	Major histocompatibility complex
MHC I	Major histocompatibility complex I
MHC II	Major histocompatibility complex II
MIATA	Minimal information about T cell assays
MIF	Migration inhibitory factor
MiHA	Minor histocompatibility antigens
MIP-3α	Macrophage inflammatory protein-3
MLL	Mixed lineage leukemia
MM	Multiple myeloma
MMAE	Monomethyl auristatin E
MMP	Matrix metalloproteinases
MP	Myeloid progenitors
MPIF-1	Myeloid progenitor inhibitory factor-1
MPL	Monophosphoryl lipid A
MR	Minor response
MRD	Minimal residual disease
MSC	Mesenchymal stem cells
MSI-H	High-level microsatellite-unstable
MTD	Maximum tolerated dose
mTOR	Mammalian target of rapamycin
MTP-PE	Muramyl tripeptide phosphatidylethanolamine
MTX	Methotrexate
MUC	Mucin
MVD	Microvessel density
N	Nodes
NA	Not available
NCI	The National Cancer Institute
NF	Nuclear factor
NF-κB	Nuclear factor-κB
NHL	Non-Hodgkin's lymphoma
NK	Natural killer
NKT	Natural killer T
NKTCs	Natural killer T cells
NLPHL	Nodular lymphocyte predominant HL
NMIBC	Nonmuscle, invasive bladder cancer
NMSCs	Non-melanocytic skin cancers
NO	Nitric oxide
NRAS	Neuroblastoma RAS oncogene
NRSTS	Non-rhabdomyosarcoma soft tissue sarcomas
NSAIDs	Nonsteroid anti-inflammatory drugs
NSCLC	Non-small cell lung carcinoma
NTS	Nuclear targeting sequence
OFA	Ofatumumab
ORR	Overall response rate
OS	Osteosarcoma
OS	Overall survival
OT	18α-Olean-12-ene-3β-23,28-triol

P	Placebo
PAI-1	Plasminogen activator inhibitor type 1
PAMPs	Pathogen-associated molecular patterns
PanINs	Pancreatic intraepithelial neoplasias
PAR-1	Protease-activated receptor-1
PBLs	Peripheral blood lymphocytes
PBMC	Peripheral blood mononuclear cells
PC	Pancreatic cancer
PD	Progressive disease
PD1	Programmed death-1
PD-1	Programmed cell death-1
PDAC	Pancreatic ductal adenocarcinoma
PDCs	Plasmacytoid dendritic cells
PDEGF	Platelet-derived endothelial cell growth factor
PDGF	Platelet-derived growth factor
PDGFR	Platelet-derived growth factor
PD-L1	Programmed cell death-1 ligand 1
PFS	Progression-free survival
PGE2	Prostaglandin E2
PGs	Prostaglandins
Phe	Phenylalanine
PI3K	Phosphatidylinositol 3-kinase
PIKC	Phosphoinositol kinase C
PK	Pharmacokinetics
PL	Placebo
PMNs	Polymorphonuclear neutrophils
PNETs	Primitive neuroectodermal tumors
Poly-A:U	Polyadenylic-polyuridylic acid
Poly-I:C	Polyinosinic-polycytidylic acid
PPARγ	Peroxisome proliferator-activated receptor-γ
PPARδ	Peroxisome proliferator-activated receptor-δ
PPV	Personalized peptide vaccination
PR	Partial regression
PR	Partial response
PR	Progesterone receptor
pRB	Retinoblastoma protein
PRRs	Pattern recognition receptors
PUFAs	Polyunsaturated fatty acids
PXA	Pleomorphic xanthoastrocytoma
R	Rituximab
RANK	Receptor activator of nuclear factor-kappa B
RARB	Retinoic acid receptor beta
RASSF1	RAS association domain family protein 1
Rb	Retinoblastoma
RCC	Renal cell cancer
RCTs	Randomized-controlled trials
RECIST	Response Evaluation Criteria In Solid Tumors
RFS	Relapse-free survival

RHAMM	Receptor for hyaluronan-mediated motility
RIC	Reduced-intensity conditioning
rIL-2	Recombinant IL-2
RMS	Rhabdomyosarcoma
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RR	Response rate
RRMM	Relapsed or refractory MM
RT	Radiation therapy
RTK	Receptor tyrosine kinase
RXRs	Retinoid X receptors
SART	Squamous cell carcinoma antigen recognized by T cells
SASP	Senescence-associated secretory phenotype
SC	Subcutaneous
SCC	Squamous cell carcinoma
SCCHN	Squamous cell carcinoma of the head and neck
sCD30	Soluble CD30
scFv	Single-chain variable-fragment
SCLCs	Small cell lung carcinomas
SCP-1	Stromal cell-derived protein
SD	Stable disease
SDF-1	Stromal cell-derived factor-1
SEREX	Serological analysis of antigens by recombinant expression cloning
SERMs	Selective estrogen response modulators
sIL-2R	Soluble interleukin-2
SIR	Standardized incidence ratio
SL	Salmonella
SLN	Sentinel lymph node
SLP	Synthetic long peptides
SPTs	Second primary lung tumors
SRE	Skeletal-related events
SSX	Synovial sarcoma X chromosome breakpoint
STAT	Signal transducer and activator of transcription
STAT-3	Signal transducer and activator of transcription-3
STS	Soft tissue sarcomas
SWOG	South West Oncology Group
T	Thickness
TA	Tumor antigen
TAA	Tumor-associated antigen
TADCs	Tumor-associated dendritic cells
TAM	Tumor-related macrophage
TAMs	Tumor-associated macrophages
TAMs	Tumor-associated macrophages/microglia
TAMs	Tumor-associated monocytes/macrophages
TANs	Tumor-associated neutrophils
TAP-1	Abnormal transport proteins
TApDCs	Tumor-associated plasmacytoid dendritic cells

TCC	Transitional cell carcinoma
TCR	T cell receptor
TDSFs	Tumor-derived soluble factors
TERT	Telomerase reverse transcriptase
TF	Tissue factor
TGF	Transforming growth factor
TGFBI	Transforming growth factor-β-inducible gene-h3
TGFβ	Transforming growth factor-β
Th	T helper
Th1	T helper 1
Th17	T helper 17
Th2	T helper 2
TIC	Tubal intraepithelial carcinoma
TIL	Tumor-infiltrating lymphocytes
TIM-3	Immunoglobulin- and mucin domain-containing protein 3
TIMP1	Tissue inhibitor of metalloproteinase 1
TKI	Tyrosine kinase inhibitor
TLR	Toll-like receptor
TLS	Tertiary lymphoid structures
TMZ	Temozolomide
TNBC	Triple-negative breast cancer
TNF	Tumor necrosis factor
TNFR	Tumor necrosis factor receptor
TNFR1	TNF receptor 1
TNF-α	Tumor necrosis factor-α
TP53	Tumor protein 53
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand
TRAILR2	TNF-related apoptosis-inducing ligand receptor 2
Treg	Regulatory T cell
TRM	Treatment-related mortality
TRUC	$Tbet^{-/-}$ and $Rag2^{-/-}$ ulcerative colitis
TSA	Tumor-specific antigens
TSP-1	Thrombospondin-1
TTP	Time to tumor progression
TTS	Time to tumor survival
TUM-CAM	Tumor chorioallantoic membrane
TUR	Transurethral resection
TX	Thromboxane
U	Unit
UCB	Umbilical cord blood
uPA	Urokinase plasminogen activator
uPAR	Urokinase plasminogen activator receptor
UPS	Undifferentiated pleomorphic sarcoma
URI	Upper respiratory tract infection
UTI	Urinary tract infection
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptors
VEGFR1	Vascular endothelial growth factor receptor 1

VH	Variable heavy
VIN	Vulvar intraepithelial neoplasia
VISTA	V-domain immunoglobulin suppressor of T cell activation
VL	Variable light
VNTR	Variable number of tandem repeats
VPF	Vascular permeability factor
WHIM	Warts, hypogammaglobulinemia, infections, myelokathexis
WHO	World Health Organization
WT1	Wilms tumor antigen
WT1	Wilms tumor gene 1
XA	Xanthoastrocytoma
YLD	Years lived with disability
YLL	Years of life lost
βHCG	Beta subunit of human chorionic gonadotropin

Cancer Immunotherapy Confers a Global Benefit

1

Zahra Aryan, Håkan Mellstedt, and Nima Rezaei

Contents

Z. Aryan, MD, MPH
Research Center for Immunodeficiencies, Children's Medical Center, Pediatrics Center of Excellence, Tehran, Iran

Endocrinology and Metabolism Research Center, Molecular Immunology Research Center, Tehran University of Medical Sciences, Tehran 14194, Iran
e-mail: aryanzahra@yahoo.com

H. Mellstedt, MD, PhD
Department of Oncology, Cancer Centre Karolinska, Karolinska University Hospital Solna, Stockholm SE-171 76, Sweden

N. Rezaei, MD, PhD (✉)
Research Center for Immunodeficiencies, Children's Medical Center, Pediatrics Center of Excellence, Tehran University of Medical Sciences, Tehran 14194, Iran

Department of Immunology, Molecular Immunology Research Center, School of Medicine, Tehran University of Medical Sciences, Tehran 14194, Iran

Universal Scientific Education and Research Network (USERN), Tehran, Iran
e-mail: Rezaei_nima@tums.ac.ir

1.1 Introduction

Cancer is a major public health issue which can affect every individual. Worldwide, cancer is one of the leading causes of mortality, morbidity, and decreased quality of life. Additionally, incidence of cancers is growing, and it would be the main

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_1, © Springer-Verlag Berlin Heidelberg 2015

source of burden on both patients and societies, particularly in low- to medium-resource countries. A total of one fifth of overall cancers can be prevented by immunization against oncogenic infections. Thus, national vaccination programs against viruses such as HPV help prevent cancers and are regarded as the primary level of prevention using immunotherapy. On the other hand, current standards of care have failed to do much for many cancer patients; hence, a new therapeutic avenue like immunotherapy is needed to improve the care of cancer patients. With regard to current status of cancers worldwide including considerable incidence, morbidity, mortality rate, and insufficiency of current mainstays of cancer management including surgical approaches, chemotherapy, and radiotherapy, immunotherapy holds great promise in combating cancers. In this chapter, a glance at overall status of morbidity, mortality, and burden of cancers worldwide has been made. Then the utility of immunotherapy at primary, secondary, and tertiary level of prevention from cancers is discussed. At the end immune-related response criteria for cancer immunotherapy as well as cost-effectiveness of cancer immunotherapy have been discussed.

1.2 Incidence, Morbidity, and Mortality of Cancers: Why Is a New Therapeutic Avenue Indicated?

Nowadays, cancer has become a global health issue with respect to its worldwide increase in incidence and burden. New cancer cases were estimated to be 12.7 million in 2008, whereas it is expected to rise to 22.2 million in 2030. This is alarming since increase in incidence of cancers outnumbers the proportional increase in population worldwide [1]. Another unpleasant fact is the high mortality of this growing issue. In 2008, about 7.6 million died from cancers, accounting for 13 % of all deaths worldwide. About 70 % of overall cancer deaths occurred in low- to medium-resource countries [1, 2]. Of 12.7 million new cancer cases in 2008, 48.1 % in Asia, 25.3 % in Europe, 12.7 % in North America, 7.2 % in Latin America and Caribbean, 5.6 % in Africa, and 1.1 % in Oceania were diagnosed. On the other hand, of 7.6 million deaths, 53.8 % in Asia, 22.7 % in Europe, 8.4 % in North America, 7.2 % in Latin America and Caribbean, 7.2 % in Africa, and 0.7 % in Oceania occurred. Considering these absolute numbers of new cases and deaths owing to cancers, low- to middle-income countries are at emergent need for appropriate heath policies to fight cancers. In the following years, it is also estimated that new cases will mostly occur in low- to medium-resource countries due to two major reasons: (1) increase in incidence of cancers associated with westernized lifestyle including colorectal, breast, and prostate cancers and (2) increase in the incidence of infection-related cancers (stomach, liver, and cervical cancers and less importantly lymphomas and Kaposi's sarcoma) owing to the dramatic increase in the prevalence of human immunodeficiency virus (HIV), hepatitis B virus (HBV), and human papillomavirus (HPV) infections, particularly in Sub-Saharan Africa and East Asia [1, 3]. These data shed light on "global cancer transition" that should be considered when establishing priorities to control cancers. One of the best strategies to control cancer pandemic, particularly in a low-resource setting, is to provide vaccination against oncogenic viruses considered to have prophylactic use in immunotherapy to fight cancers [4–7].

1.2.1 Cancer Incidence

Worldwide incidence of overall cancers was estimated to be 180.8 per 100,000 in 2008, and it is predicted to grow in the future. In 2008, the highest age-standardized incidence of overall cancers per 100,000 was estimated for Denmark with 326.1, followed by Ireland with 317.0, Australia and New Zealand with 313.3, Belgium with 306.8, and France with 300.4. By contrast, the least incidence was estimated for countries of Gaza Strip and West Bank with 54.9, Syrian Arab Republic with 72.2, Namibia with 78.3, Sudan with 81.5, and Botswana with 85.7 (Fig. 1.1). With respect to these data, incidence of cancer is the highest in high-resource countries, possibly due to westernized lifestyle and exposure to a wide range of pollutants and carcinogens.

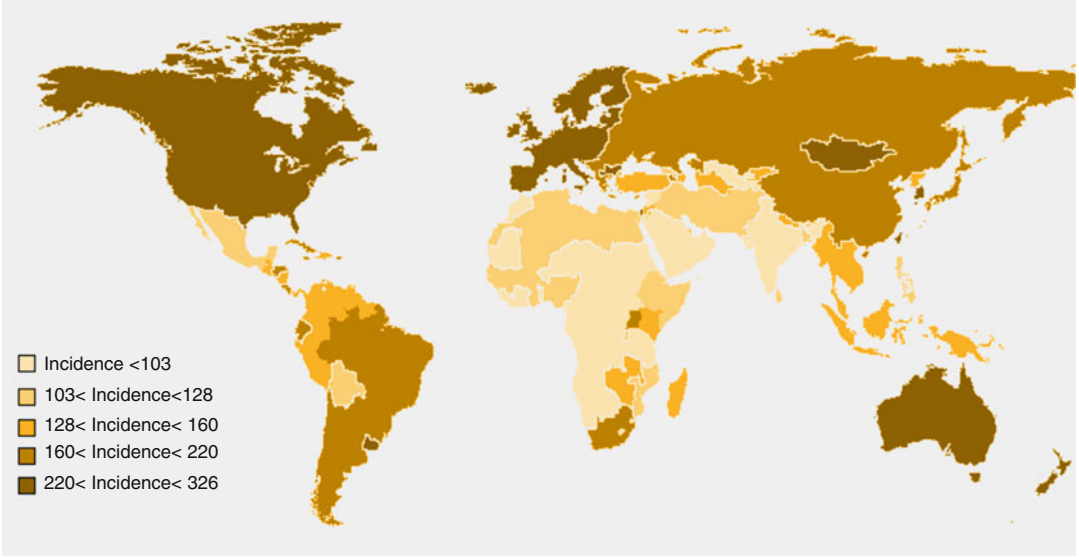


Fig. 1.1 Estimated age-standardized incidence of overall cancers per 100,000 in different countries (Extracted from)

However, it does not mean that cancers are of less importance in restricted resource regions as explained by several reasoning: (1) An absolute number of new cancer cases are higher in less developed countries with respect to overall population, and (2) underestimation of cancers in restricted resource setting is a possibility considering the worldwide inequality in health system facilities.

More than the environment, host-related characteristics are also major determinants of cancer incidence. Since cancer development is the result of immune system defeat in the war against tumor cells, immune-deficient states are believed to predispose subjects to several cancers. The most common form of immune deficiency is secondary types, though; both primary and secondary forms are associated with cancer development [8–11]. Infection-related cancers, but not other cancers, have increased incidence in these subjects [9, 11]. Acquired immunodeficiency syndrome (AIDS), as one of the most important causes of secondary immunodeficiency, is associated with increased malignancies [9]. AIDS-defining cancers encompass Kaposi's sarcoma, cervical cancer, and non-hodgkin lymphoma (NHL) with a standardized incidence ratio (SIR) of 3640.0, 5.8, and 76.7, respectively. Interestingly, Kaposi's sarcoma risk of incidence is up to 3,640 times higher in patients with AIDS compared to the normal population [9]. More interestingly, subjects who received transplantation and are immunosuppressed with drugs have SIR of 208.0 for Kaposi's sarcoma and are also at increased risk for other infection-related cancers [9]. Hence, immunodeficiency is an important risk factor for cancer development. All the interventions targeting immune system directly or indirectly to improve immunity will reduce infections in immune-deficient patients and thereby prevent infection-related cancers [10, 11]. In addition to immunodeficiency, chronic inflammatory states and autoimmunities predispose individuals to cancer development [12–14]. Incidence of cancers also varies in different age groups. Total cancer incidence increases by aging either in men or women (Fig. 1.2a) [15]. Overall cancer incidence per 100,000 in adults is 202.8 in men and 164.4 in women, while the corresponding figure is only 9.4 in children below 18 years. It is suggestive of up to 20 times increase in incidence of cancers by aging. Accumulating genetic alterations in a long period of time and gradual deterioration of immune system by aging, regarded as immunosenescence, are all attributed to increased incidence of cancers with increase in age [16, 17].

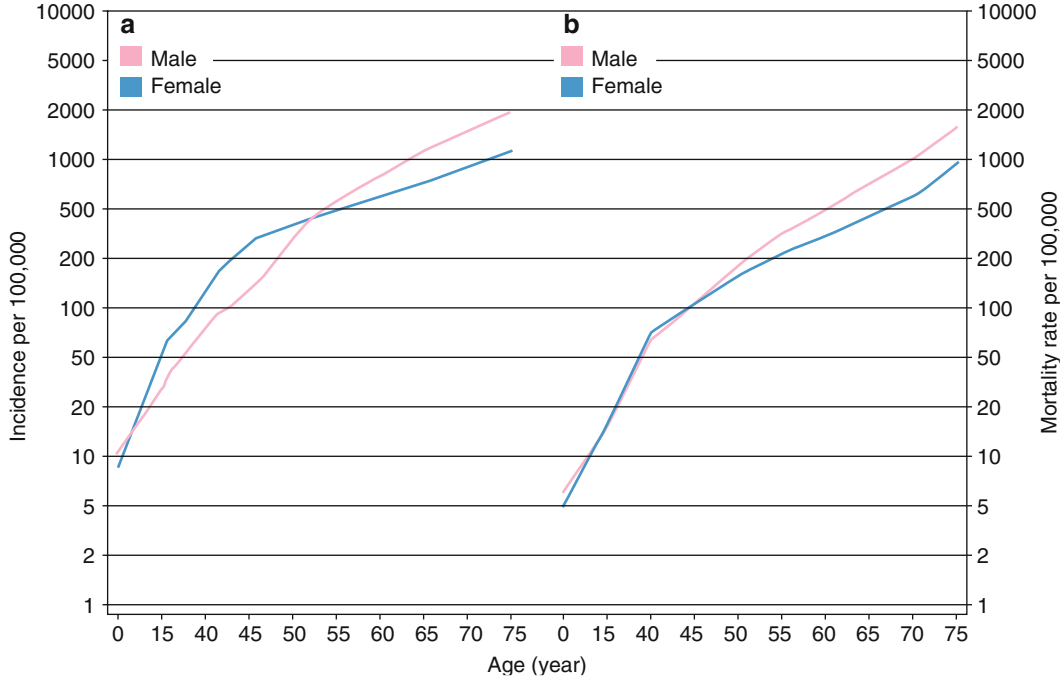


Fig. 1.2 (**a**) Worldwide overall cancer incidence estimation for different ages; by increasing age, incidence of cancers will increase both in men and women. (**b**) Worldwide overall cancer mortality rate estimation for different ages; by increasing age, mortality rate of cancers will increase either in men or women

Interestingly, types and the course of cancers are also different between children and adults. Leukemia is the most common cancer in childhood with incidence of 2.9 per 100,000, while lung, breast, prostate, and colorectal cancers with incidence of more than 20 per 100,000 are the most common cancers in adults. With respect to rapid expansion of elder population all around the world, there is an emerging need for novel strategies to prevent and treat cancers [18].

In addition to environmental factors and host characteristics, incidence of cancers varies with respect to cancer sites. Some cancers are so common, while some are relatively uncommon (Fig. 1.3). The highest incidence of cancer is attributed to breast, prostate, colorectal, lung, and cervical cancers with incidence of 38.9, 27.9, 22.9, 17.2, and 15.2 per 100,000, respectively. Beyond the range, testis cancers, multiple myeloma, nasopharyngeal cancers, Hodgkin lymphoma, and Kaposi's sarcoma are the least common cancers worldwide with incidence of 1.5, 1.4, 1.2, 1.0, and 0.4 per 100,000, respectively. As described in the following, fatality of cancers with high incidence is not low; notably, some like lung cancers have also the highest mortality rates among cancers. As current surgical, radiotherapeutic, and chemotherapeutic approaches failed to improve the outcome of cancers, novel approaches like immunotherapy may be the solution.

1.2.2 Cancer Mortality Rate

Worldwide mortality rate of overall cancers was estimated to be 105.6 per 100,000 in 2008, and it is also predicted to grow in the future if health systems do not improve worldwide. In contrast to higher incidence of cancers in developed high-resource countries, the mortality rate of cancers is higher in low-resource countries (Fig. 1.4). The highest age-standardized mortality rates of cancers per 100,000 are recorded in Mongolia with 185.2, followed by Hungary with 166.1, Armenia with 154.2, Uruguay with 150.6, and

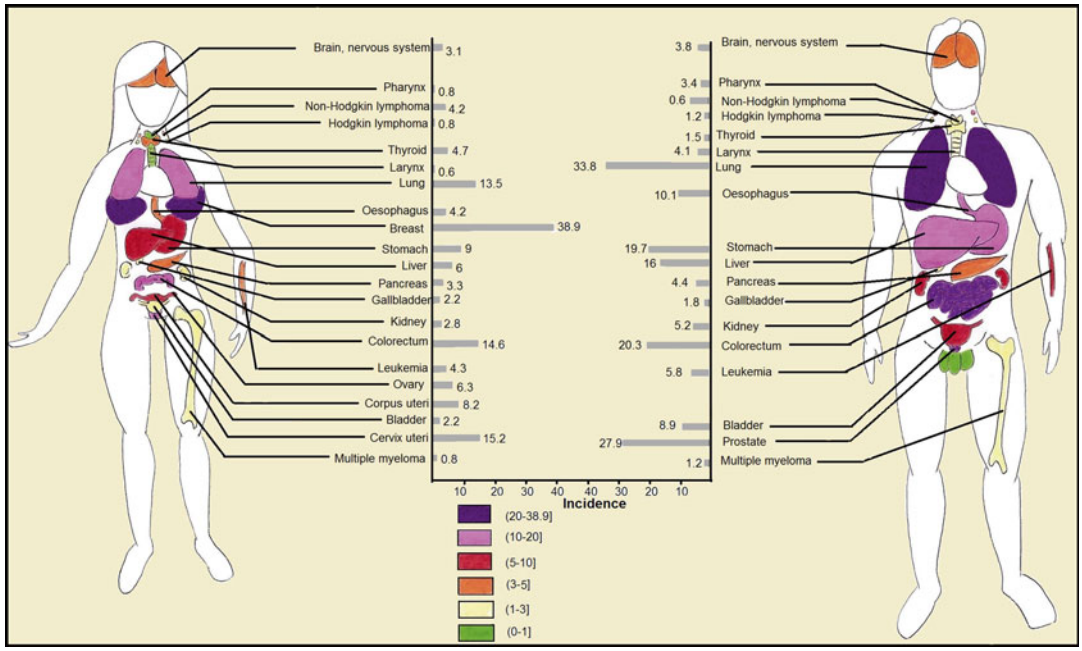


Fig. 1.3 Worldwide age-standardized estimate of incidence of each cancer type per 100,000

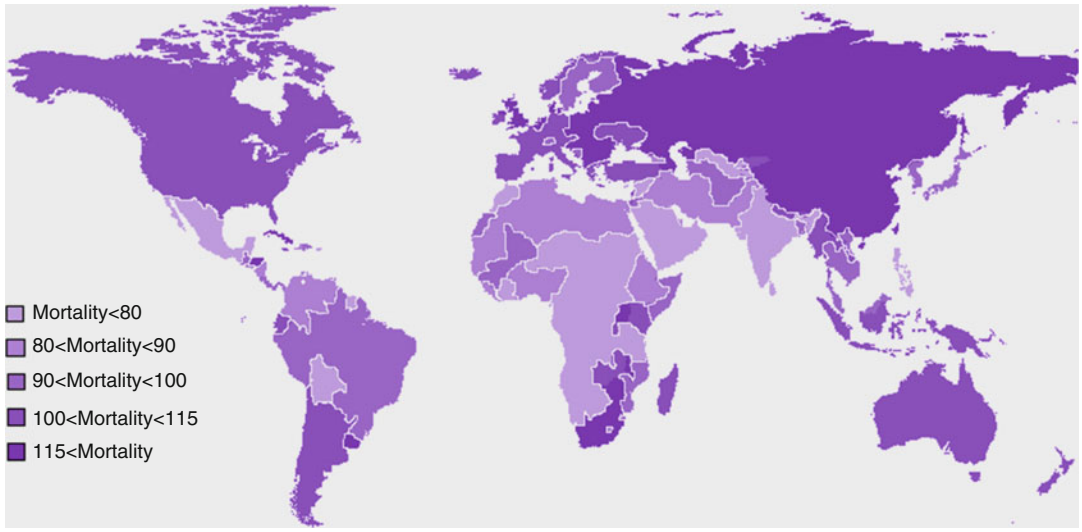


Fig. 1.4 Estimated age-standardized mortality rate of overall cancers per 100,000 in different countries

Serbia with 142.6. As evident, area of residence affects cancer status. Population composition particularly mean age of people, lifestyle, environmental factors including pollutants and status of infectious diseases in that area, and vaccination and screening programs are determinants of cancer incidence in each geographic area. However, mortality rate of cancers is affected by access to health facilities together with natural course of disease and its fatality. Proportion of cancer death to new cases is the highest in Africa; conversely the least proportional death has been recorded in

North America. Disparities in receiving immunotherapy also exist in nationwide perspective in which patients with lower socioeconomic status are less likely to benefit from novel efficacious therapeutic modalities [19]. Access to current standards of care as well as novel treatments affects the outcome of cancers. It implies that our interventions are efficacious in changing the outcome of cancers but should be spread worldwide equally. To reach this goal, spread of knowledge about new therapeutic modalities as well as investment of international and national organizations on cancer are needed.

Similar to incidence, patient's characteristics affect the outcome of cancer. Immunodeficient patients are at increased risk of cancers and also have higher risk for cancer-related mortality. However, investigations indicate that cancer-related mortality will dramatically decrease by interventions aimed to preserve immune system functions [20]. As expected, mortality rate of cancers also increases with aging (Fig. 1.2b), which may be explained by higher fatality of cancers occurring in elderly as well as deterioration of immune functions in this group of patients [21].

The greatest determinant of cancer mortality is the site of the tumor and its stage at diagnosis. A five-year survival of less than 10 % in patients with pancreatic, liver, esophageal, and lung cancers warrants the need for novel, more efficacious therapeutic modalities. In other words, more than 90 % of patients with these fatal cancers will be deceased in only 5 years. Regardless of sex, the highest mortality rates are seen in patients with lung, breast, stomach, liver, and colorectal cancers with age-standardized mortality rates of 19.3, 12.4, 10.2, 9.9, and 8.2 deaths per 100,000. Age-standardized mortality rate of different cancers in both sexes is depicted in Fig. 1.5.

1.2.3 Burden of Cancers

Worldwide, it was estimated that about 169.3 million healthy life years were lost due to cancer in 2008 [2]. Distribution of disability-adjusted

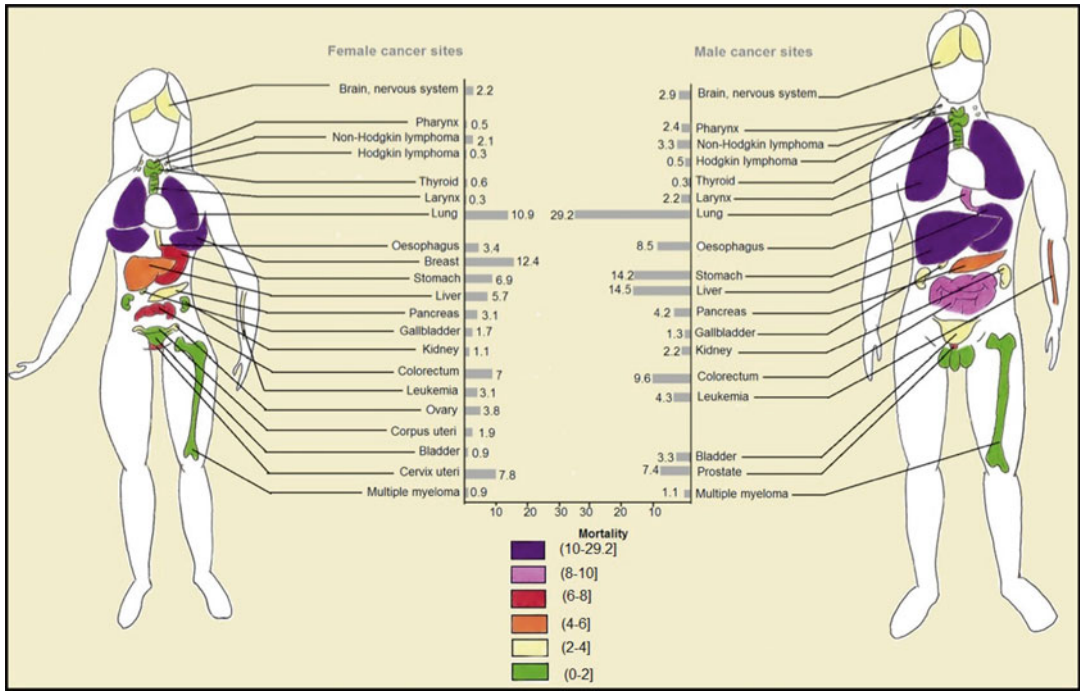


Fig. 1.5 Worldwide age-standardized estimate of age-standardized mortality rate of each cancer type per 100,000

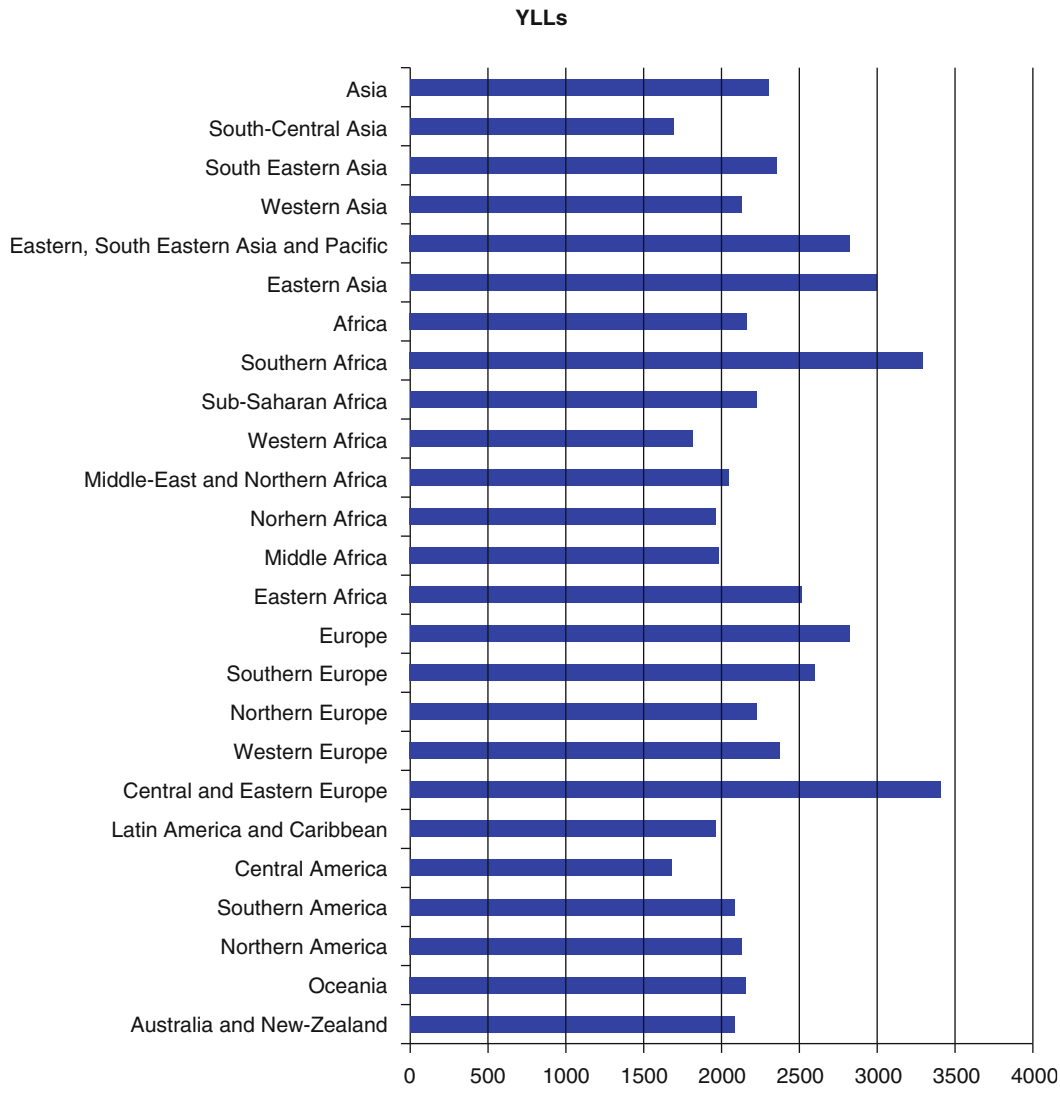


Fig. 1.6 Estimate of age-standardized rate of years of life lost (YLLs) per 100.000 for different areas

life year (DALY) of cancers was similar to distribution of cancer death worldwide since years of life lost (YLL) accounts for higher proportion of DALY than years lived with disability (YLD) [2]. The highest YLLs are seen in Central and Eastern Europe, Southern Africa, and Eastern Asia, respectively (Fig. 1.6). Higher YLD is seen in countries with very high human development index (HDI), while YLD total is lower in countries with low HDI [2] (Fig. 1.7). As in high-resource settings, life expectancy of patients with cancers is higher; thus, patients will remain alive but with cancer-related morbidities. With respect to greater contribution of YLLs to DALYs, approximately 70 % of DALY of overall cancers is attributable to less developed countries (Fig. 1.8). The highest DALY of cancers in men is recorded in Hungary with 4,756 per 100,000, followed by Mongolia with 4,526 per 100,000, Armenia with 4,243 per 100,000, Belarus with 4,171 per 100,000, and Uruguay with 3,891 per 100,000. This figure is a bit different for DALY

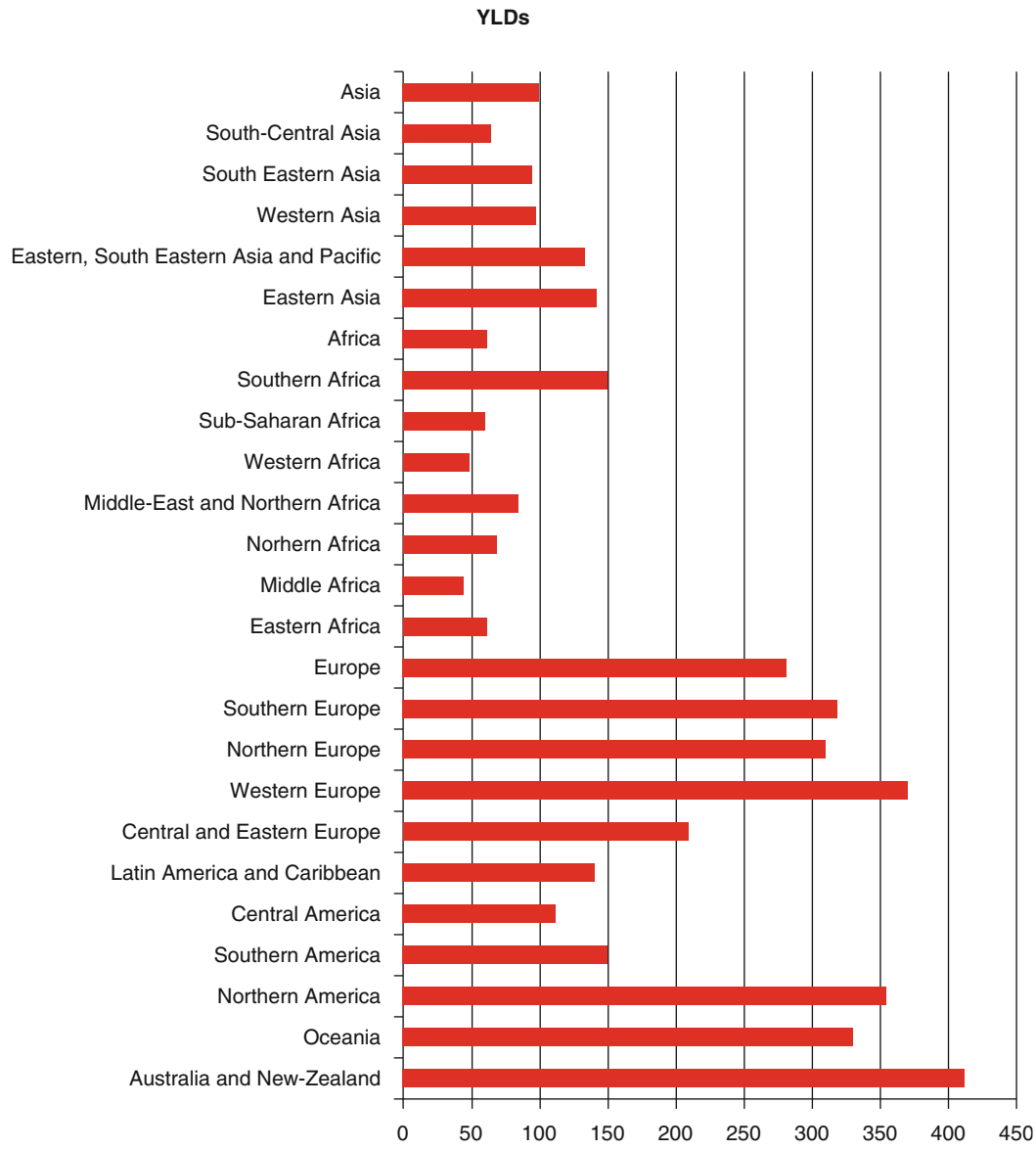


Fig. 1.7 Estimate of age-standardized rate of years lived with disability (YLDs) per 100.000 for different areas

in women, as the highest DALY denotes to Malawi with 4,416 per 100,000, Uganda with 4,331 per 100,000, Zimbabwe with 3,594 per 100,000, Mali with 3,384 per 100,000, and Zambia with 3,357 per 100,000. Worldwide, lung cancer in men and breast cancer in women have the highest DALY; however, in Sub-Saharan Africa, infection-related cancers have the highest DALY in both sexes [2]. In Sub-Saharan Africa, infection-related cancers of Kaposi's sarcoma, liver and cervical cancers, and NHL had the 36 % of overall DALYs, which are dramatically higher compared to other regions. Figure 1.9 shows specific standardized DALY of each cancer site in both sexes. Of note, DALYs of overall cancers are 6 % higher in men compared to women. It is in consistence with higher incidence of cancers observed in men with a rate difference of 38.4.

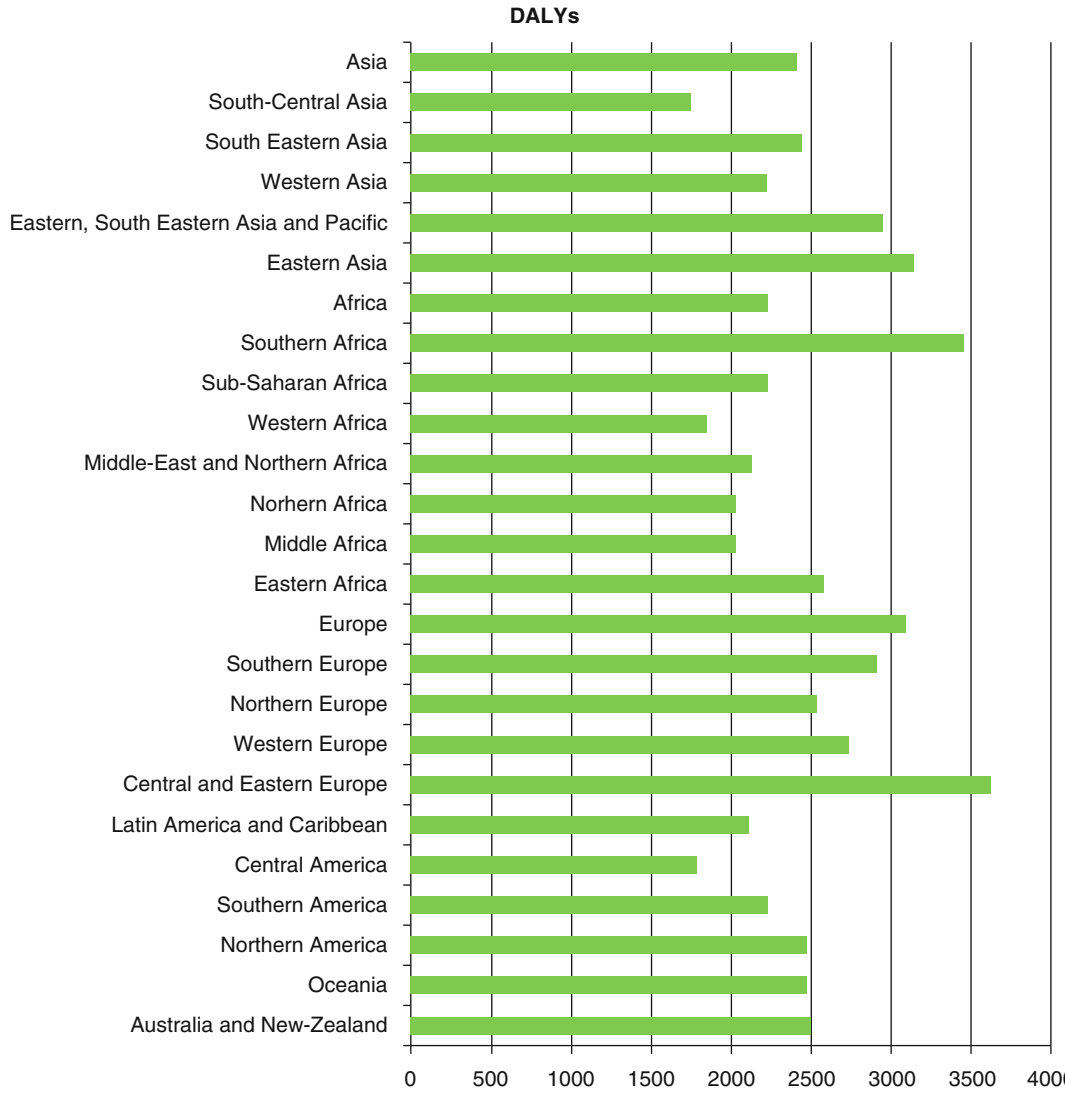


Fig. 1.8 Estimate of age-standardized rate of disability-adjusted life years (DALYs) per 100,000 for different areas

1.3 History of Immunotherapy of Cancers

First experience of cancer immunotherapy dates back to 1898 when William B. Coley succeeded to treat inoperable sarcomas by intratumoral injections of *Streptococcus pyogenes* and *Serratia marcescens* toxins [22]. This challenging observation of administration of bacterial products to already cancer patients with weakened immune system constructed the cornerstones of today's cancer immunotherapy. For ensuing 50 years, the progress of cancer immunotherapy was slow with only sporadic documents of successful treatments that were mostly irreproducible. However, further studies paved the road to immunotherapy of cancers. In this way, another important step was the attempt of Maurice Hilleman to invent hepatitis B vaccine. Hepatitis B vaccine prevents the spread of hepatitis B virus (HBV) infection and its consequences such as development of hepatocellular carcinoma (HCC) by induction of

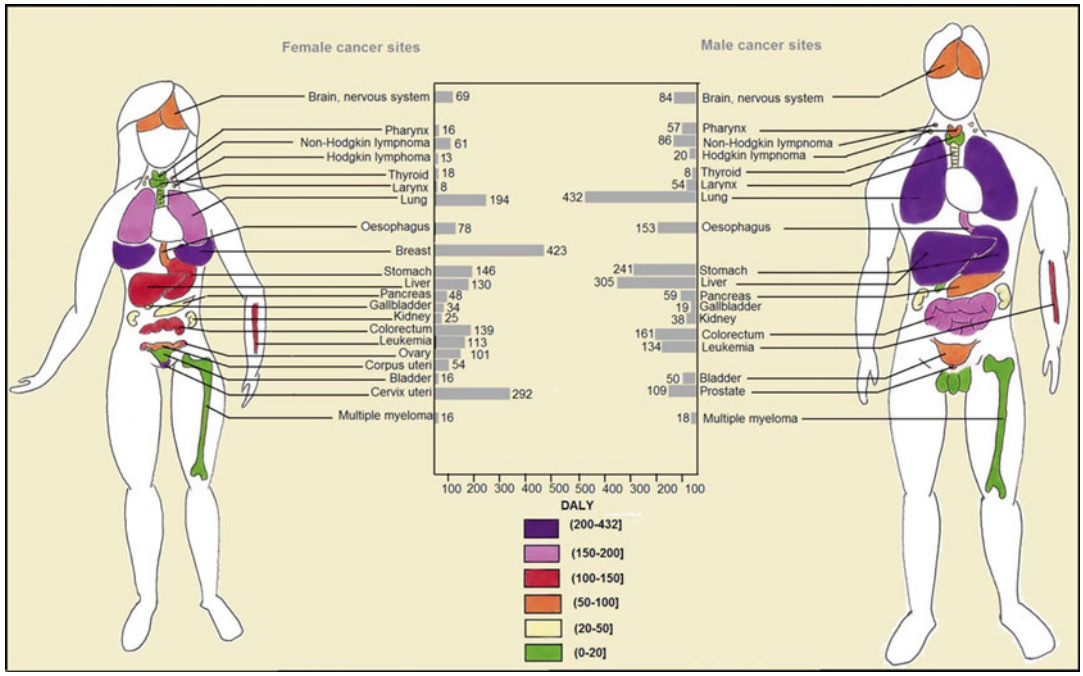


Fig. 1.9 Worldwide estimate of age-standardized rate of disability-adjusted life years (DALYs) per 100,000 of each cancer type

active immunity against HBV. Concurrent with these investigations, in 1976, post-resection intravesical instillation of bacillus Calmette-Guérin (BCG) was shown to prolong survival of patients with bladder cancer. Indeed, cancer immunotherapy was evolving in both prophylactic and therapeutic approaches. Since the 1980s, emerging field of cancer immunotherapy was revolutionized by introduction of cytokines, monoclonal antibodies (mAbs), and adoptive cell therapy in treatment of cancers. Since then, cytokines and mAbs were tested not only as standalone therapeutic modalities but also in combinational schedules with chemotherapy [23–25] and radiotherapy [26]. Interferon (IFN)-α was approved for hairy cell leukemia in 1986, and it was the first immunotherapeutic drug approved for use in melanoma patients in 1995, owing to comprehensive studies by Kirkwood and his colleagues [27–29]. Rituximab is the first mAb which received Food and Drug Administration (FDA) approval for NHL in 1997 [30]. Immunotherapy also showed efficacy in postsurgical management of patients with cancers. One of the first reports dates back to 1988 when Grohn et al. employed levamisole adjuvant immunotherapy in patients with breast cancer with equivocal results [31].

Nearby these events, in 1986, recombinant HBV vaccine was developed, and efforts to constructing human papillomavirus (HPV) vaccine were initiated. The first HPV vaccine was approved by the FDA in 2007. Similar to HBV vaccine, HPV vaccine prevents viral-induced cancers, in particular cervical cancers of the genital area and anus and oropharyngeal cancers. Despite common concept about vaccines, cancer vaccination can be applied to treat neoplastic lesions as secondary line of prevention. HPV vaccination was used to treat vulvar intraepithelial neoplasia (VIN) with promising results in 2009 [32]. In addition, dendritic cell (DC) based, peptide based, and combined vaccines were introduced to treat cancer patients in the recent decade [33].

Gene therapy, by providing the opportunity to manipulate the immune system, holds a great promise for cancer immunotherapy [33]. Gene

transfer with novel biologic or nonbiologic delivery vehicles enabled scientists to genetically modulate T cells to combat tumors [33]. Combination of adoptive T cell therapy with gene transfer was one the most important steps in the field of cancer immunotherapy. In this way, T cell receptor (TCR) gene transfer was one of the greatest achievements in treating cancers, reached in 2001 [34]. Interestingly, further investigations suggest hopes for combination of cancer vaccines with current mainstays of cancer treatments. One of the most interesting studies was conducted by Antonia et al. on patients with extensive-stage small cell lung cancer in 2007 [35]. The patients received dendritic cells transduced with the full-length wild-type p53 gene delivered via an adenoviral vector as cancer vaccine prior to chemotherapy. Significant clinical response was observed in more than half of the patients owing to pre-chemotherapeutic stimulation of the immune system by cancer vaccine [35]. Another progress was the combination of adoptive T cell therapy with radiotherapy made in 2005. In this experience, combined radiotherapy with intratumoral injection of the cancer vaccine was promising in patients with refractory hepatoma [36]. Vaccine-based therapeutics aim to enhance endogenous immune response against cancers, while adoptive T cell therapy is based on infusion of primed tumor-specific T cells. Finally, sipuleucel-T (Provenge), an active autologous dendritic cell-based vaccine, received FDA approval for patients with castration-resistant prostate cancer in 2010 [37]. Sipuleucel-T is the first and the sole FDA-approved therapeutic cancer vaccine. Unfortunately, no adoptive T cell therapy has yet obtained FDA approval maybe due to its obstacles in providing sufficient amounts of primed specific T cells [38]. However, cancer-testis antigens of MAGE family with restricted expression in tumor cells hold promise for the future of not only cancer vaccines but also adoptive T cell therapies [39, 40].

Almost all the progresses in immunotherapy are owned to progresses in understanding the immunopathology of cancers. This is well reflected in development of novel monoclonal antibodies and immune adjuvants through the past decade. Ipilimumab (also known as MDX-010-20 in clinical trials) received FDA approval for treatment of patients with advanced metastatic melanoma in 2011. Ipilimumab blocks the inhibitory effects of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) on presenting the tumor antigens and improves cytotoxic T lymphocyte function [41, 42]. Pegylated IFN-α has been approved for treating patients with advanced melanoma in March 2011 [33]. Another progress was the discovery of pattern recognition receptors and targeting them in cancer immunotherapy; imiquimod (Aldara), a Toll-like receptor 7 agonist, was employed in the treatment of VIN since 2008 [43]. Imiquimod has been approved by the FDA for external genital warts, papilloma, superficial basal cell carcinoma, and actinic keratosis [44]. Imiquimod administration results in endogenous induction of IFN production [44]. Figure 1.10 shows a timeline of important events related to understanding either the immunopathology of cancers or immunotherapy of cancers.

Through more than a century of experience with immunotherapy, scientists and health-care providers aimed to reinstate immune surveillance against tumor cells in either primary lesions or metastases by immunotherapy. Immunotherapy provides a dynamic and specific formation of adaptive immune response that fights tumor-mediated immunoediting. This effect of immunotherapy promises long-term protection against relapse of cancers, while it may not be efficacious as other drugs, radiotherapy, and surgery in immediate debulking of tumor masses. Hence, combinational therapies may be the key to improve both tumor progression and overall survival of the patients. Despite brilliant progresses in the field of cancer immunotherapy, it is still in its infancy and may provide definite treatment for all cancers in its maturity.

1.4 Immunotherapy Is Going Upstream to Combat Cancers

Immunotherapy helps health-care providers prevent not only cancer development but also prevent its further progression and cancer-related

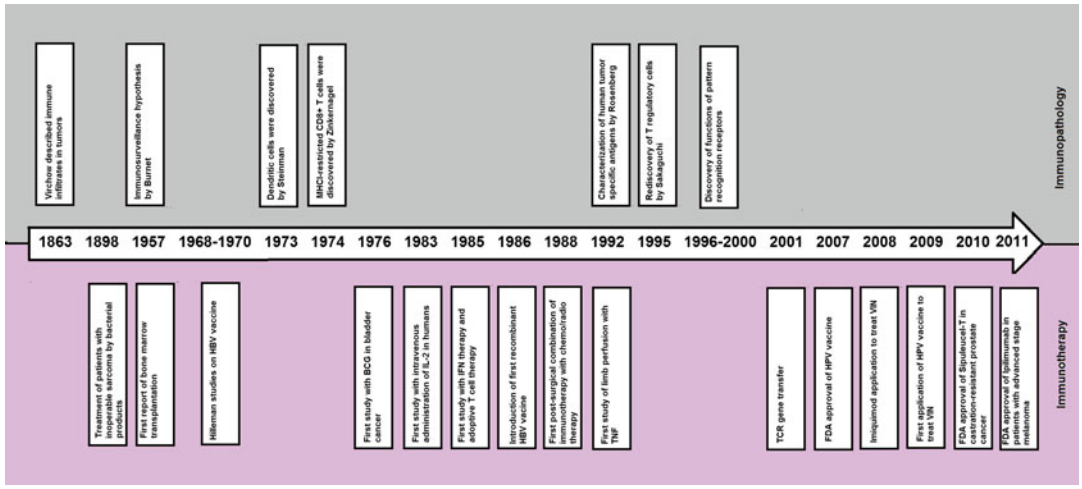


Fig. 1.10 Timeline of important events related to immunopathology and immunotherapy of cancers. *Upper side* of the *arrow* shows events related to immunopathology, whereas events related to immunotherapy are shown in the *bottom* of the figure. *TCR* T cell receptor, *IL-2* interleukin-2, *IFN* interferon, *TNF* tumor necrosis factor, *BCG* bacillus Calmette-Guérin, *HBV* hepatitis B virus, *HPV* human papilloma virus, *MHCI* major histocompatibility complex-1, *FDA* Food and Drug Administration, *VIN* vulvar intraepithelial neoplasia

complications offering prevention from cancers at all levels. Modulation of immune responses in favor of enhancing tumor cell detection and immune clearance of these cells is what immunotherapy does. In addition, immunotherapy helps recover an injured or completely destroyed immune system after intensive cancer therapies as occurred in intensive chemotherapy schedules.

Chronic inflammation owing to infectious etiologies or continuous sterile inflammation appears to cause cancers of variable origins [45]. Targeting the immune system to control infections known as causes of variable cancer as well as conditions associated with chronic inflammation (i.e., autoimmunities) results in a dramatic decrease in incidence of cancers [46]. This application of immunotherapy prevents cancers at the primary level. Interestingly, not all subjects with predisposing chronic inflammation state develop cancers. For instance, the human herpesvirus 8 (HHV8) causes Kaposi's sarcoma in the context of HIV caused by immunodeficiency or drug-induced immunodeficiency. Hence, immunotherapeutic approaches prevent the spread of HIV in the community and can be regarded as primordial level of prevention from cancers. A considerable numbers of vaccines and immune adjuvants as well as monoclonal antibodies are developed to combat cancers at primary and primordial stages.

On the other hand, a broad spectrum of immunotherapeutic medications have been developed to treat patients with cancers. At this stage, immunotherapy acts as the second level of prevention from cancers. Adoptive cell therapy, therapeutic cancer vaccines, immune adjuvants, cytokines, monoclonal antibodies, and gene therapies are already established to treat different cancers. Treatment of an already diagnosed patient with any type of cancer is designated as the secondary level of prevention from cancer. This is the most known kind of use of immunotherapy to combat cancers; however, it acts lately after establishing the cancer and usually can eliminate cancer in a limited number of patients. Today, immunotherapy is considered as a first line of treatment of a wide range of cancers. Immunotherapy also offers hope for patients failed with other available therapeutics and patients with end-stage cancers. In addition, combination of immunotherapy with almost all available therapeutic approaches has been tested and holds promises at least to increase progression-free survival of patients. Many new

immunotherapeutic drugs are now under development, and many of them are in clinical trials.

Finally, immunotherapy can ameliorate the toxic effects of other available therapeutic modalities, known as supportive immunotherapy. At this stage, immunotherapy prevents further disabilities due to cancer progression or therapies and improves quality of life of patients. Accordingly, immunotherapy also provides a tertiary level of prevention from different cancers.

1.4.1 Prophylactic Implication of Immunotherapy

It is best to prevent cancer development than to prevent cancer progression and its related complications. Immunotherapy is defined by treatment of disease using enhancement or suppression of immune responses. Accordingly, it can be used to treat cancers as well as treat underlying diseases that cause cancers. The latter is prophylactic use of immunotherapy for cancers. Elimination of infections known to cause cancers and improving the immune system by eliminating chronic inflammation states and immunodeficiency are the bases of prophylactic cancer immunotherapy. Despite complex expensive immunotherapeutic approaches employed at other levels of prevention from cancers, immunotherapy at primary level includes simple inexpensive methods. To follow a healthy lifestyle, daily intake of anti-inflammatory drugs, vaccination against oncogenic viruses, and finally immunotherapies aimed to control spread of HIV are all acting to prevent cancers at primary or primordial level.

Increased body mass index (BMI) is believed to increase the risk of several cancers [3]. Correction of lifestyle by doing regular exercise, eating fresh foods full of antioxidants, restricting calorie intake, and eating low-fat foods leads to prevention of cancers [47–49]. The main mechanism is inhibiting tumorigenesis and providing inappropriate microenvironment for tumor growth; however, a healthy lifestyle improves immune function [47, 48, 50]. Indeed, the main antitumor mechanism of having a healthy lifestyle is to prevent tumorigenesis, but combating chronic inflammation state as well as enhancing the function of effector innate cells to eliminate tumor cells should also be acknowledged [50–54]. From this perspective, a healthy lifestyle acts as an old, inexpensive, simple immunomodulatory way of preventing cancers at primary level [47, 48]. Thus, healthy lifestyle can be regarded as the first ancient immunotherapy employed against cancers.

Infection-related cancers are caused by a variety of viral and bacterial agents. Chronic inflammation, mutagenesis by integration of pathogen genes with host genome, and induction of immunodeficiency are all believed to be mechanisms by which infectious agents can cause cancers. HBV is one of the most important agents known to cause HCC. The HBV vaccine is able to induce immunity against HBV infection in more than 95 % of vaccinated subjects. Thereby, it can prevent HBV-caused HCC [55]. In endemic areas of HBV infection like Taiwan, it has been shown that HBV vaccination has resulted in decrease of HCC incidence from 1.08 per 100,000 to 0.49 per 100,000, suggestive of a 50 % decrease in HCC incidence [56]. Interestingly, in some areas like Alaska, known as an endemic area of HBV infection in the United States, HBV vaccination has eliminated HCC in children [57]. In addition, in coinfected patients with HBV and hepatitis C virus (HCV), immunotherapy with IFN-α reduces the risk of HCC development [58]. Hence, active immunotherapy against HBV infection leads to significant reduction in HCC occurrence, and it is logical to assume HBV vaccine as a prophylactic immunotherapy against cancers.

HPV vaccine is another golden step in history of cancer immunotherapy. HPV-16 and HPV-18 are responsible for more than 70 % of cervical cancers worldwide. However, HPV-6 and HPV-11 are also targeted in newer generations of HPV vaccine. The best target group of vaccine administration is young girls who are not still sexually active. Indeed, the vaccine is beneficial for someone who is not infected with the virus. Quadrivalent vaccination against HPV (16, 18, 6, and 11) results in 98 % (95 % confidence interval of 86–100 %) protection against HPV-16 or HPV-18 cancer-related lesions. It also confers weak

protection against other cervical neoplastic lesions [59]. As the cervical cancer holds the second place in incidence and DALYs of cancers of women all around the world, its prevention is of utmost importance. HPV vaccine is a cost-effective approach to reduce cervical cancer incidence worldwide [60, 61]. In addition to cervical cancer, HPV-16 and HPV-18 are implicated in the development of other perineal and perianal neoplastic lesions as well as squamous cell carcinoma of the head and neck and oropharyngeal cancers. HPV vaccination can reduce risk of these cancers but with a lesser extent [62]. Vaccination of women also offers protection against HPV-related cancers in men owing to herd immunity; however, covering boys in vaccination programs is not without clinical benefits and needs further investigations [62]. Interestingly, vaccination of women with high-grade VIN with a mix of oncoproteins E6 and E7 from HPV-16 resulted in relief of VIN-related symptoms in 60 % of patients, highlighting the important role of HPV vaccine for cervical cancers [32].

By contrast to HBV and HPV vaccine, other immunotherapeutic approaches to control HIV and Epstein-Barr virus (EBV) are on the way. In the development of anti-HIV agents, targeting the adaptive immune system failed due to progressive involvement of the adaptive system. However, recent studies herald promises in targeting the innate immunity by targeting DCs, pattern recognition receptors, and alarmins [63]. Prevention from spread of HIV and progression of HIV infection to AIDS averts AIDS-associated syndromes and AIDS-defining cancers (Kaposi's sarcoma, cervical cancer, and NHL). Immunotherapeutic approaches combat HIV spread, and others used to prevent infections in immunodeficient patients whether primary or secondary are regarded as prophylactic implication of cancer immunotherapy [10, 63]. Autosomal recessive hyper-IgE syndrome, X-linked agammaglobulinemia, common variable immunodeficiency, X-linked lymphoproliferative disease, IL-2-inducible T cell kinase (ITK) deficiency, epidermodysplasia verruciformis, and warts, hypogammaglobulinemia, infections, myelokathexis (WHIM) syndrome are primary immunodeficiency diseases with increased risk of infection-related cancers. Granulocyte-macrophage colony-stimulating factor (GM-CSF), intravenous immunoglobulin (IVIG) administration, and allogeneic hematopoietic stem cell transplantation (HSCT) provide benefits for these patients [10]. EBV is one of the most common viral infections with more than 90 % seropositivity worldwide [64]. It is implicated in the development of several cancers including Burkitt's lymphoma, NK or T cell lymphoma, Hodgkin lymphoma, and nasopharyngeal carcinomas particularly in subjects with incompetent immune system [64]. EBV-associated lymphoproliferative disorders are of utmost clinical importance in patients who undergone transplantation. Immunotherapy with adoptive T cell transfer specific for EBV antigens promises hope to prevent EBV-associated lymphoproliferative disorders in vulnerable subjects with EBV viremia [65–67].

Sterile chronic inflammation and immune dysregulation states like autoimmunities also predispose individuals to cancer development. Sterile chronic inflammation is strongly associated with colorectal cancer development. Patients with ulcerative colitis are at increased risk of colorectal cancers that might be prevented by anti-inflammatory drugs like 5-aminosalicylic acid (5-ASA) [68]. Another group of patients suffering from familial adenomatous polyposis (FAP) has chronic inflammation state and 100 % increased risk of colorectal cancers. Celecoxib, a cyclooxygenase-2 (COX-2) inhibitor, is approved to be used for prevention of colorectal cancer in this group of patients [68]. In subjects with no underlying inflammatory disease of the gastrointestinal tract, nonsteroidal anti-inflammatory drugs (NSAIDs) reduce the risk of colorectal cancer by 18–39 %; however, to date no NSAIDs have been approved to prevent sporadic tumor prevention [69–71]. Similarly, monoclonal antibodies used in the treatment of patients with autoimmunities that predispose patients to malignancies can be regarded as another prophylactic immunotherapeutic approach [72].

Altogether, immunotherapy can be used to prevent development of cancers. Prophylactic use of immunotherapy, also regarded as immunoprevention, offers benefits for a wide range of cancers particularly infection-related cancers. Both active immunizations with vaccines and passive immunizations with monoclonal antibodies and cytokines are employed in prophylactic immunotherapy. Many other prophylactic immunotherapeutic modalities may be introduced in the future.

1.4.2 Therapeutic Implication of Immunotherapy

Immunotherapy currently has been set as a key component of therapeutic regimens of many cancers [33]. Bone marrow transplantation (BMT) following ablative/non-myeloablative bone marrow therapies is now the standard of care of many hematological malignancies. Similarly donor lymphocyte infusion following failed BMT is an accepted immunotherapy for treatment of relapsed hematological malignancies [73–75]. Once cancer develops, immunotherapy helps the patient's immune system fight with tumor cells to prevent cancer progression and finally elimination of cancer [76]. Benefits of immunotherapy are not only restricted to patients with advanced stages of cancers, and by contrast, patients with early stages of cancers are good candidates for immunotherapy. Bacillus Calmette-Guérin (BCG) for early-stage bladder carcinoma [77–79] and sipuleucel-T immunotherapy for castration-resistant prostate cancer are all examples of approved immunotherapies employed at different stages of urological cancers [37, 80, 81]. In addition, immunotherapy offers hope for approximately all types of cancers. FDA-approved immunotherapeutic drugs are now available for chronic lymphocytic leukemia (CLL) [82, 83], NHL [30, 84, 85], Hodgkin lymphoma (HL) [86, 87], acute leukemia [88], breast cancer [89], lung cancer [90], colorectal cancers [69, 91–93], bladder cancer [78, 79], prostate cancer [80, 81], renal cell carcinoma [29, 94], basal cell carcinoma [44, 95], melanoma [96–98], cervical cancer [5, 32, 59, 60], hepatocellular carcinoma [55, 56, 58], and soft tissue tumors [99, 100]. Promisingly, a large number of immunotherapies are also under investigation. Table 1.1 shows current FDA-approved immunotherapies to treat different cancers.

Immunotherapeutic weapons are of wide categories: immunomodulator monoclonal antibodies whether agonistic or blocking, cytokines [interleukin (IL)-2, IFN-α, IL-12, GM-CSF, and tumor necrosis factor-α (TNF)-α], therapeutic cancer vaccines particularly DC vaccines, adoptive T cell transfer, gene therapy, and novel immune adjuvant and delivery vehicles are all available to help cancer patients. Elimination of immunosuppression and boosting of the immune responses against tumor cells are what immunotherapy does. These effects of immunotherapy offer long-term antitumor immune response that fights with already established cancer, prevents its progression, and prevents new metastases. Accordingly, immunotherapy should not logically become restricted to patients with advanced and metastatic cancers. Despite initial experiences with immunotherapy on patients who failed with other therapeutics, today, immunotherapy is set to become the first-line treatment either in combination with other therapeutic modalities or as stand-alone therapy. In addition, to accurately measure the immunotherapy-induced tumor destruction, immune-related response criteria have been developed and should be used in clinical practice and future researches. In the following, different aspects of cancer immunotherapy as therapeutic (second level of cancer prevention) have been described.

1.5 Strategies of Cancer Immunotherapy

Two main strategies of cancer immunotherapy to treat cancer patients are (1) reduction of immunosuppressive milieu and (2) boosting the antitumor responses.

Table 1.1 FDA-approved immunotherapeutic agents to treat cancers

Category	Drug/vaccine[a]	Recommended schedule	Indications in cancers	Side effects[c]	Mechanism of action	Year of FDA approval
Immunomodulator mAb	Ipilimumab (MDX-010, Yervoy) [96–98]	(3 mg/kg IV/3 weeks)×4	Metastatic melanoma	Autoimmune reactions, gastrointestinal manifestations, colitis, hepatotoxicity rash, pruritus	Blockade of CTLA-4	2011
Immunomodulator mAb	Brentuximab vedotin (Adcetris) [86, 87, 267, 268]	(1.8 mg/kg IV/3 weeks)×4	Relapsed or refractory Hodgkin lymphoma and anaplastic large cell lymphoma	Cytopenia (all lineages), peripheral sensory neuropathy, nausea and vomiting, fatigue, URI, pyrexia	Targeting cells bearing CD30 to deliver toxic chemicals	2011
DC vaccine	Sipuleucel-T (Provenge) [37, 269, 270]	A single course with autologous DCs activated with PA2024 and GM-CSF	Castration-resistant prostate cancer	Cardiovascular events, chills, fever, fatigue, nausea, and headache	Increase tumor antigen presentation	2010
mAb	Denosumab (Prolia, Xgeva) [271]	120 mg SC/4 weeks	Bony solid's tumors, bone metastases	URI, UTI, cataracts, rash, gastrointestinal manifestations	Inhibitor of osteoclast maturation by RANK ligand inhibition	2010
Immunomodulator mAb	Ofatumumab (Arzerra) [82, 272–274]	Dose 1 = 300 mg; doses 2–8 = 500 or 1,000 or 2,000 mg, IV	Refractory chronic lymphocytic lymphoma, phase I/II for follicular lymphoma	Cytopenia (all lineages)	Targeting CD20, complement activation, and ADCC	2009
mAb	Panitumumab (Vectibix) [92, 93, 275–278]	9 mg/kg IV on day 1 with combined chemotherapy or 6 mg/kg/2 weeks IV with combined chemotherapy, 6 mg/kg twice per week IV in a monotherapy schedule	Colorectal cancer, refractory esophageal cancer, refractory ovarian cancer, and biliary tract cancer	Skin toxicities most commonly dry skin, rash, acne	Targeting epidermal growth factor receptor	2006
mAb	Cetuximab (Erbitux) or doxorubicin-loaded cetuximab [279–281]	400 mg/m^b initial dose, then 250 mg/m^b per week	Colorectal cancer, head and neck cancers, phase II study for lung cancer	Skin toxicities most commonly acne	Targeting epidermal growth factor receptor	2004

mAb	Bevacizumab (Avastin) [89, 232, 233, 282]	5 mg/kg every 2 week	Colorectal cancers, lung cancer, renal cell carcinoma, and glioblastoma multiforme[b]	Hypertension, hemorrhage, ischemic heart disease	Inhibitor of VEGF-A	2004
Immunomodulator mAb, radioimmunotherapy	Tositumomab or ^{131}I-tositumomab (Bexxar) [283, 284]	Dosimetric dose of 5 mCi on day 19 and therapeutic dose of 0.75 Gy on day 12	Non-Hodgkin lymphoma, diffuse large B cell lymphoma, follicular lymphoma	Hypersensitivity reactions, thrombocytopenia	Targeting cells bearing CD20 for delivery of radiotherapeutic agent (^{131} I) or complement activation and ADCC	2003
mAb, radioimmunotherapy	Ibritumomab tiuxetan (Zevalin), ^{90}yttrium, ^{111}I-ibritumomab [285, 286]	0.4 mCi/kg following rituximab or chemotherapy	Non-Hodgkin lymphoma	Hypersensitivity reactions, pancytopenia	Targeting cells bearing CD20 for delivery of radiotherapeutic agents, complement activation, and ADCC	2002
mAb	Alemtuzumab (Campath) [287]	30 mg three times a week	Chronic lymphocytic leukemia	Infections, pancytopenia	Targeting CD52 bearing cells, ADCC	2001
mAb	Gemtuzumab linked to calicheamicins (Mylotarg) [288–290]	3–5 mg/m[b] at 2 doses with a 3-week interval	Acute myeloid leukemia	Pancytopenia, respiratory disorders, tumor lysis syndrome, hypersensitivity	Targeting CD33-bearing cells to deliver cytotoxic agents	2000
mAb	Trastuzumab (Herceptin) [89, 291, 292]	4 mg/kg loading, then 2 mg/kg/week	Breast cancer	Gastrointestinal side effects, hepatotoxicity, metabolic effects	Targeting ErbB2	1998
Cytokine	Recombinant IL-2 (aldesleukin) [196, 197, 293]	1,000 U/ml for a course of therapy	Metastatic renal cell carcinoma and advanced-stage melanoma	Vascular leak syndrome	Booster of antitumor immune responses, T cell growth factor	1998
TLR7 agonist	Imiquimod (Aldara) [294, 295]	Cream of Aldara three times a week	Basal cell carcinoma, actinic keratosis	Hypopigmentation, redness, scarring	Booster of antitumor immune responses	1997
mAb	Rituximab (Rituxan, Mabthera) or ^{131}I-rituximab [226, 296, 297]	375 mg/m 2 single dose	Non-Hodgkin lymphoma	Neutropenia	Targeting cells bearing CD20 for delivery of radiotherapeutic agents, complement activation, and ADCC	1997

(continued)

Table 1.1 (continued)

Category	Drug/vaccine[a]	Recommended schedule	Indications in cancers	Side effects[c]	Mechanism of action	Year of FDA approval
Cytokine	IFN-α2B, recently pegylated (Peg) form [298, 299]	Peg-IFN-α2b 3 μg/kg/week	Hairy cell leukemia, advanced-stage melanoma	Depression, pancytopenia, bleeding, fatigue, flu-like symptoms	Booster of antitumor immune responses	1986–1997
Adjuvant	BCG [300, 301]	Weekly intravesical instillation of BCG×6	Superficial bladder cancer	BCG-osis in case of immunosuppression	Booster of antitumor immune responses	1980s

FDA Food and Drug Administration, *IV* intravenous, *SC* subcutaneous, *mg* milligram, *kg* kilogram, *U* unit, *CTLA-4* cytotoxic T lymphocyte-associated antigen 4, *mAb* monoclonal antibody, *URI* upper respiratory tract infection, *UTI* urinary tract infection, *RANK* receptor activator of nuclear factor-kappa B, *VEGF* vascular endothelial growth factor, *PDGFR* platelet-derived growth factor, *GM-CSF* granulocyte-macrophage colony-stimulating factor, *BCG* bacillus Calmette-Guérin

[a]Other drug names as well as trade names are in parenthesis

[b]Bevacizumab was approved for breast cancer but the approval was revoked in 2011 due to severe side effects including severe hypertension and hemorrhage. In addition, no evidence was found on the efficacy of bevacizumab in extending overall survival or improving quality of life of patients with breast cancers. Recently, it is used in combination with chemotherapy in recurrent metastatic HER2-positive breast cancer patients with promising results

[c]Infusion-related adverse events are other side effects of drugs administered intravenously particularly mAbs. These side effects include hypotension, shortness of breath, bronchospasm, rigors, fever, chills, and/or rash

1.5.1 Immunotherapy Acts to Eliminate Immunosuppression

Immunotherapy reduces immunosuppression by blocking the negative regulatory receptors and inhibitory checkpoints [CTLA-4 and programmed death-1 (PD-1)], blocking immunosuppressive cytokines [transforming growth factor-β (TGF-β), IL-10, and TNF], blocking immunosuppressive enzymes [indoleamine 2,3-dioxygenase (IDO)], and targeting the T regulatory cells. Blockade or inhibition of negative regulators of the immune system enhances endogenous antitumor responses as well as antitumor activity of the immune system following other therapeutic approaches.

CTLA-4 (CD152) is expressed on T cells in combination with a wide variety of other immune cells [101]. It acts as a negative immune regulatory receptor that switches off T cell attacks to tumor cells. Indeed, CTLA-4 is one of the main players in establishing peripheral tolerance [101]. It competes with CD28 to bind to B7-1/B7-2 with higher affinity and avidity [101]. Two mAbs have been developed to block CTLA-4: ipilimumab (MDX-010) and tremelimumab (CP-675,206). Both of these antibodies are under investigation for a wide range of cancers. Tremelimumab has been tested in treatment regimen of patients with metastatic or refractory melanoma, colorectal cancer, and prostate cancer. Unfortunately, favorable response in terms of tumor regression and improvement of survival was only detected in less than 10 % of patients [97, 102–104]. Results of trials with ipilimumab were more positive, and ipilimumab has received FDA approval for patients with metastatic melanoma [41, 98]. In addition, ipilimumab is also underway for patients with lung cancer [105, 106] and castration-resistant prostate cancer [107]. PD-1 is an inhibitory receptor present on activated T cells and B cells and has two main ligands PD-L1 and PD-L2. PD-L1 has a broad expression on not only immune cells but also nonimmune and tumor cells, whereas PD-L2 expression is restricted to antigen-presenting cells (APCs). PD-L1 is one of the most important actors in the maintenance of immunosuppressive microenvironment around tumor cells. PD-1 blocking antibody, CT-011, is under investigation for patients with advanced hematological malignancies and multiple myeloma [108, 109].

Anti-inflammatory cytokines IL-10 and TGF-β are produced by tumor cells and suppress antitumor responses. Anti-TGF-β antibodies are now in development for cancer immunotherapy. Initial experiences on animal models of osteosarcoma reduced T regulatory cell numbers and increased number of cytotoxic T lymphocyte nearby prevention from growth of new metastases [110]. Fresolimumab, a fully human anti-TGF-β, is now produced and may be tested in the treatment of cancer patients [111]. Anti-IL-10 antibodies and anti-IL-10 receptor antibodies have potential antitumor activity, but they have not currently entered into clinical trials for cancer patients [112, 113]. In addition, infliximab, an anti-TNF-α antibody, was tested in patients with RCC and resulted in 16 % partial response and 16 % stable disease among recipients [114]. TNF-α is an inflammatory cytokine, and its increased levels has been associated with poor prognosis in cancer patients [114].

IDO catalyzes degradation of essential L-tryptophan amino acid and keeps it away from activated T cells needing it for clonal expansion. Tumor cells as well as plasmacytoid DCs present in tumor-draining lymph nodes express high amounts of IDO leading to indirect suppression of antitumor responses. 1-Methyl tryptophan (1MT) inhibits IDO and prevents tumor cell growth of variable origins, but it is still under laboratory investigations [115–117].

T regulatory cells are a strong source of inhibitory signals, preventing the effect of endogenous antitumor responses and inhibiting sufficient response to immunotherapeutic agents boosting immune system. Denileukin diftitox, a conjugate of diphtheria toxin and IL-2, has efficacy in the treatment of patients with T cell lymphoma, B cell NHL, and melanoma. Further studies demonstrated its efficacy in enhancing cancer vaccine responses by depletion of T regulatory cells. Indeed, denileukin diftitox is a targeted therapy to kill T regulatory cells [84, 118–120]. Other

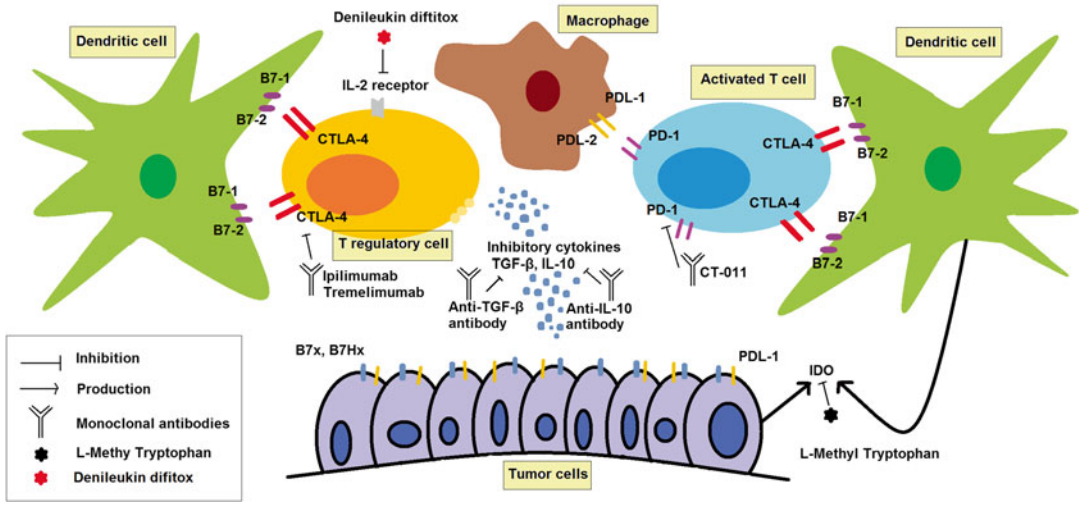


Fig. 1.11 Cancer immunotherapy eliminates immunosuppressive milieu of cancer patients. Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed death-1 (PD-1) are expressed by activated T cells and act as T cell checkpoint blockers that inhibit T cell functions. These are blocked by anti-CTLA-4 and anti-PD-1 monoclonal antibodies. Interleukin-10 (IL-10) and transforming growth factor-β (TGF-β) are inhibitory cytokines produced by T regulatory cells and tumor cells. These inhibitory cytokines are blocked by specific monoclonal antibodies that are under investigations in laboratory. Denileukin diftitox, a conjugate of IL-2 and diphtheria toxin, kills T regulatory cells and enhances endogenous or induced antitumor responses. L-Methyl tryptophan inhibits indoleamine 2,3-dioxygenase (IDO). IDO inhibits T cell expansion by degradation of essential amino acid of tryptophan

potential targets to weaken T regulatory responses include IL-35 and MFG-E8 that block T regulatory functions [121, 122]. Figure 1.11 summarizes the immunotherapies aimed at inhibiting immunosuppression to treat cancers.

1.5.2 Immunotherapy Boosts the Antitumor Immune Responses and Enhances Killing of the Tumor Cell

1.5.2.1 Activated DCs and T Cells Are Pivotal in Cancer Immunotherapy

Immunotherapy boosts the immune responses against cancers by providing primed T cells either *in vivo* or ex *vivo*. Therapeutic cancer vaccines provide the opportunity to priming the T cells *in vivo*, while adoptive T cell transfer gifts ex *vivo* primed T cells to the immune system of cancer patients. DC vaccines constitute the most popular therapeutic cancer vaccines developed to treat a wide variety of cancers. First, DCs should be cultured from patients' peripheral blood mononuclear cells (PBMCs). Then they should be matured (most commonly with inflammatory cytokine cocktails) and loaded with tumor antigens. Finally, they are reintroduced to the patient's body to activate T cells and enhance antitumor responses. Some researchers prefer *in vivo* maturation of DCs by injection of these cells into an inflamed tissue as a simple, inexpensive, and physiologic way of maturation that enhances migration of DCs to draining lymph nodes [123]. Furthermore, antigens can also be loaded *in vivo* using antibodies that bind DC surface like DEC205 [124, 125]. DC vaccines are under investigation (phase I/II clinical trial) for high-grade glioma [126, 127], glioblastoma [128], hepatocellular carcinoma [129], pancreatic cancer [130], colorectal cancer [131], metastatic melanoma [132, 133], multiple myeloma [134–136], acute leukemia [137, 138], breast cancer [139], ovarian cancer [140, 141], RCC [142], and non-small cell lung cancer [143].

On the other hand, adoptive T cell transfer relies on *in vitro* expansion of T cells harvested from cancer patients and reintroducing these manipulated and primed T cells into the patient's circulation. These T cells can be harvested from four major sites: (1) PBMC, (2) resections from draining lymph nodes, (3) malignant effusions, and (4) directly from tumor biopsies. However, the quantity and quality of harvested T cells from each site differ significantly; PBMCs are an easy site to obtain T cells, while biopsy-derived T cells are more reactive against tumor antigens [76, 144]. Thereafter, T cells will be engineered to express T cell receptors (TCR) necessary for tumor recognition or to express T bodies (a chimeric antigen receptor that directly binds tumor antigens) [145]. Finally, T cells can be expanded with exposure to relevant tumor antigens, activating mAbs and T cell growth factors like IL-15 [146]. $CD8^+$ cytotoxic T lymphocyte constitute the main cells produced and transferred in adoptive T cell therapy [147]. Adoptive T cell therapy is now underway for neuroblastoma [148], hepatocellular cancers [149], gastric cancer [150], metastatic melanoma [151–153], hematological malignancies [154–156], colorectal cancer [157, 158], posttransplant lymphoproliferative diseases [159, 160], breast cancer [161, 162], ovarian cancer [163, 164], advanced lung cancer [165, 166], RCC [167], and nasopharyngeal carcinoma [168].

1.5.2.2 Materials of Activating DCs and T Cells

Tumor-specific and tumor-associated antigens as well as immunostimulatory cytokines and immune adjuvants help in activating DCs and priming the T or natural killer (NK) cells *in vivo* or ex *vivo*. Cancer-testis antigens are mainly expressed in germ cells and also appear on tumors of variable origin. However, these antigens are rarely expressed by other human cell types under physiologic conditions. Of all cancer-testis antigens, MAGE-1 family has obtained growing interest as potential target for different cancers at variable stages [169–173]. MAGE-A3 and NY-ESO-1, two cancer-testis antigens, have been used to develop cancer vaccines against melanoma [174–176], lung cancer [177], ovarian carcinoma [178], and prostate cancer [179]. In addition to tumor-specific antigens, tumor-associated antigens with narrow distribution in tumor cells are widely used in cancer immunotherapy like tumor lysate antigens in DC-loaded vaccines [37, 180, 181]. Interestingly, antigens of oncogenic viruses also exert immunostimulatory effects able to induce strong antitumor responses [182]. Tumor cells of EBV-associated nasopharyngeal carcinoma express EB nuclear antigen 1 (EBNA1) and latent membrane protein 2 (LMP2) which are EBV antigens [182]. Intradermal administration MVA-EL vaccinations, which encodes an EBNA1/LMP2 fusion protein, results in boosting T cell response against tumors [182]. Indeed, EBV-targeted immunotherapy promises hopes for patients with refractory or metastatic EBV-associated cancers [183].

Antigens can be delivered to DCs via different ways including fusion with tumor cells, loading of tumor lysates, long overlapping peptide mixtures or specific antigenic peptides, exposure with recombinant proteins, and transfection with genes encoding tumor antigens [37, 130, 139, 184, 185]. Recombinant antigens offer specific targeting of tumor cells with high safety and efficacy. Sipuleucel-T is a DC-approved vaccine for prostate cancer which contains ex *vivo* primed DCs with recombinant PA2024 protein fused with GM-CSF [37]. Interestingly, specific tumor antigens can be selectively delivered to patients to enhance *in vivo* DC uptake and presentation of tumor antigens. This direct delivery of tumor antigens is known as peptide vaccination. Subcutaneous injection of modified 9-mer WT1 peptides to patients with Wilms' tumor results in increased frequency of $CD14^+$, $CD16^+$, $CD33^+$, and CD85+ DCs [186]. However, for improving the efficacy of peptide vaccines, addition of cytokines particularly GM-CSF is of significant benefit. GM-CSF acts mainly via enhancement of antigen presentation by promotion of recruitment and maturation of DCs. Autologous or allogeneic irradiated tumor cells engineered to produce GM-CSF (GVAX) have been tested in some cancers like metastatic melanoma, colorectal cancers, non-small cell lung cancer, pancreatic

cancer, and castration-resistant prostate cancer with promising results [187–194]. This approach is expensive and technically difficult; thus, it did not gain popularity among researchers and clinicians.

In addition to therapeutic cancer vaccines, cytokine monotherapy is used for the promotion of tumor death and boosting T cell responses. Isolated limb perfusion with TNF-α is approved for treatment of patients with locally advanced soft tissue tumors [99, 100]. TNF-α is a proinflammatory cytokine involved in systemic inflammation, acute phase reaction, and constitutional symptoms of cancer patients like cachexia, whereas it also inhibits tumor growth and promotes apoptotic cell death. To overcome systemic unpleasant effects of TNF-α together with selective use of its antitumor effects, it is approved for local delivery in tumor repertoire [99, 100]. Other immunostimulatory cytokines acting as T cell growth factors, increasing survival of T cells, and enhancing T cell responses to tumor antigens are used in cancer immunotherapy. IL-2 and IFN-α have received FDA approval for patients with unresectable metastatic melanoma and renal cell carcinoma (RCC) [94, 153, 195–197].

With the discovery of pattern recognition receptors, novel immune adjuvants gained considerable popularity among researchers [199]. Toll-like receptor (TLR) agonists have received FDA approval for use as an immune adjuvant for cancer immunotherapy. Monophosphoryl lipid A (MPL), a TLR4 agonist, has been used in Cervarix®. Cervarix® is a vaccine against HPV-16 and HPV-18 and prevents HPV-related cancers [95]. Imiquimod, a TLR7 agonist, received FDA approval for basal cell carcinoma, external genital warts, and actinic keratosis [95]. In addition, the development of new immunotherapeutic agents using other TLR agonists like CpG oligonucleotides continues to be an area of active research [199]. PF-3512676, a TLR9 agonist, is now in phase II clinical trial for patients with metastatic melanoma. Intravenous or intradermal administration of PF-3512676 in melanoma patients results in activation of DCs in sentinel lymph nodes and expansion of cancer-reactive cytotoxic $CD8^+$ T cells [200, 201]. These immunological changes were in association with partial clinical response in 10 % and stable disease in 15 % of treated melanoma patients with PF-3512676 monotherapy [202]. Similarly, PF-3512676 is in phase I clinical trial for patients with basal cell carcinoma and NHL [203, 204]. PF-3512676 is also under evaluation for cutaneous T cell lymphoma, chronic lymphocytic leukemia, metastatic esophageal squamous cell carcinoma, and non-small cell lung cancer [205–210].

Finally, immunomodulator mAbs can act as agonists of stimulatory receptors on immune cells. Stimulatory mAbs have been developed for glucocorticoid-induced tumor necrosis factor receptor (GITR), OX 40 (CD 134), CD 40, and CD 137. These mAbs are now underway for a wide range of cancers from hematological malignancies to solid tumors, but these have not yet entered into clinical trials [211, 212]. Of note that, mAbs act as immunomodulators, trigger complement activation, induce Antibody-dependent cell-mediated cytotoxicity (ADCC) and also are able to provide opportunity for targeted delivery of cytotoxic materials to malignant cells (for instance ^{131}I-tositumomab, known as radioimmunotherapy) [213]. Figure 1.12 summarizes immunotherapies aimed at stimulating antitumor immune responses.

1.6 At Which Line of Treatment?

Since the introduction of cancer immunotherapy, it has been used mainly as the last line of treatment of patients with advanced disease. It does not mean that cancer immunotherapy is restricted to patients who relapsed with other standards of care. As this emerging field is still in its infancy and its safety and efficacy is not well evaluated, patients who accept to enter into trials are usually at advanced stages, have several metastases, and are inoperable. Indeed, immunotherapy provides hope for who is disappointed from other therapeutic approaches. Nonetheless, the question why it cannot be considered as a first-line therapy of cancer patients at different stages remains to be answered.

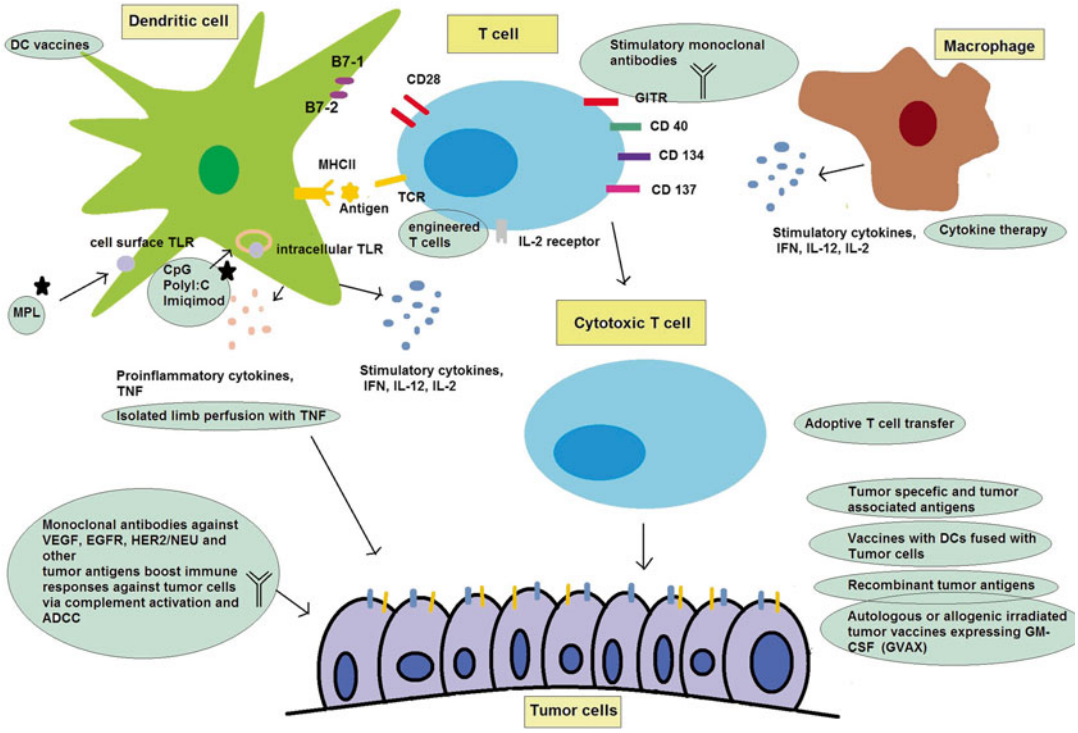


Fig. 1.12 Immunotherapy boosts antitumor immune responses and kills tumor cells. Dendritic cell (DC) vaccines and adoptive T cell transfer are two main ways of enhancing antitumor responses. Tumor antigens, immune adjuvants, and stimulatory cytokines help improve DC functions and provide cytotoxic T lymphocyte. Tumor cells themselves are the best source of antigens to produce cancer-reactive T cells. Monoclonal antibodies act as immunostimulatory agents to direct stimulation of T cells or bind tumor cells and activate complement system and Antibody-dependent cell-mediated cytotoxicity (ADCC). *TLR* Toll-like receptor, *MPL* monophosphoryl lipid A, *MHC II* major histocompatibility complex II, *TNF* tumor necrosis factor, *IFN* interferon, *IL* interleukin, *GM-CSF* granulocyte-macrophage colony-stimulating factor, *VEGF* vascular endothelial growth factor, *EGFR* epidermal growth factor receptor, *HER2/NEU* human epidermal growth factor receptor 2, *GITR* glucocorticoid-induced tumor necrosis factor receptor

For different cancers, it has been shown that considering immunotherapy as the first-line treatment did not compromise patients' prognosis and their quality of life, but inversely improved progression-free survival and sometimes overall survival of patients. For metastatic colorectal cancer, addition of panitumumab to standard chemotherapy as first-line therapy resulted in significant improvement in progression-free survival of patients without deterioration of patients' quality of life [214]. Panitumumab is approved for patients with metastatic refractory colorectal cancers, but it has several benefits (at least improvement of progression-free survival) for patients who have not received previous chemotherapy [91]. Similarly, use of ipilimumab in combination with paclitaxel and carboplatin improved progression-free survival of patients with non-small cell lung cancer who had not previously taken any medication [105]. On the other hand, immunotherapy is associated with better results in patients with early stages of cancers. BCG for superficial bladder cancer is a historical example of this claim. In addition, vaccination with HPV-16 oncoproteins in women with high-grade VIN resulted in relief of VIN-related symptoms in 60 % of patients [32]. Accordingly, immunotherapy is of benefit as the first-line treatment of patients with advanced or early stages of cancer. However, this hypothesis should be assessed in future studies.

1.7 Monotherapy or Combined Therapy?

For many years there was a dogma that chemotherapy and radiotherapy have deleterious effects on immunity and thereby effects of combined immunotherapy may be subsided. Initial experiences opposed to this dogma dating back to animal studies in the 1970s when intratumoral injection of cytotoxic drugs enhanced systemic immune response against tumors, cleared distant metastases, and promoted protective immunity with rechallenge with tumor cells [215]. In addition, systemic delivery of chemotherapy enhanced antitumor responses without induction of T regulatory cell depletion [216]. These observations suggested that cytotoxic chemotherapy may not be always immunosuppressive. Further studies unveiled that immunostimulatory or immunosuppressive effects of chemotherapeutic drugs depend on drug/dosage and schedule of treatment [38]. In addition, radiotherapy breaks immunosuppressive tumor microenvironment and enhances tumor antigen presentation. Accordingly radiotherapy with nonfatal doses for the immune system may enhance efficacy of cancer treatment to be combined with immunotherapy [217]. Under schedule that does not suppress effector cytotoxic T lymphocyte, induction of apoptotic death of tumor cells by chemotherapy/radiotherapy results in enhanced tumor antigen presentation and subsequent T cell activation. This is known as immunogenic cell death and constructs the basis of combined immunotherapy with chemotherapy/radiotherapy [218]. In addition, this combined therapy reduces the chance of tumor escape and resistance similar to multidrug therapy. Combined therapies are now on the way for wide varieties of cancers. Immunotherapy can be combined with radiotherapy, chemotherapy, targeted therapy (like tyrosine kinase inhibitors), and surgery [219–223]. Interestingly, radioimmunotherapy is an emerging field with introduction of mAbs bearing radioactive agents. Yttrium-90-ibritumomab tiuxetan is a mAb against CD20 conjugated to yttrium-90 and is used to treat relapsed B cell malignancies [224]. ^{131}I-rituximab and ^{131}I-tositumomab are other radiolabeled mAbs against CD20 [223, 225].

One of the most famous combined chemotherapies/immunotherapies is used in the treatment of hematological malignancies. Combined CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) therapy with rituximab as first-line treatment in non-hodgkin lymphoma [226], CHOP plus ^{131}I-tositumomab in non-hodgkin lymphoma [223], and CHOP plus rituximab in untreated mantle cell lymphoma [224, 227] are examples of combined chemotherapy with immunotherapy in hematological malignancies. Other promising results have been obtained in pancreatic cancer, one of the most lethal cancers worldwide. Algenpantucel-L immunotherapy combined with gemcitabine and 5-fluorouracil-based chemoradiotherapy improves progression-free survival of patients with resected pancreatic cancer [228]. Furthermore, yttrium-90-labeled humanized clivatuzumab tetraxetan with gemcitabine resulted in partial response in 16 % and stable disease in 42 % of patients with advanced pancreatic cancer [229]. However, combined immunotherapy with chemotherapy does not always improve clinical outcome. In esophageal cancer patients, intratumoral administration of ^{111}In-labeled dendritic cells (DC) in combination with preoperative chemotherapy did not improve immune nor clinical response [230].

Targeted therapy with tyrosine kinase inhibitors particularly those inhibiting vascular endothelial growth factor receptors (VEGFR) promises hope for treatment of several cancers. Both small molecules inhibiting this receptor like axitinib [231] (received FDA approval for refractory RCC in 2012) and mAbs targeting VEGFR like bevacizumab (received FDA approval for metastatic colorectal cancer, RCC, and glioblastoma multiform in 2004) are now available. Despite axitinib which is a chemotherapeutics, bevacizumab belongs to immunotherapeutic agents due to activation of complement system and ADCC when binding VEGFR [89, 232, 233]. Interestingly, combination of targeted therapy with immunotherapy has also been evaluated in patients with RCC. Combination of SU5416, VEGFR inhibitor, and IFN-α2B (received FDA approval for hairy cell leukemia and

advanced-stage melanoma in the 1990s) was tested in patients with RCC with no beneficial effects. By contrast, this combination leads to fatal events in 6.5 % of treated patients and thereby is discouraged [234].

Finally, immunotherapy confers benefits to patients who undergone surgery for complete resection or debulking of cancer. IFN-α2B after resection of melanoma in patients with high risk of relapse improves survival and decreases risk of relapse but jeopardizes quality of life of patients owing to IFN toxicities [235]. In addition to post-surgical benefits, immunotherapy can be employed prior to surgery and resection of tumor masses. Neoadjuvant chemotherapy like induction of resistance cells, difficulty in resection, and false shrinkage of tumor on imaging results in fast growth of residual tumor cells after resection; thus, neoadjuvant immunotherapy is more advantageous compared to neoadjuvant or induction chemotherapy [236]. Neoadjuvant immunotherapy has been tested for several cancers; carcinoembryogenic antigen (CEA)-derived MHCII-loaded DC vaccination prior to resection of colorectal metastases was promising [131]. In addition, adoptive T cell transfer prior or after surgery/radiotherapy of glioblastoma multiforme patients is an emerging solution for this lethal cancer [237, 238].

1.8 Monitoring the Immunological and Clinical Responses to Immunotherapy

It should be borne in mind that immunotherapy-induced tumor destruction may appear with delay after a period of tumor progression and metastasis. By contrast, response to other standards of care of cancer patients including surgery, radiotherapy, and chemotherapy appears early with obvious reduction of tumor size and metastasis [239]. It highlights the significance of the development of immune-related response criteria nearby classic World Health Organization (WHO) criteria and Response Evaluation Criteria In Solid Tumors (RECIST). WHO criteria and RECIST measure response to cancer therapy mostly with respect to tumor shrinkage and appearance of new metastases [240–243]. However, immune-related response criteria should be applied to measure response to cancer immunotherapy considering the distinct biology of tumor cell killing using each cancer therapy approach [242]. One of the evidences of this distinction is immune infiltrate of tumor mass caused by immunotherapy that is responsible for delayed but effective antitumor responses [239]. Major determinants of safety and efficacy of cancer immunotherapy encompass specificity of therapy or quality of primed T cells, quantity of primed T cells against cancer, and half-life of induced response against cancer [33]. This also underscores the significance of a reliable unique assay to measure immunological response to immunotherapy. Such universal standard assays let us compare immune responses in different trials. The minimal information about T cell assays (MIATA) project aims at establishing universal criteria to assess immunological response to cancer immunotherapy [244, 245]. Of note is that the best site of assessment for immune response is tumor microenvironment rather than peripheral blood or distant sites from tumor origin due to immunosuppressive effect of tumor microenvironment [244, 245].

1.9 Limitations of Cancer Immunotherapy

Several obstacles limit the implication of cancer immunotherapy including technical obstacles, side effects of immunotherapeutic drugs, and lack of broad availability of approved immunotherapeutic drugs. Harvesting sufficient amounts of T cells or DCs from cancer patients and activating them are not always easy and inversely are associated with technical difficulties, high costs, and different interindividual efficacies. Similarly, provision of autologous whole-tumor cell vaccines expressing GM-CSF is also difficult and expensive [76, 239]. In addition, novel adjuvants are needed to optimize therapy with cancer vaccines. Despite considerable investment on cancer

vaccine development, less is paid on the development of vaccine adjuvant components [246].

On the other hand, bypass of tumor tolerance is inevitably associated with break of peripheral tolerance to self-antigens. Accordingly, autoimmune manifestations are the most common adverse events of cancer immunotherapy. In addition some drugs such as IFN induce fatal toxicities leading to drug discontinuation. As immunotherapy is a systemic treatment, adverse events may appear all over the body. Various side effects are observed with the administration of various immunotherapeutic drugs including gastrointestinal involvement with colitis, nausea, vomiting, and hepatotoxicity; skin involvement with rash and pruritus; endocrine involvement like adrenalitis and hypophysitis; hematological manifestations from pancytopenia to isolated neutropenia; and respiratory or urinary tract infections [39, 96, 247–249]. On the other hand, some drugs are associated with specific toxicities: peripheral sensory neuropathy with brentuximab vedotin [250], skin toxicities with panitumumab and cetuximab [251], and hypertension and hemorrhage with bevacizumab [252]. These side effects restrict the use of immunotherapy as FDA revoked bevacizumab approval for breast cancer due to its fatal side effects.

1.10 Supportive Therapy

Despite inevitable side effects of immunotherapy to treat cancers, immunotherapy also offers hope for rehabilitation and reconstruction of destroyed nonmalignant tissues during cancer treatment. This use of immunotherapy is tertiary level of prevention from cancers which completes the treatise of cancer immunotherapy. The most famous one is GM-CSF following myelosuppressive chemotherapy. Not only GM-CSF but also granulocyte-CSF (G-CSF) increases the maturation and release of myeloid linage including DCs and neutrophils. These functions of G(M)-CSF on DCs is used to construct more efficient therapeutic cancer vaccines (known as GVAX); however, increase of neutrophil count is pivotal in supportive therapy of cancer patients. Neutropenia predisposes cancer patients to a wide range of bacterial infections and increases mortality of patients. G(M)-CSF efficiently reduces the risk of infection-related morbidity and mortality [253–255]. GM-CSF has FDA approval for recovery following HSCT of several hematological malignancies and is part of the guidelines of supportive therapy of many other countries other than the United States [256]. However, it has supportive implication in several malignancies like breast cancer [255]. In breast cancer patients that received chemotherapy, use of GM-CSF reduces asthenia, anorexia, stomatitis, myalgia, dysgeusia, and nail disorders [257].

Vitamins also can act as supportive immunotherapeutic agents. High-dose methotrexate (MTX) which inhibits dihydrofolate reductase (DHFR) is used in chemotherapy of a wide range of lymphoproliferative diseases as well as breast cancer [258]. DHFR is a pivotal enzyme in folic acid metabolism and is required for thymidine synthesis and cell replication [258]. Accordingly, folic acid derivatives can be used to rescue bone marrow as well as gut epithelial cells. The beneficial effects of folic acid supplementation are more chargeable to nonmalignant cells justifying use of folic acid even concomitant with chemotherapy [258].

1.11 Effect of Immunotherapy on Health-Related Quality of Life of Cancer Patients

Symptomatic treatment is one essential component of care of cancer patients. Cancer patients have constitutional symptoms like decreased appetite and fever owing to systemic inflammation. Psychiatric complaints like depression and insomnia also are common among the patients. In addition, chemotherapy and radiotherapy as well as surgical resection of tumor masses exacerbate general condition of patients at least for a short period of time surrounding the therapy. In this manner mastectomy is one of the overwhelming events in care of breast cancer patients with undeniable psychiatric effects like depression. Interestingly, depression reduces the overall survival of breast cancer patients controlling for

other variables [259]. Similarly, immunotherapy is not free of side effects and also may cause toxicities for several organs. However, it is shown that certain immunotherapeutic drugs improve health-related quality of life of patients. Cytokine-induced killer (CIK) cell transfer for patients with several cancers including hepatocellular carcinoma and gastric cancer homological malignancies improves patients' quality of life. CIK improves appetite and sleep, in addition to relieving pain [260]. Sometimes immunotherapy has equivocal effects on quality of life. Addition of cetuximab in chemotherapeutic regimen of patients with metastatic colorectal cancer does not impact patients' quality of life but improves overall survival [261]. Similarly, use of ipilimumab for advanced melanoma or panitumumab for metastatic colorectal cancer does not impact quality of life of treated patients [214, 262]. On the other hand, immunotherapy may negatively impact the quality of life of treated patients. IL-2 or IFN-α2B therapy for RCC and melanoma patients may cause depressive but not anxiety symptoms in the first week of treatment [195].

1.12 Cost-Effectiveness of Cancer Immunotherapy

Not all cancer immunotherapeutic drugs are expensive or technically difficult to be developed. Vaccination against oncogenic viruses like HPV offers a cost-effective solution to prevent cancers more prominently in limited resource settings [263]. Several studies from African low-income countries confirmed the cost-effectiveness of girl vaccination against HPV [263]. Prevention from cancer development is of utmost importance in countries without organized programs of cervical cancer screening [263]. In addition to low-income countries, developed countries which provide access to both screening and therapeutic programs for cervical cancer benefit from HPV vaccination [61]. Cost-effectiveness of cancer prophylaxis in such a setting underscores the importance of primary prevention. In this way, both the health system and people will benefit as people experience better status of health while spending less.

On the other hand, immunotherapy aiming at cancer treatment is usually expensive considering either therapeutic cancer vaccines or adoptive T cell therapy. However, it should be borne in mind that other therapies for cancers are not inexpensive and predispose patients to life-threatening side effects. Hence, cost-effectiveness of other therapies for cancer patients is also under question, but these are all that can be done to save patients. Immunotherapy provides hopes for patients disappointed from other drugs or have metastatic advanced stages of disease. In addition, immunotherapy is now available for pancreatic cancer, esophageal cancer, liver cancer, and lung cancer which have a 5-year mortality of more than 90 % [16, 57, 90, 105, 129, 192, 193, 208, 210, 230, 264, 265]. It suggests that immunotherapy for treatment of cancer patients is unlikely to be cost-effective but is the only hope of several patients to live 1 day more.

Considering supportive immunotherapy, administration of GM-CSF is shown to be cost-effective in the treatment of neutropenia and prevention of cancer-/chemotherapy-related sequelae on bone marrow. Increase of neutrophil count is associated with reduction in infection-related morbidity and mortality of cancer patients concurrent with improvement in their health-related quality of life [253, 266]. Indeed, cancer immunotherapy has approved economic benefit at primary and tertiary level of care, and at second level, it provides hope for patients who have recurrent/relapsed end-stage disease.

1.13 Concluding Remarks

Immunotherapy can be active or passive, rapid in onset of effects, or associated with delayed response, specific or nonspecific. Active immunization against cancers includes different vaccines, cell-based therapies, peptide-based therapies, cytokines, and gene therapies, while the passive immunization against cancers is also provided by developing variable mAbs. These antibodies exert their effects via complement activation and ADCC that are rapid in onset. Of note, monoclonal antibodies also exert

immunomodulator effects by targeting the immune system rather than tumor-associated antigens. Innate immune actors are more important in induction of passive immunization against cancers, while adaptive immunity plays the central role in active immunization. Despite limited number of immunotherapeutic drugs that obtained FDA approval, a large number are in the way to get permission to be used in routine practice.

Today, cancer is an important global issue with high incidence, mortality, and considerable burden on the health system. Considering failure of chemotherapy, radiotherapy, or surgery in treatment of many cancer patients, a new therapeutic avenue is indicated. Immunotherapy could be the solution providing protection against cancer at all levels of care. Prophylactic use of immunotherapy with immunomodulation to treat diseases predisposes individuals to cancer, and vaccination against oncogenic viruses is beneficial for both health-care providers and people. Therapeutic use of immunotherapy offers hope for those who are disappointed from other therapies, and supportive immunotherapy helps rescue patients following intensive therapies. Accordingly, cancer immunotherapy confers a global benefit, and everybody all around the world has the right to benefit from this novel therapeutic avenue.

References

1. Bray F, Jemal A, Grey N et al. Global cancer transitions according to the human development index (2008–2030): a population-based study. Lancet Oncol. 2012;13(8):790–801.
2. Soerjomataram I, Lortet-Tieulent J, Parkin DM et al. Global burden of cancer in 2008: a systematic analysis of disability-adjusted life-years in 12 world regions. Lancet. 2012;380(9856):1840–50.
3. Renehan AG, Tyson M, Egger M, et al. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet. 2008;371(9612):569–78.
4. Goldie S. A public health approach to cervical cancer control: considerations of screening and vaccination strategies. Int J Gynaecol Obstet. 2006;94 Suppl 1:S95–105.
5. Tsu V, Murray M, Franceschi S. Human papillomavirus vaccination in low-resource countries: lack of evidence to support vaccinating sexually active women. Br J Cancer. 2012;107(9):1445–50.
6. Edmunds W, Dejene A, Mekonnen Y, et al. The cost of integrating hepatitis B virus vaccine into national immunization programmes: a case study from Addis Ababa. Health Policy Plan. 2000;15(4):408–16.
7. Eholie SP, Aoussi FE, Ouattara IS, et al. HIV treatment and care in resource-constrained environments: challenges for the next decade. J Int AIDS Soc. 2012;15(2):1734.
8. Grönhagen C, Fored C, Granath F, et al. Increased risk of cancer among 3663 patients with cutaneous lupus erythematosus: a Swedish nationwide cohort study. Br J Dermatol. 2012;166(5):1053–9.
9. Grulich AE, van Leeuwen MT, Falster MO, et al. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. Lancet. 2007;370(9581):59–67.
10. Rezaei N, Hedayat M, Aghamohammadi A, et al. Primary immunodeficiency diseases associated with increased susceptibility to viral infections and malignancies. J Allergy Clin Immunol. 2011;127(6):1329–41. e2.
11. Patel P, Hanson DL, Sullivan PS, et al. Incidence of types of cancer among HIV-infected persons compared with the general population in the United States, 1992–2003. Ann Int Med. 2008;148(10):728–36.
12. Shacter E, Weitzman SA. Chronic inflammation and cancer. Oncology. 2002;16(2):217–30.
13. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420(6917):860–7.
14. Achenza MS, Selmi C. Autoimmunity and cancer. Asian Pac J Cancer Prev. 2012;13:29–40.
15. Hakim FT, Flomerfelt FA, Boyiadzis M, et al. Aging, immunity and cancer. Curr Opin Immunol. 2004;16(2):151–6.
16. Lang P, Mitchell W, Lapenna A, et al. Immunological pathogenesis of main age-related diseases and frailty: role of immunosenescence. Eur Ger Med. 2010;1(2):112–21.
17. de Magalhães JP. How ageing processes influence cancer. Nat Rev Cancer. 2013;13(5):357–65.
18. Akushevich I, Kravchenko J, Ukraintseva S, et al. Age patterns of incidence of geriatric disease in the US elderly population: medicare based analysis. J Am Geriatr Soc. 2012;60(2):323–7.
19. Flowers CR, Fedewa SA, Chen AY, et al. Disparities in the early adoption of chemoimmunotherapy for diffuse large B-cell lymphoma in the United States. Cancer Epidemiol Biomarkers Prev. 2012;21(9):1520–30.
20. Simard EP, Engels EA. Cancer as a cause of death among people with AIDS in the United States. Clin Infect Dis. 2010;51(8):957–62.
21. Moller H, Flatt G, Moran A. High cancer mortality rates in the elderly in the UK. Cancer Epidemiol. 2011;35(5):407–12.

22. Coley WB. The treatment of inoperable sarcoma by bacterial toxins (the mixed toxins of the streptococcus erysipelas and the bacillus prodigiosus). Proc R Soc Med. 1910;3(Surg Sect):1–48.
23. Czuczman M, Grillo-Lopez A, White C, et al. Treatment of patients with low-grade B-cell lymphoma with the combination of chimeric anti-CD20 monoclonal antibody and CHOP chemotherapy. J Clin Oncol. 1999;17(1):268.
24. Lake RA, Robinson BW. Immunotherapy and chemotherapy—a practical partnership. Nat Rev Cancer. 2005;5(5):397–405.
25. Huncharek M, Caubet J, McGarry R. Single-agent DTIC versus combination chemotherapy with or without immunotherapy in metastatic melanoma: a meta-analysis of 3273 patients from 20 randomized trials. Melanoma Res. 2001; 11(1):75–81.
26. Demaria S, Bhardwaj N, McBride WH, et al. Combining radiotherapy and immunotherapy: a revived partnership. Int J Radiat Oncol Biol Phys. 2005;63(3):655–66.
27. Kirkwood JM, Ernstoff MS. Interferons in the treatment of human cancer. J Clin Oncol. 1984;2(4):336–52.
28. Kirkwood JM, Ernstoff M. Potential applications of the interferons in oncology: lessons drawn from studies of human melanoma. Semin Oncol. 1986;13(3 Suppl 2):48–56.
29. Kirkwood JM. Systemic adjuvant treatment of high-risk melanoma: the role of interferon alfa-2b and other immunotherapies. Eur J Cancer. 1998;34 Suppl 3:S12–7.
30. Maloney DG, Grillo-Lopez AJ, White CA, et al. IDEC-C2B8 (rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-hodgkin lymphoma. Blood. 1997;90(6):2188–95.
31. Gröhn P, Heinonen E, Klefström P, et al. Adjuvant postoperative radiotherapy, chemotherapy, and immunotherapy in stage III breast cancer. Cancer. 1984;54(4):670–4.
32. Kenter GG, Welters MJ, Valentijn ARP, et al. Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. N Engl J Med. 2009;361(19):1838–47.
33. Kirkwood JM, Butterfield LH, Tarhini AA, et al. Immunotherapy of cancer in 2012. CA Cancer J Clin. 2012;62(5):309–35.
34. Kessels HW, Wolkers MC, van den Boom MD, et al. Immunotherapy through TCR gene transfer. Nat Immunol. 2001;2(10):957–61.
35. Gabrilovich DI. Combination of chemotherapy and immunotherapy for cancer: a paradigm revisited. Lancet Oncol. 2007;8(1):2–3.
36. Chi K-H, Liu S-J, Li C-P, et al. Combination of conformal radiotherapy and intratumoral injection of adoptive dendritic cell immunotherapy in refractory hepatoma. J Immunother. 2005;28(2): 129–35.
37. Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010;363(5):411–22.
38. Lesterhuis WJ, Haanen JB, Punt CJ. Cancer immunotherapy – revisited. Nat Rev Drug Discov. 2011;10(8):591–600.
39. Stauss HJ, Morris EC. Immunotherapy with gene-modified T cells: limiting side effects provides new challenges. Gene Ther. 2013;20(11):1029–32.
40. Huang CJ, Lowe AJ, Batt CA. Recombinant immunotherapeutics: current state and perspectives regarding the feasibility and market. Appl Microbiol Biotechnol. 2010;87(2):401–10.
41. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711–23.
42. Ozao-Choy J, Carvajal R, Hamid O. Ipilimumab for metastatic melanoma. Drugs Today (Barc, Spain: 1998). 2012;48(6):381.
43. van Seters M, van Beurden M, ten Kate FJ, et al. Treatment of vulvar intraepithelial neoplasia with topical imiquimod. N Engl J Med. 2008;358(14):1465–73.
44. Stern PL, van der Burg SH, Hampson IN, et al. Therapy of human papillomavirus-related disease. Vaccine. 2012;30 Suppl 5:F71–82.
45. Deeks SG. HIV infection, inflammation, immunosenescence, and aging. Ann Rev Med. 2011;62:141–55.
46. Malaguarnera M, Cristaldi E, Romano G, et al. Autoimmunity in the elderly: implications for cancer. J Cancer Res Ther. 2012;8(4):520.
47. Rogers CJ, Colbert LH, Greiner JW, et al. Physical activity and cancer prevention: pathways and targets for intervention. Sports Med. 2008;38(4):271–96.
48. Lampe JW. Dairy products and cancer. J Am Coll Nutr. 2011;30(5 Suppl 1):464S–70.
49. Bhardwaj P, Du B, Zhou XK, et al. Caloric restriction reverses obesity-induced mammary gland inflammation in mice. Cancer Prev Res (Phila). 2013;6(4):282–9.
50. Ericksen RE, Rose S, Westphalen CB, et al. Obesity accelerates Helicobacter felis-induced gastric carcinogenesis by enhancing immature myeloid cell trafficking and TH17 response. Gut. 2013;63(3):385–94.
51. Wang H, Chan YL, Li TL, et al. Reduction of splenic immunosuppressive cells and enhancement of antitumor immunity by synergy of fish oil and selenium yeast. PLoS One. 2013;8(1):e52912.
52. Cardeno A, Sanchez-Hidalgo M, de la Lastra AC. An up-date of olive oil phenols in inflammation and cancer: molecular mechanisms and clinical implications. Curr Med Chem. 2013;20(37):4758–76.
53. Burris RL, Ng HP, Nagarajan S. Soy protein inhibits inflammation-induced VCAM-1 and inflammatory cytokine induction by inhibiting the NF-kappaB and AKT signaling pathway in apolipoprotein E-deficient mice. Eur J Nutr. 2013;53(1):135–48.
54. Wimberly AL, Forsyth CB, Khan MW, et al. Ethanol-induced mast cell-mediated inflammation leads to increased susceptibility of intestinal tumorigenesis in the APC Delta468 min mouse model of

colon cancer. Alcohol Clin Exp Res. 2013;37 Suppl 1:E199–208.
55. Chang MH. Cancer prevention by vaccination against hepatitis B. Recent Results Cancer Res. 2009;181:85–94.
56. Chang MH, Shau WY, Chen CJ, et al. Hepatitis B vaccination and hepatocellular carcinoma rates in boys and girls. JAMA. 2000;284(23):3040–2.
57. McMahon BJ, Bulkow LR, Singleton RJ, et al. Elimination of hepatocellular carcinoma and acute hepatitis B in children 25 years after a hepatitis B newborn and catch-up immunization program. Hepatology. 2011;54(3):801–7.
58. Hung CH, Lu SN, Wang JH, et al. Sustained HCV clearance by interferon-based therapy reduces hepatocellular carcinoma in hepatitis B and C dually-infected patients. Antivir Ther. 2011;16(7):959–68.
59. Group FIS. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. N Engl J Med. 2007;356(19):1915–27.
60. Wigle J, Coast E, Watson-Jones D. Human papillomavirus (HPV) vaccine implementation in low and middle-income countries (LMICs): health system experiences and prospects. Vaccine. 2013;31(37):3811–7.
61. Demarteau N, Van Kriekinge G, Simon P. Incremental cost-effectiveness evaluation of vaccinating girls against cervical cancer pre- and post-sexual debut in Belgium. Vaccine. 2013;31:3962–71.
62. Psyrri A, Sasaki C, Vassilakopoulou M, et al. Future directions in research, treatment and prevention of HPV-related squamous cell carcinoma of the head and neck. Head Neck Pathol. 2012;6 Suppl 1:S121–8.
63. Ahlers JD, Belyakov IM. Strategies for recruiting and targeting dendritic cells for optimizing HIV vaccines. Trends Mol Med. 2009;15(6):263–74.
64. Grywalska E, Markowicz J, Grabarczyk P, et al. Epstein-Barr virus-associated lymphoproliferative disorders. Postepy Hig Med Dosw (Online). 2013;67:481–90.
65. Eiz-Vesper B, Maecker-Kolhoff B, Blasczyk R. Adoptive T-cell immunotherapy from third-party donors: characterization of donors and set up of a T-cell donor registry. Front Immunol. 2012;3:410.
66. Ramirez N, Olavarria E. Viral-specific adoptive immunotherapy after allo-SCT: the role of multimer-based selection strategies. Bone Marrow Transplant. 2013;48(10):1265–70.
67. Icheva V, Kayser S, Wolff D, et al. Adoptive transfer of epstein-barr virus (EBV) nuclear antigen 1-specific t cells as treatment for EBV reactivation and lymphoproliferative disorders after allogeneic stem-cell transplantation. J Clin Oncol. 2013;31(1):39–48.
68. Velayos FS, Terdiman JP, Walsh JM. Effect of 5-aminosalicylate use on colorectal cancer and dysplasia risk: a systematic review and metaanalysis of observational studies. Am J Gastroenterol. 2005;100(6):1345–53.
69. Rostom A, Dube C, Lewin G, et al. Nonsteroidal anti-inflammatory drugs and cyclooxygenase-2 inhibitors for primary prevention of colorectal cancer: a systematic review prepared for the U.S. Preventive Services Task Force. Ann Intern Med. 2007;146(5):376–89.
70. Rothwell PM. Aspirin in prevention of sporadic colorectal cancer: current clinical evidence and overall balance of risks and benefits. Recent Results Cancer Res. 2013;191:121–42.
71. Dube C, Rostom A, Lewin G, et al. The use of aspirin for primary prevention of colorectal cancer: a systematic review prepared for the U.S. Preventive Services Task Force. Ann Intern Med. 2007;146(5):365–75.
72. Brennan FR, Morton LD, Spindeldreher S, et al. Safety and immunotoxicity assessment of immunomodulatory monoclonal antibodies. MAbs. 2010;2(3):233–55.
73. Spitzer TR, Dey BR, Chen YB, et al. The expanding frontier of hematopoietic cell transplantation. Cytometry B Clin Cytom. 2012;82(5):271–9.
74. Bensinger WI. Allogeneic transplantation: peripheral blood vs. bone marrow. Curr Opin Oncol. 2012;24(2):191–6.
75. Bar M, Sandmaier BM, Inamoto Y, et al. Donor lymphocyte infusion for relapsed hematological malignancies after allogeneic hematopoietic cell transplantation: prognostic relevance of the initial CD3+ T cell dose. Biol Blood Marrow Transplant. 2013;19(6):949–57.
76. Dougan M, Dranoff G. Immune therapy for cancer. Annu Rev Immunol. 2009;27:83–117.
77. Ojea A, Nogueira JL, Solsona E, et al. A multicentre, randomised prospective trial comparing three intravesical adjuvant therapies for intermediate-risk superficial bladder cancer: low-dose bacillus Calmette-Guerin (27 mg) versus very low-dose bacillus Calmette-Guerin (13.5 mg) versus mitomycin C. Eur Urol. 2007;52(5):1398–406.
78. Babjuk M, Burger M, Zigeuner R, et al. EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2013. Eur Urol. 2013;64(4):639–53.
79. Brausi M, Witjes JA, Lamm D, et al. A review of current guidelines and best practice recommendations for the management of nonmuscle invasive bladder cancer by the International Bladder Cancer Group. J Urol. 2011;186(6):2158–67.
80. Cookson MS, Roth BJ, Dahm P, et al. Castration-resistant prostate cancer: AUA guideline. J Urol. 2013;190(2):429–38.
81. Agarwal N, Sonpavde G, Sternberg CN. Novel molecular targets for the therapy of castration-resistant prostate cancer. Eur Urol. 2012;61(5):950–60.
82. Coiffier B, Lepretre S, Pedersen LM, et al. Safety and efficacy of ofatumumab, a fully human monoclonal anti-CD20 antibody, in patients with relapsed or refractory B-cell chronic lymphocytic leukemia: a phase 1–2 study. Blood. 2008;111(3):1094–100.

83. Wierda WG, Kipps TJ, Durig J, et al. Chemoimmunotherapy with O-FC in previously untreated patients with chronic lymphocytic leukemia. Blood. 2011;117(24):6450–8.
84. Ansell SM, Tang H, Kurtin PJ, et al. Denileukin diftitox in combination with rituximab for previously untreated follicular B-cell non-hodgkin lymphoma. Leukemia. 2012;26(5):1046–52.
85. Shih YC, Elting LS, Pavluck AL, et al. Immunotherapy in the initial treatment of newly diagnosed cancer patients: utilization trend and cost projections for non-hodgkin lymphoma, metastatic breast cancer, and metastatic colorectal cancer. Cancer Invest. 2010;28(1):46–53.
86. Gopal AK, Ramchandren R, O'Connor OA, et al. Safety and efficacy of brentuximab vedotin for Hodgkin lymphoma recurring after allogeneic stem cell transplantation. Blood. 2012;120(3):560–8.
87. Younes A, Gopal AK, Smith SE, et al. Results of a pivotal phase II study of brentuximab vedotin for patients with relapsed or refractory Hodgkin's lymphoma. J Clin Oncol. 2012;30(18):2183–9.
88. Bross PF, Beitz J, Chen G, et al. Approval summary: gemtuzumab ozogamicin in relapsed acute myeloid leukemia. Clin Cancer Res. 2001;7(6):1490–6.
89. Gianni L, Romieu GH, Lichinitser M, et al. AVEREL: a randomized phase III Trial evaluating bevacizumab in combination with docetaxel and trastuzumab as first-line therapy for HER2-positive locally recurrent/metastatic breast cancer. J Clin Oncol. 2013;31(14):1719–25.
90. Barlesi F, Scherpereel A, Rittmeyer A, et al. Randomized phase III trial of maintenance bevacizumab with or without pemetrexed after first-line induction with bevacizumab, cisplatin, and pemetrexed in advanced nonsquamous non-small-cell lung cancer: AVAPERL (MO22089). J Clin Oncol. 2013;25(5):1044–52.
91. Douillard JY, Siena S, Cassidy J, et al. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. J Clin Oncol. 2010;28(31):4697–705.
92. Giusti RM, Cohen MH, Keegan P, et al. FDA review of a panitumumab (Vectibix) clinical trial for first-line treatment of metastatic colorectal cancer. Oncologist. 2009;14(3):284–90.
93. Peeters M, Price TJ, Cervantes A, et al. Randomized phase III study of panitumumab with fluorouracil, leucovorin, and irinotecan (FOLFIRI) compared with FOLFIRI alone as second-line treatment in patients with metastatic colorectal cancer. J Clin Oncol. 2010;28(31):4706–13.
94. Motzer RJ, Bacik J, Murphy BA, et al. Interferon-alfa as a comparative treatment for clinical trials of new therapies against advanced renal cell carcinoma. J Clin Oncol. 2002;20(1):289–96.
95. Vacchelli E, Galluzzi L, Eggermont A, et al. Trial watch: FDA-approved Toll-like receptor agonists for cancer therapy. Oncoimmunology. 2012;1(6):894–907.
96. Ribas A, Hodi FS, Callahan M, et al. Hepatotoxicity with combination of vemurafenib and ipilimumab. N Engl J Med. 2013;368(14):1365–6.
97. Ribas A, Kefford R, Marshall MA, et al. Phase III randomized clinical trial comparing tremelimumab with standard-of-care chemotherapy in patients with advanced melanoma. J Clin Oncol. 2013;31(5):616–22.
98. Di Giacomo AM, Ascierto PA, Pilla L, et al. Ipilimumab and fotemustine in patients with advanced melanoma (NIBIT-M1): an open-label, single-arm phase 2 trial. Lancet Oncol. 2012;13(9):879–86.
99. Lans TE, Grunhagen DJ, de Wilt JH, et al. Isolated limb perfusions with tumor necrosis factor and melphalan for locally recurrent soft tissue sarcoma in previously irradiated limbs. Ann Surg Oncol. 2005;12(5):406–11.
100. Bonvalot S, Rimareix F, Causeret S, et al. Hyperthermic isolated limb perfusion in locally advanced soft tissue sarcoma and progressive desmoid-type fibromatosis with TNF 1 mg and melphalan (T1-M HILP) is safe and efficient. Ann Surg Oncol. 2009;16(12):3350–7.
101. Peggs KS, Quezada SA, Korman AJ, et al. Principles and use of anti-CTLA4 antibody in human cancer immunotherapy. Curr Opin Immunol. 2006;18(2):206–13.
102. McNeel DG, Smith HA, Eickhoff JC, et al. Phase I trial of tremelimumab in combination with short-term androgen deprivation in patients with PSA-recurrent prostate cancer. Cancer Immunol Immunother. 2012;61(7):1137–47.
103. Chung KY, Gore I, Fong L, et al. Phase II study of the anti-cytotoxic T-lymphocyte-associated antigen 4 monoclonal antibody, tremelimumab, in patients with refractory metastatic colorectal cancer. J Clin Oncol. 2010;28(21):3485–90.
104. Kirkwood JM, Lorigan P, Hersey P, et al. Phase II trial of tremelimumab (CP-675,206) in patients with advanced refractory or relapsed melanoma. Clin Cancer Res. 2010;16(3):1042–8.
105. Lynch TJ, Bondarenko I, Luft A, et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non-small-cell lung cancer: results from a randomized, double-blind, multicenter phase II study. J Clin Oncol. 2012;30(17):2046–54.
106. Reck M, Bondarenko I, Luft A, et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line therapy in extensive-disease-small-cell lung cancer: results from a randomized, double-blind, multicenter phase 2 trial. Ann Oncol. 2013;24(1):75–83.
107. van den Eertwegh AJM, Versluis J, van den Berg HP, et al. Combined immunotherapy with

granulocyte-macrophage colony-stimulating factor-transduced allogeneic prostate cancer cells and ipilimumab in patients with metastatic castration-resistant prostate cancer: a phase 1 dose-escalation trial. Lancet Oncol. 2012;13(5):509–17.
108. Berger R, Rotem-Yehudar R, Slama G, et al. Phase I safety and pharmacokinetic study of CT-011, a humanized antibody interacting with PD-1, in patients with advanced hematologic malignancies. Clin Cancer Res. 2008;14(10):3044–51.
109. Benson Jr DM, Bakan CE, Mishra A, et al. The PD-1/PD-L1 axis modulates the natural killer cell versus multiple myeloma effect: a therapeutic target for CT-011, a novel monoclonal anti-PD-1 antibody. Blood. 2010;116(13):2286–94.
110. Kawano M, Itonaga I, Iwasaki T, et al. Anti-TGF-beta antibody combined with dendritic cells produce antitumor effects in osteosarcoma. Clin Orthop Relat Res. 2012;470(8):2288–94.
111. Chopra A. 89Zr-labeled fresolimumab (human anti-transforming growth factor-beta monoclonal antibody). Bethesda: Molecular Imaging and Contrast Agent Database (MICAD); 2004.
112. Bockholt NA, Knudson MJ, Henning JR, et al. Anti-interleukin-10R1 monoclonal antibody enhances bacillus Calmette-Guerin induced T-helper type 1 immune responses and antitumor immunity in a mouse orthotopic model of bladder cancer. J Urol. 2012;187(6):2228–35.
113. Baj-Krzyworzeka M, Baran J, Szatanek R, et al. Prevention and reversal of tumor cell-induced monocyte deactivation by cytokines, purified protein derivative (PPD), and anti-IL-10 antibody. Cancer Immun. 2004;4:8.
114. Harrison ML, Obermueller E, Maisey NR, et al. Tumor necrosis factor alpha as a new target for renal cell carcinoma: two sequential phase II trials of infliximab at standard and high dose. J Clin Oncol. 2007;25(29):4542–9.
115. Zeng J, Cai S, Yi Y, et al. Prevention of spontaneous tumor development in a ret transgenic mouse model by ret peptide vaccination with indoleamine 2,3-dioxygenase inhibitor 1-methyl tryptophan. Cancer Res. 2009;69(9):3963–70.
116. Qian F, Villella J, Wallace PK, et al. Efficacy of levo-1-methyl tryptophan and dextro-1-methyl tryptophan in reversing indoleamine-2,3-dioxygenase-mediated arrest of T-cell proliferation in human epithelial ovarian cancer. Cancer Res. 2009;69(13):5498–504.
117. Yang HJ, Yen MC, Lin CC, et al. A combination of the metabolic enzyme inhibitor APO866 and the immune adjuvant L-1-methyl tryptophan induces additive antitumor activity. Exp Biol Med (Maywood). 2010;235(7):869–76.
118. Dannull J, Su Z, Rizzieri D, et al. Enhancement of vaccine-mediated antitumor immunity in cancer patients after depletion of regulatory T cells. J Clin Invest. 2005;115(12):3623–33.
119. Duvic M, Geskin L, Prince HM. Duration of response in cutaneous T-cell lymphoma patients treated with denileukin diftitox: results from 3 phase III studies. Clin Lymphoma Myeloma Leuk. 2013;13(4):377–84.
120. Telang S, Rasku MA, Clem AL, et al. Phase II trial of the regulatory T cell-depleting agent, denileukin diftitox, in patients with unresectable stage IV melanoma. BMC Cancer. 2011;11:515.
121. Collison LW, Workman CJ, Kuo TT, et al. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. Nature. 2007;450(7169):566–9.
122. Jinushi M, Nakazaki Y, Dougan M, et al. MFG-E8-mediated uptake of apoptotic cells by APCs links the pro- and antiinflammatory activities of GM-CSF. J Clin Invest. 2007;117(7):1902–13.
123. Nair S, McLaughlin C, Weizer A, et al. Injection of immature dendritic cells into adjuvant-treated skin obviates the need for ex vivo maturation. J Immunol. 2003;171(11):6275–82.
124. Jiang W, Swiggard WJ, Heufler C, et al. The receptor DEC-205 expressed by dendritic cells and thymic epithelial cells is involved in antigen processing. Nature. 1995;375(6527):151–5.
125. Lahoud MH, Ahmet F, Kitsoulis S, et al. Targeting antigen to mouse dendritic cells via Clec9A induces potent CD4 T cell responses biased toward a follicular helper phenotype. J Immunol. 2011;187(2):842–50.
126. Akiyama Y, Oshita C, Kume A, et al. Alpha-type-1 polarized dendritic cell-based vaccination in recurrent high-grade glioma: a phase I clinical trial. BMC Cancer. 2012;12:623.
127. Chang CN, Huang YC, Yang DM, et al. A phase I/II clinical trial investigating the adverse and therapeutic effects of a postoperative autologous dendritic cell tumor vaccine in patients with malignant glioma. J Clin Neurosci. 2011;18(8):1048–54.
128. Ardon H, Van Gool SW, Verschuere T, et al. Integration of autologous dendritic cell-based immunotherapy in the standard of care treatment for patients with newly diagnosed glioblastoma: results of the HGG-2006 phase I/II trial. Cancer Immunol Immunother. 2012;61(11):2033–44.
129. Tada F, Abe M, Hirooka M, et al. Phase I/II study of immunotherapy using tumor antigen-pulsed dendritic cells in patients with hepatocellular carcinoma. Int J Oncol. 2012;41(5):1601–9.
130. Rong Y, Qin X, Jin D, et al. A phase I pilot trial of MUC1-peptide-pulsed dendritic cells in the treatment of advanced pancreatic cancer. Clin Exp Med. 2012;12(3):173–80.
131. Lesterhuis WJ, De Vries IJ, Schreibelt G, et al. Immunogenicity of dendritic cells pulsed with CEA peptide or transfected with CEA mRNA for vaccination of colorectal cancer patients. Anticancer Res. 2010;30(12):5091–7.
132. Oshita C, Takikawa M, Kume A, et al. Dendritic cell-based vaccination in metastatic melanoma patients: phase II clinical trial. Oncol Rep. 2012;28(4):1131–8.
133. Wilgenhof S, Van Nuffel AM, Corthals J, et al. Therapeutic vaccination with an autologous mRNA

electroporated dendritic cell vaccine in patients with advanced melanoma. J Immunother. 2011;34(5):448–56.
134. Zahradova L, Mollova K, Ocadlikova D, et al. Efficacy and safety of Id-protein-loaded dendritic cell vaccine in patients with multiple myeloma—phase II study results. Neoplasma. 2012;59(4):440–9.
135. Rollig C, Schmidt C, Bornhauser M, et al. Induction of cellular immune responses in patients with stage-I multiple myeloma after vaccination with autologous idiotype-pulsed dendritic cells. J Immunother. 2011;34(1):100–6.
136. Yi Q, Szmania S, Freeman J, et al. Optimizing dendritic cell-based immunotherapy in multiple myeloma: intranodal injections of idiotype-pulsed CD40 ligand-matured vaccines led to induction of type-1 and cytotoxic T-cell immune responses in patients. Br J Haematol. 2010;150(5):554–64.
137. Kitawaki T, Kadowaki N, Fukunaga K, et al. A phase I/IIa clinical trial of immunotherapy for elderly patients with acute myeloid leukaemia using dendritic cells co-pulsed with WT1 peptide and zoledronate. Br J Haematol. 2011;153(6):796–9.
138. Van Tendeloo VF, Van de Velde A, Van Driessche A, et al. Induction of complete and molecular remissions in acute myeloid leukemia by Wilms' tumor 1 antigen-targeted dendritic cell vaccination. Proc Natl Acad Sci U S A. 2010;107(31):13824–9.
139. Qi CJ, Ning YL, Han YS, et al. Autologous dendritic cell vaccine for estrogen receptor (ER)/progestin receptor (PR) double-negative breast cancer. Cancer Immunol Immunother. 2012;61(9):1415–24.
140. Chu CS, Boyer J, Schullery DS, et al. Phase I/II randomized trial of dendritic cell vaccination with or without cyclophosphamide for consolidation therapy of advanced ovarian cancer in first or second remission. Cancer Immunol Immunother. 2012;61(5):629–41.
141. Rahma OE, Ashtar E, Czystowska M, et al. A gynecologic oncology group phase II trial of two p53 peptide vaccine approaches: subcutaneous injection and intravenous pulsed dendritic cells in high recurrence risk ovarian cancer patients. Cancer Immunol Immunother. 2012;61(3):373–84.
142. Baek S, Kim CS, Kim SB, et al. Combination therapy of renal cell carcinoma or breast cancer patients with dendritic cell vaccine and IL-2: results from a phase I/II trial. J Transl Med. 2011;9:178.
143. Perroud Jr MW, Honma HN, Barbeiro AS, et al. Mature autologous dendritic cell vaccines in advanced non-small cell lung cancer: a phase I pilot study. J Exp Clin Cancer Res. 2011;30:65.
144. Shirasu N, Kuroki M. Functional design of chimeric T-cell antigen receptors for adoptive immunotherapy of cancer: architecture and outcomes. Anticancer Res. 2012;32(6):2377–83.
145. Turtle CJ, Hudecek M, Jensen MC, et al. Engineered T cells for anti-cancer therapy. Curr Opin Immunol. 2012;24(5):633–9.
146. Liu D, Song L, Wei J, et al. IL-15 protects NKT cells from inhibition by tumor-associated macrophages and enhances antimetastatic activity. J Clin Invest. 2012;122(6):2221–33.
147. Klebanoff CA, Gattinoni L, Restifo NP. Sorting through subsets: which T-cell populations mediate highly effective adoptive immunotherapy? J Immunother. 2012;35(9):651–60.
148. Grupp SA, Prak EL, Boyer J, et al. Adoptive transfer of autologous T cells improves T-cell repertoire diversity and long-term B-cell function in pediatric patients with neuroblastoma. Clin Cancer Res. 2012;18(24):6732–41.
149. Zhou P, Liang P, Dong B, et al. Phase clinical study of combination therapy with microwave ablation and cellular immunotherapy in hepatocellular carcinoma. Cancer Biol Ther. 2011;11(5):450–6.
150. Shi L, Zhou Q, Wu J, et al. Efficacy of adjuvant immunotherapy with cytokine-induced killer cells in patients with locally advanced gastric cancer. Cancer Immunol Immunother. 2012;61(12):2251–9.
151. Bernatchez C, Radvanyi LG, Hwu P. Advances in the treatment of metastatic melanoma: adoptive T-cell therapy. Semin Oncol. 2012;39(2):215–26.
152. Radvanyi LG, Bernatchez C, Zhang M, et al. Specific lymphocyte subsets predict response to adoptive cell therapy using expanded autologous tumor-infiltrating lymphocytes in metastatic melanoma patients. Clin Cancer Res. 2012;18(24):6758–70.
153. Verdegaal EM, Visser M, Ramwadhdoebe TH, et al. Successful treatment of metastatic melanoma by adoptive transfer of blood-derived polyclonal tumor-specific CD4+ and CD8+ T cells in combination with low-dose interferon-alpha. Cancer Immunol Immunother. 2011;60(7):953–63.
154. Linn YC, Niam M, Chu S, et al. The anti-tumour activity of allogeneic cytokine-induced killer cells in patients who relapse after allogeneic transplant for haematological malignancies. Bone Marrow Transplant. 2012;47(7):957–66.
155. Rapoport AP, Aqui NA, Stadtmauer EA, et al. Combination immunotherapy using adoptive T-cell transfer and tumor antigen vaccination on the basis of hTERT and survivin after ASCT for myeloma. Blood. 2011;117(3):788–97.
156. Warren EH, Fujii N, Akatsuka Y, et al. Therapy of relapsed leukemia after allogeneic hematopoietic cell transplantation with T cells specific for minor histocompatibility antigens. Blood. 2010;115(19):3869–78.
157. Nicol AJ, Tokuyama H, Mattarollo SR, et al. Clinical evaluation of autologous gamma delta T cell-based immunotherapy for metastatic solid tumours. Br J Cancer. 2011;105(6):778–86.
158. Parkhurst MR, Yang JC, Langan RC, et al. T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis. Mol Ther. 2011;19(3):620–6.
159. Bollard CM, Rooney CM, Heslop HE. T-cell therapy in the treatment of post-transplant lymphoproliferative disease. Nat Rev Clin Oncol. 2012;9(9):510–9.

160. Moosmann A, Bigalke I, Tischer J, et al. Effective and long-term control of EBV PTLD after transfer of peptide-selected T cells. Blood. 2010;115(14):2960–70.
161. Wright SE. Immunotherapy of breast cancer. Expert Opin Biol Ther. 2012;12(4):479–90.
162. Hardy NM, Mossoba ME, Steinberg SM, et al. Phase I trial of adoptive cell transfer with mixed-profile type-I/type-II allogeneic T cells for metastatic breast cancer. Clin Cancer Res. 2011;17(21):6878–87.
163. Kandalaft LE, Powell Jr DJ, Coukos G. A phase I clinical trial of adoptive transfer of folate receptor-alpha redirected autologous T cells for recurrent ovarian cancer. J Transl Med. 2012;10:157.
164. Geller MA, Cooley S, Judson PL, et al. A phase II study of allogeneic natural killer cell therapy to treat patients with recurrent ovarian and breast cancer. Cytotherapy. 2011;13(1):98–107.
165. Iwai K, Soejima K, Kudoh S, et al. Extended survival observed in adoptive activated T lymphocyte immunotherapy for advanced lung cancer: results of a multicenter historical cohort study. Cancer Immunol Immunother. 2012;61(10):1781–90.
166. Sakamoto M, Nakajima J, Murakawa T, et al. Adoptive immunotherapy for advanced non-small cell lung cancer using zoledronate-expanded gammadelta T cells: a phase I clinical study. J Immunother. 2011;34(2):202–11.
167. Kobayashi H, Tanaka Y, Yagi J, et al. Phase I/II study of adoptive transfer of gammadelta T cells in combination with zoledronic acid and IL-2 to patients with advanced renal cell carcinoma. Cancer Immunol Immunother. 2011;60(8):1075–84.
168. Louis CU, Straathof K, Bollard CM, et al. Adoptive transfer of EBV-specific T cells results in sustained clinical responses in patients with locoregional nasopharyngeal carcinoma. J Immunother. 2010;33(9):983–90.
169. Podberezin M, Wen J, Chang CC. Cancer stem cells: a review of potential clinical applications. Arch Pathol Lab Med. 2012;137(8):1111–6.
170. Chiriva-Internati M, Pandey A, Saba R, et al. Cancer testis antigens: a novel target in lung cancer. Int Rev Immunol. 2012;31(5):321–43.
171. Kulkarni P, Shiraishi T, Rajagopalan K, et al. Cancer/testis antigens and urological malignancies. Nat Rev Urol. 2012;9(7):386–96.
172. Meek DW, Marcar L. MAGE-A antigens as targets in tumour therapy. Cancer Lett. 2012;324(2):126–32.
173. Pandey A, Kurup A, Shrivastava A, et al. Cancer testes antigens in breast cancer: biological role, regulation, and therapeutic applicability. Int Rev Immunol. 2012;31(5):302–20.
174. Odunsi K, Matsuzaki J, Karbach J, et al. Efficacy of vaccination with recombinant vaccinia and fowlpox vectors expressing NY-ESO-1 antigen in ovarian cancer and melanoma patients. Proc Natl Acad Sci U S A. 2012;109(15):5797–802.
175. Adams S, O'Neill DW, Nonaka D, et al. Immunization of malignant melanoma patients with full-length NY-ESO-1 protein using TLR7 agonist imiquimod as vaccine adjuvant. J Immunol. 2008;181(1):776–84.
176. Robbins PF, Morgan RA, Feldman SA, et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. J Clin Oncol. 2011;29(7):917–24.
177. McCormack E, Adams KJ, Hassan NJ, et al. Bi-specific TCR-anti CD3 redirected T-cell targeting of NY-ESO-1- and LAGE-1-positive tumors. Cancer Immunol Immunother. 2013;62(4):773–85.
178. Brossart P, Wirths S, Stuhler G, et al. Induction of cytotoxic T-lymphocyte responses in vivo after vaccinations with peptide-pulsed dendritic cells. Blood. 2000;96(9):3102–8.
179. Sonpavde G, Wang M, Peterson LE, et al. HLA-restricted NY-ESO-1 peptide immunotherapy for metastatic castration resistant prostate cancer. Invest New Drugs. 2014;32:235–42.
180. Simon T, Fonteneau JF, Gregoire M. Requirement of tumor-associated antigen-specific CD4(+) T cells for an efficient dendritic cell vaccine in antitumor immunotherapy. Immunotherapy. 2013;5(6):565–7.
181. Kamigaki T, Kaneko T, Naitoh K, et al. Immunotherapy of autologous tumor lysate-loaded dendritic cell vaccines by a closed-flow electroporation system for solid tumors. Anticancer Res. 2013;33(7):2971–6.
182. Hui EP, Taylor GS, Jia H, et al. Phase I trial of recombinant modified vaccinia ankara encoding Epstein-Barr viral tumor antigens in nasopharyngeal carcinoma patients. Cancer Res. 2013;73(6):1676–88.
183. Smith C, Khanna R. A new approach for cellular immunotherapy of nasopharyngeal carcinoma. Oncoimmunology. 2012;1(8):1440–2.
184. Van Nuffel AM, Benteyn D, Wilgenhof S, et al. Dendritic cells loaded with mRNA encoding full-length tumor antigens prime CD4+ and CD8+ T cells in melanoma patients. Mol Ther. 2012;20(5):1063–74.
185. Van Nuffel AM, Tuyaerts S, Benteyn D, et al. Epitope and HLA-type independent monitoring of antigen-specific T-cells after treatment with dendritic cells presenting full-length tumor antigens. J Immunol Methods. 2012;377(1–2):23–36.
186. Ohno S, Takano F, Ohta Y, et al. Frequency of myeloid dendritic cells can predict the efficacy of Wilms' tumor 1 peptide vaccination. Anticancer Res. 2011;31(7):2447–52.
187. Suh KW, Piantadosi S, Yazdi HA, et al. Treatment of liver metastases from colon carcinoma with autologous tumor vaccine expressing granulocyte-macrophage colony-stimulating factor. J Surg Oncol. 1999;72(4):218–24.
188. Soiffer R, Hodi FS, Haluska F, et al. Vaccination with irradiated, autologous melanoma cells engineered to secrete granulocyte-macrophage colony-stimulating factor by adenoviral-mediated gene transfer augments antitumor immunity in patients with metastatic melanoma. J Clin Oncol. 2003;21(17):3343–50.

189. Salgia R, Lynch T, Skarin A, et al. Vaccination with irradiated autologous tumor cells engineered to secrete granulocyte-macrophage colony-stimulating factor augments antitumor immunity in some patients with metastatic non-small-cell lung carcinoma. J Clin Oncol. 2003;21(4):624–30.
190. Leong SP, Enders-Zohr P, Zhou YM, et al. Recombinant human granulocyte macrophage-colony stimulating factor (rhGM-CSF) and autologous melanoma vaccine mediate tumor regression in patients with metastatic melanoma. J Immunother. 1999;22(2):166–74.
191. Soiffer R, Lynch T, Mihm M, et al. Vaccination with irradiated autologous melanoma cells engineered to secrete human granulocyte-macrophage colony-stimulating factor generates potent antitumor immunity in patients with metastatic melanoma. Proc Natl Acad Sci U S A. 1998;95(22):13141–6.
192. Lutz E, Yeo CJ, Lillemoe KD, et al. A lethally irradiated allogeneic granulocyte-macrophage colony stimulating factor-secreting tumor vaccine for pancreatic adenocarcinoma. A phase II trial of safety, efficacy, and immune activation. Ann Surg. 2011;253(2):328–35.
193. Jaffee EM, Hruban RH, Biedrzycki B, et al. Novel allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: a phase I trial of safety and immune activation. J Clin Oncol. 2001;19(1):145–56.
194. Michael A, Ball G, Quatan N, et al. Delayed disease progression after allogeneic cell vaccination in hormone-resistant prostate cancer and correlation with immunologic variables. Clin Cancer Res. 2005;11(12):4469–78.
195. Capuron L, Ravaud A, Dantzer R. Early depressive symptoms in cancer patients receiving interleukin 2 and/or interferon alfa-2b therapy. J Clin Oncol. 2000;18(10):2143–51.
196. Atkins MB, Kunkel L, Sznol M, et al. High-dose recombinant interleukin-2 therapy in patients with metastatic melanoma: long-term survival update. Cancer J Sci Am. 2000;6 Suppl 1:S11–4.
197. Mekhail T, Wood L, Bukowski R. Interleukin-2 in cancer therapy: uses and optimum management of adverse effects. BioDrugs. 2000;14(5):299–318.
198. Napolitani G, Rinaldi A, Bertoni F, et al. Selected Toll-like receptor agonist combinations synergistically trigger a T helper type 1-polarizing program in dendritic cells. Nat Immunol. 2005;6(8):769–76.
199. Valmori D, Souleimanian NE, Tosello V, et al. Vaccination with NY-ESO-1 protein and CpG in Montanide induces integrated antibody/Th1 responses and CD8 T cells through cross-priming. Proc Natl Acad Sci U S A. 2007;104(21):8947–52.
200. Molenkamp BG, van Leeuwen PA, Meijer S, et al. Intradermal CpG-B activates both plasmacytoid and myeloid dendritic cells in the sentinel lymph node of melanoma patients. Clin Cancer Res. 2007;13(10):2961–9.
201. Fourcade J, Kudela P, Andrade Filho PA, et al. Immunization with analog peptide in combination with CpG and montanide expands tumor antigen-specific CD8+ T cells in melanoma patients. J Immunother. 2008;31(8):781–91.
202. Pashenkov M, Goess G, Wagner C, et al. Phase II trial of a toll-like receptor 9-activating oligonucleotide in patients with metastatic melanoma. J Clin Oncol. 2006;24(36):5716–24.
203. Hofmann MA, Kors C, Audring H, et al. Phase 1 evaluation of intralesionally injected TLR9-agonist PF-3512676 in patients with basal cell carcinoma or metastatic melanoma. J Immunother. 2008;31(5):520–7.
204. Leonard JP, Link BK, Emmanouilides C, et al. Phase I trial of toll-like receptor 9 agonist PF-3512676 with and following rituximab in patients with recurrent indolent and aggressive non Hodgkin's lymphoma. Clin Cancer Res. 2007;13(20):6168–74.
205. Belani CP, Nemunaitis JJ, Chachoua A, et al. Phase 2 trial of erlotinib with or without PF-3512676 (CPG 7909, a toll-like receptor 9 agonist) in patients with advanced recurrent EGFR-positive non-small cell lung cancer. Cancer Biol Ther. 2013;14(7):557–63.
206. Millward M, Underhill C, Lobb S, et al. Phase I study of tremelimumab (CP-675 206) plus PF-3512676 (CPG 7909) in patients with melanoma or advanced solid tumours. Br J Cancer. 2013;108(10):1998–2004.
207. Zent CS, Smith BJ, Ballas ZK, et al. Phase I clinical trial of CpG oligonucleotide 7909 (PF-03512676) in patients with previously treated chronic lymphocytic leukemia. Leuk Lymphoma. 2012;53(2):211–7.
208. Hirsh V, Paz-Ares L, Boyer M, et al. Randomized phase III trial of paclitaxel/carboplatin with or without PF-3512676 (Toll-like receptor 9 agonist) as first-line treatment for advanced non-small-cell lung cancer. J Clin Oncol. 2011;29(19):2667–74.
209. Matsushita H, Ohta S, Shiraishi H, et al. Endotoxin tolerance attenuates airway allergic inflammation in model mice by suppression of the T-cell stimulatory effect of dendritic cells. Int Immunol. 2010;22(9):739–47.
210. Iwahashi M, Katsuda M, Nakamori M, et al. Vaccination with peptides derived from cancer-testis antigens in combination with CpG-7909 elicits strong specific CD8+ T cell response in patients with metastatic esophageal squamous cell carcinoma. Cancer Sci. 2010;101(12):2510–7.
211. Tomillero A, Moral MA. Gateways to clinical trials. Methods Find Exp Clin Pharmacol. 2008;30(7):543–88.
212. Molckovsky A, Siu LL. First-in-class, first-in-human phase I results of targeted agents: highlights of the 2008 American society of clinical oncology meeting. J Hematol Oncol. 2008;1:20.
213. Mauerer R, Gruber R. Monoclonal antibodies for the immunotherapy of solid tumours. Curr Pharm Biotechnol. 2012;13(8):1385–98.
214. Bennett L, Zhao Z, Barber B, et al. Health-related quality of life in patients with metastatic colorectal

cancer treated with panitumumab in first- or second-line treatment. Br J Cancer. 2011;105(10):1495–502.
215. Bast Jr RC, Segerling M, Ohanian SH, et al. Regression of established tumors and induction of tumor immunity by intratumor chemotherapy. J Natl Cancer Inst. 1976;56(4):829–32.
216. Turk JL, Parker D, Poulter LW. Functional aspects of the selective depletion of lymphoid tissue by cyclophosphamide. Immunology. 1972;23(4):493–501.
217. Durante M, Reppingen N, Held KD. Immunologically augmented cancer treatment using modern radiotherapy. Trends Mol Med. 2013;19(9):565–82.
218. Kroemer G, Galluzzi L, Kepp O, et al. Immunogenic cell death in cancer therapy. Annu Rev Immunol. 2013;31:51–72.
219. Weng D, Song B, Koido S, et al. Immunotherapy of radioresistant mammary tumors with early metastasis using molecular chaperone vaccines combined with ionizing radiation. J Immunol. 2013;191(2):755–63.
220. Harris M, Wang XG, Jiang Z, et al. Combined treatment of the experimental human papilloma virus-16-positive cervical and head and neck cancers with cisplatin and radioimmunotherapy targeting viral E6 oncoprotein. Br J Cancer. 2013;108(4):859–65.
221. Formenti SC, Demaria S. Combining radiotherapy and cancer immunotherapy: a paradigm shift. J Natl Cancer Inst. 2013;105(4):256–65.
222. Yang Y, Liu C, Peng W, et al. Antitumor T-cell responses contribute to the effects of dasatinib on c-KIT mutant murine mastocytoma and are potentiated by anti-OX40. Blood. 2012;120(23):4533–43.
223. Press OW, Unger JM, Rimsza LM, et al. Phase III randomized intergroup trial of CHOP plus rituximab compared with CHOP chemotherapy plus (131) iodine-tositumomab for previously untreated follicular non-Hodgkin lymphoma: SWOG S0016. J Clin Oncol. 2013;31(3):314–20.
224. Smith MR, Li H, Gordon L, et al. Phase II study of rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone immunochemotherapy followed by yttrium-90-ibritumomab tiuxetan in untreated mantle-cell lymphoma: Eastern Cooperative Oncology Group study E1499. J Clin Oncol. 2012;30(25):3119–26.
225. Kruger PC, Cooney JP, Turner JH. Iodine-131 rituximab radioimmunotherapy with BEAM conditioning and autologous stem cell transplant salvage therapy for relapsed/refractory aggressive non-Hodgkin lymphoma. Cancer Biother Radiopharm. 2012;27(9):552–60.
226. Cunningham D, Hawkes EA, Jack A, et al. Rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisolone in patients with newly diagnosed diffuse large B-cell non-Hodgkin lymphoma: a phase 3 comparison of dose intensification with 14-day versus 21-day cycles. Lancet. 2013;381(9880):1817–26.
227. Delarue R, Haioun C, Ribrag V, et al. CHOP and DHAP plus rituximab followed by autologous stem cell transplantation in mantle cell lymphoma: a phase 2 study from the Groupe d'Etude des Lymphomes de l'Adulte. Blood. 2013;121(1):48–53.
228. Hardacre JM, Mulcahy M, Small W, et al. Addition of algenpantucel-L immunotherapy to standard adjuvant therapy for pancreatic cancer: a phase 2 study. J Gastrointest Surg. 2013;17(1):94–100.
229. Ocean AJ, Pennington KL, Guarino MJ, et al. Fractionated radioimmunotherapy with (90) Y-clivatuzumab tetraxetan and low-dose gemcitabine is active in advanced pancreatic cancer: a phase 1 trial. Cancer. 2012;118(22):5497–506.
230. Fujiwara S, Wada H, Miyata H, et al. Clinical trial of the intratumoral administration of labeled DC combined with systemic chemotherapy for esophageal cancer. J Immunother. 2012;35(6):513–21.
231. Motzer RJ, Escudier B, Tomczak P, et al. Axitinib versus sorafenib as second-line treatment for advanced renal cell carcinoma: overall survival analysis and updated results from a randomised phase 3 trial. Lancet Oncol. 2013;14(6):552–62.
232. Atmaca A, Al-Batran SE, Werner D, et al. A randomised multicentre phase II study with cisplatin/docetaxel vs oxaliplatin/docetaxel as first-line therapy in patients with advanced or metastatic non-small cell lung cancer. Br J Cancer. 2013;108(2):265–70.
233. Heng DY, Mackenzie MJ, Vaishampayan UN, et al. Primary anti-vascular endothelial growth factor (VEGF)-refractory metastatic renal cell carcinoma: clinical characteristics, risk factors, and subsequent therapy. Ann Oncol. 2012;23(6):1549–55.
234. Lara Jr PN, Quinn DI, Margolin K, et al. SU5416 plus interferon alpha in advanced renal cell carcinoma: a phase II California cancer consortium study with biological and imaging correlates of angiogenesis inhibition. Clin Cancer Res. 2003;9(13):4772–81.
235. Cole BF, Gelber RD, Kirkwood JM, et al. Quality-of-life-adjusted survival analysis of interferon alfa-2b adjuvant treatment of high-risk resected cutaneous melanoma: an Eastern Cooperative Oncology Group study. J Clin Oncol. 1996;14(10):2666–73.
236. Glynne-Jones R, Grainger J, Harrison M, et al. Neoadjuvant chemotherapy prior to preoperative chemoradiation or radiation in rectal cancer: should we be more cautious? Br J Cancer. 2006;94(3):363–71.
237. Lillehei KO, Mitchell DH, Johnson SD, et al. Long-term follow-up of patients with recurrent malignant gliomas treated with adjuvant adoptive immunotherapy. Neurosurgery. 1991;28(1):16–23.
238. Dillman RO, Duma CM, Ellis RA, et al. Intralesional lymphokine-activated killer cells as adjuvant therapy for primary glioblastoma. J Immunother. 2009;32(9):914–9.
239. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. Nature. 2011;480(7378):480–9.
240. Hunter R. WHO handbook for reporting results of cancer treatment. Int J Radiat Biol. 1980;38(4):481.
241. Nishino M, Jackman DM, Hatabu H, et al. New Response Evaluation Criteria in Solid Tumors (RECIST) guidelines for advanced non-small cell lung

cancer: comparison with original RECIST and impact on assessment of tumor response to targeted therapy. AJR Am J Roentgenol. 2010;195(3):W221–8.
242. Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res. 2009;15(23):7412–20.
243. Shim JH, Lee HC, Kim SO, et al. Which response criteria best help predict survival of patients with hepatocellular carcinoma following chemoembolization? A validation study of old and new models. Radiology. 2012;262(2):708–18.
244. Britten CM, Janetzki S, van der Burg SH, et al. Minimal information about T cell assays: the process of reaching the community of T cell immunologists in cancer and beyond. Cancer Immunol Immunother. 2011;60(1):15–22.
245. Janetzki S, Britten CM, Kalos M, et al. "MIATA"-minimal information about T cell assays. Immunity. 2009;31(4):527–8.
246. Cheever MA. Twelve immunotherapy drugs that could cure cancers. Immunol Rev. 2008;222:357–68.
247. Fecher LA, Agarwala SS, Hodi FS, et al. Ipilimumab and its toxicities: a multidisciplinary approach. Oncologist. 2013;18(6):733–43.
248. Palumbo MO, Kavan P, Miller Jr WH, et al. Systemic cancer therapy: achievements and challenges that lie ahead. Front Pharmacol. 2013;4:57.
249. Alatrash G, Jakher H, Stafford PD, et al. Cancer immunotherapies, their safety and toxicity. Expert Opin Drug Saf. 2013;12:631–45.
250. de Claro RA, McGinn K, Kwitkowski V, et al. U.S. food and drug administration approval summary: brentuximab vedotin for the treatment of relapsed Hodgkin lymphoma or relapsed systemic anaplastic large-cell lymphoma. Clin Cancer Res. 2012;18(21):5845–9.
251. Ocvirk J, Heeger S, McCloud P, et al. A review of the treatment options for skin rash induced by EGFR-targeted therapies: evidence from randomized clinical trials and a meta-analysis. Radiol Oncol. 2013;47(2):166–75.
252. Letarte N, Bressler LR, Villano JL. Bevacizumab and central nervous system (CNS) hemorrhage. Cancer Chemother Pharmacol. 2013;71(6):1561–5.
253. Lee S, Knox A, Zeng IS, et al. Primary prophylaxis with granulocyte colony-stimulating factor (GCSF) reduces the incidence of febrile neutropenia in patients with non-hodgkin lymphoma (NHL) receiving CHOP chemotherapy treatment without adversely affecting their quality of life: cost-benefit and quality of life analysis. Support Care Cancer. 2013;21(3):841–6.
254. Younis T, Rayson D, Thompson K. Primary G-CSF prophylaxis for adjuvant TC or FEC-D chemotherapy outside of clinical trial settings: a systematic review and meta-analysis. Support Care Cancer. 2012;20(10):2523–30.
255. Chan A, Fu WH, Shih V, et al. Impact of colony-stimulating factors to reduce febrile neutropenic events in breast cancer patients receiving docetaxel plus cyclophosphamide chemotherapy. Support Care Cancer. 2011;19(4):497–504.
256. Aapro MS, Bohlius J, Cameron DA, et al. 2010 update of EORTC guidelines for the use of granulocyte-colony stimulating factor to reduce the incidence of chemotherapy-induced febrile neutropenia in adult patients with lymphoproliferative disorders and solid tumours. Eur J Cancer. 2011;47(1):8–32.
257. Martin M, Lluch A, Segui MA, et al. Toxicity and health-related quality of life in breast cancer patients receiving adjuvant docetaxel, doxorubicin, cyclophosphamide (TAC) or 5-fluorouracil, doxorubicin and cyclophosphamide (FAC): impact of adding primary prophylactic granulocyte-colony stimulating factor to the TAC regimen. Ann Oncol. 2006;17(8):1205–12.
258. Robien K, Schubert MM, Yasui Y, et al. Folic acid supplementation during methotrexate immunosuppression is not associated with early toxicity, risk of acute graft-versus-host disease or relapse following hematopoietic transplantation. Bone Marrow Transplant. 2006;37(7):687–92.
259. Giese-Davis J, Collie K, Rancourt KM, et al. Decrease in depression symptoms is associated with longer survival in patients with metastatic breast cancer: a secondary analysis. J Clin Oncol. 2011;29(4):413–20.
260. Hontscha C, Borck Y, Zhou H, et al. Clinical trials on CIK cells: first report of the international registry on CIK cells (IRCC). J Cancer Res Clin Oncol. 2011;137(2):305–10.
261. Lang I, Kohne CH, Folprecht G, et al. Quality of life analysis in patients with KRAS wild-type metastatic colorectal cancer treated first-line with cetuximab plus irinotecan, fluorouracil and leucovorin. Eur J Cancer. 2013;49(2):439–48.
262. Revicki DA, van den Eertwegh AJ, Lorigan P, et al. Health related quality of life outcomes for unresectable stage III or IV melanoma patients receiving ipilimumab treatment. Health Qual Life Outcomes. 2012;10:66.
263. Fesenfeld M, Hutubessy R, Jit M. Cost-effectiveness of human papillomavirus vaccination in low and middle income countries: a systematic review. Vaccine. 2013;31(37):3786–804.
264. Avella DM, Li G, Schell TD, et al. Regression of established hepatocellular carcinoma is induced by chemoimmunotherapy in an orthotopic murine model. Hepatology. 2012;55(1):141–52.
265. Xie F, Zhang X, Li H, et al. Adoptive immunotherapy in postoperative hepatocellular carcinoma: a systemic review. PLoS One. 2012;7(8):e42879.
266. Hershman D, Hurley D, Wong M, et al. Impact of primary prophylaxis on febrile neutropenia within community practices in the US. J Med Econ. 2009;12(3):203–10.
267. Pro B, Advani R, Brice P, et al. Brentuximab vedotin (SGN-35) in patients with relapsed or refractory systemic anaplastic large-cell lymphoma: results of a phase II study. J Clin Oncol. 2012;30(18):2190–6.

268. Younes A, Bartlett NL, Leonard JP, et al. Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas. N Engl J Med. 2010;363(19):1812–21.
269. Higano CS, Schellhammer PF, Small EJ, et al. Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. Cancer. 2009;115(16):3670–9.
270. Small EJ, Schellhammer PF, Higano CS, et al. Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer. J Clin Oncol. 2006;24(19):3089–94.
271. Smith MR, Saad F, Coleman R, et al. Denosumab and bone-metastasis-free survival in men with castration-resistant prostate cancer: results of a phase 3, randomised, placebo-controlled trial. Lancet. 2012;379(9810):39–46.
272. Czuczman MS, Fayad L, Delwail V, et al. Ofatumumab monotherapy in rituximab-refractory follicular lymphoma: results from a multicenter study. Blood. 2012;119(16):3698–704.
273. Wierda WG, Padmanabhan S, Chan GW, et al. Ofatumumab is active in patients with fludarabine-refractory CLL irrespective of prior rituximab: results from the phase 2 international study. Blood. 2011;118(19):5126–9.
274. Hagenbeek A, Gadeberg O, Johnson P, et al. First clinical use of ofatumumab, a novel fully human anti-CD20 monoclonal antibody in relapsed or refractory follicular lymphoma: results of a phase 1/2 trial. Blood. 2008;111(12):5486–95.
275. Waddell T, Chau I, Cunningham D, et al. Epirubicin, oxaliplatin, and capecitabine with or without panitumumab for patients with previously untreated advanced oesophagogastric cancer (REAL3): a randomised, open-label phase 3 trial. Lancet Oncol. 2013;14(6):481–9.
276. Thaler J, Karthaus M, Mineur L, et al. Skin toxicity and quality of life in patients with metastatic colorectal cancer during first-line panitumumab plus FOLFIRI treatment in a single-arm phase II study. BMC Cancer. 2012;12:438.
277. Lacouture ME, Mitchell EP, Piperdi B, et al. Skin toxicity evaluation protocol with panitumumab (STEPP), a phase II, open-label, randomized trial evaluating the impact of a pre-emptive skin treatment regimen on skin toxicities and quality of life in patients with metastatic colorectal cancer. J Clin Oncol. 2010;28(8):1351–7.
278. Peeters M, Siena S, Van Cutsem E, et al. Association of progression-free survival, overall survival, and patient-reported outcomes by skin toxicity and KRAS status in patients receiving panitumumab monotherapy. Cancer. 2009;115(7):1544–54.
279. Mamot C, Ritschard R, Wicki A, et al. Tolerability, safety, pharmacokinetics, and efficacy of doxorubicin-loaded anti-EGFR immunoliposomes in advanced solid tumours: a phase 1 dose-escalation study. Lancet Oncol. 2012;13(12):1234–41.
280. Van Cutsem E, Tejpar S, Vanbeckevoort D, et al. Intrapatient cetuximab dose escalation in metastatic colorectal cancer according to the grade of early skin reactions: the randomized EVEREST study. J Clin Oncol. 2012;30(23):2861–8.
281. Schmid-Bindert G, Gebbia V, Mayer F, et al. Phase II study of pemetrexed and cisplatin plus cetuximab followed by pemetrexed and cetuximab maintenance therapy in patients with advanced nonsquamous non-small cell lung cancer. Lung Cancer. 2013;81(3):428–32.
282. Chen XL, Lei YH, Liu CF, et al. Angiogenesis inhibitor bevacizumab increases the risk of ischemic heart disease associated with chemotherapy: a meta-analysis. PLoS One. 2013;8(6):e66721.
283. Vose JM, Carter S, Burns LJ, et al. Phase III randomized study of rituximab/carmustine, etoposide, cytarabine, and melphalan (BEAM) compared with iodine-131 tositumomab/BEAM with autologous hematopoietic cell transplantation for relapsed diffuse large B-cell lymphoma: results from the BMT CTN 0401 trial. J Clin Oncol. 2013;31(13):1662–8.
284. Amro H, Wilderman SJ, Dewaraja YK, et al. Methodology to incorporate biologically effective dose and equivalent uniform dose in patient-specific 3-dimensional dosimetry for non-hodgkin lymphoma patients targeted with 131I-tositumomab therapy. J Nucl Med. 2010;51(4):654–9.
285. Witzig TE, Wiseman GA, Maurer MJ, et al. A phase I trial of immunostimulatory CpG 7909 oligodeoxynucleotide and yttrium ibritumomab tiuxetan radioimmunotherapy for relapsed B-cell non-Hodgkin lymphoma. Am J Hematol. 2013;88:589–93.
286. Provencio M, Cruz Mora MA, Gomez-Codina J, et al. Consolidation treatment with Yttrium-90 ibritumomab tiuxetan after new induction regimen in patients with intermediate- and high-risk follicular lymphoma according to the follicular lymphoma international prognostic index: a multicenter, prospective phase II trial of the Spanish Lymphoma Oncology Group. Leuk Lymphoma. 2013;32:5156–64.
287. Pettitt AR, Jackson R, Carruthers S, et al. Alemtuzumab in combination with methylprednisolone is a highly effective induction regimen for patients with chronic lymphocytic leukemia and deletion of TP53: final results of the national cancer research institute CLL206 trial. J Clin Oncol. 2012;30(14):1647–55.
288. Castaigne S, Pautas C, Terre C, et al. Effect of gemtuzumab ozogamicin on survival of adult patients with de-novo acute myeloid leukaemia (ALFA-0701): a randomised, open-label, phase 3 study. Lancet. 2012;379(9825):1508–16.
289. Hasle H, Abrahamsson J, Forestier E, et al. Gemtuzumab ozogamicin as postconsolidation therapy does not prevent relapse in children with AML: results from NOPHO-AML 2004. Blood. 2012;120(5):978–84.
290. Burnett AK, Russell NH, Hills RK, et al. Addition of gemtuzumab ozogamicin to induction chemotherapy

improves survival in older patients with acute myeloid leukemia. J Clin Oncol. 2012;30(32):3924–31.
291. Swain SM, Kim SB, Cortes J, et al. Pertuzumab, trastuzumab, and docetaxel for HER2-positive metastatic breast cancer (CLEOPATRA study): overall survival results from a randomised, double-blind, placebo-controlled, phase 3 study. Lancet Oncol. 2013;14(6):461–71.
292. Rimawi MF, Mayer IA, Forero A, et al. Multicenter phase II study of neoadjuvant lapatinib and trastuzumab with hormonal therapy and without chemotherapy in patients with human epidermal growth factor receptor 2-overexpressing breast cancer: TBCRC 006. J Clin Oncol. 2013;31(14):1726–31.
293. Ma C, Armstrong A. Severe adverse events from the treatment of advanced melanoma: a systematic review of severe side effects associated with ipilimumab, vemurafenib, interferon alfa-2b, dacarbazine, and interleukin-2. J Dermatol Treat. 2014;25:401–8.
294. Richel O, de Vries HJ, van Noesel CJ, et al. Comparison of imiquimod, topical fluorouracil, and electrocautery for the treatment of anal intraepithelial neoplasia in HIV-positive men who have sex with men: an open-label, randomised controlled trial. Lancet Oncol. 2013;14(4):346–53.
295. Nakuci M, Bassukas ID. Office-based treatment of basal cell carcinoma with immunocryosurgery: feasibility and efficacy. Acta Dermatovenerol Alp Panonica Adriat. 2013;22(2):35–8.
296. Delarue R, Tilly H, Mounier N, et al. Dose-dense rituximab-CHOP compared with standard rituximab-CHOP in elderly patients with diffuse large B-cell lymphoma (the LNH03-6B study): a randomised phase 3 trial. Lancet Oncol. 2013;14(6):525–33.
297. Leahy MF, Turner JH. Radioimmunotherapy of relapsed indolent non-hodgkin lymphoma with 131I-rituximab in routine clinical practice: 10-year single-institution experience of 142 consecutive patients. Blood. 2011;117(1):45–52.
298. Kirkwood JM, Lee S, Moschos SJ, et al. Immunogenicity and antitumor effects of vaccination with peptide vaccine+/-granulocyte-monocyte colony-stimulating factor and/or IFN-alpha2b in advanced metastatic melanoma: Eastern Cooperative Oncology Group phase II trial E1696. Clin Cancer Res. 2009;15(4):1443–51.
299. Egberts F, Gutzmer R, Ugurel S, et al. Sorafenib and pegylated interferon-alpha2b in advanced metastatic melanoma: a multicenter phase II DeCOG trial. Ann Oncol. 2011;22(7):1667–74.
300. Zhu S, Tang Y, Li K, et al. Optimal schedule of bacillus calmette-guerin for non-muscle-invasive bladder cancer: a meta-analysis of comparative studies. BMC Cancer. 2013;13(1):332.
301. Jones G, Cleves A, Wilt TJ et al. Intravesical gemcitabine for non-muscle invasive bladder cancer. Cochrane Database Syst Rev. 2012;1:CD009294.

Vaccination in Human Solid Tumors: Recent Progress in the Clinical Arena

2

Giorgio Parmiani, Carolina Cimminiello, Cristina Maccalli, and Vincenzo Russo

Contents

G. Parmiani, MD (✉) • C. Cimminiello, MD
C. Maccalli, PhD
Unit of Immuno-biotherapy of Melanoma and Solid Tumors, San Raffaele Foundation Scientific Institute, Via Olgettina 58, Milan 20132, Italy

Department of Medical Oncology, San Raffaele Scientific Institute, Milan, Italy
e-mail: parmiani.giorgio@hsr.it

V. Russo, MD
Unit of Cancer Gene Therapy, San Raffaele Foundation Scientific Institute, Via Olgettina 58, Milan 20132, Italy

Department of Medical Oncology, San Raffaele Scientific Institute, Milan, Italy

2.1 Introduction

2.1.1 Preclinical Rationale

The first descriptions of the tumor-associated antigens (TAAs) in rodent models clearly suggested that chemically UV- and/or virus-induced tumors may express individual, non-cross-reacting TAAs, unique for each tumor, and are possibly derived from non-synonymous somatic mutations [1, 2]. However, the use of such TAAs in a therapeutic context could not be adequately explored for three to four decades owing to the lack of molecular knowledge about these TAAs and of the technology to purify and synthesize them. This lack of therapeutic approaches based on unique TAAs was also due to the limited information of the features of the immune response, particularly of T cell response against tumors. In fact, the first molecularly characterized human TAA (i.e., MAGE-1) shown to be specifically recognized by patient's T cells has been reported in 1991 [3]. MAGE-1, however, was shown to be encoded by a cancer germline gene as a normal protein expressed even in a subset of testis and/or placental cells (thereby designated as the cancer/testis antigen). However, these cells usually do not express MHC molecules, thus preventing their recognition and destruction by T cells. This TAA family now includes several members with a potential immunogenicity and immunosensitivity [4, 5]. The report by Boon's group in

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_2, © Springer-Verlag Berlin Heidelberg 2015

Table 2.1 Tumor-associated antigens (TAAs) recognized by T cells

1	Shared/self-/differentiation TAAs (e.g., Melan-A, PSA, CEA)
2	Shared/self-/cancer testis or germinal TAAs (e.g., MAGE, NY-ESO-1)
3	Universal TAAs (e.g., survivin, hIERI)
4	Mutated, unique TAAs

1991 led to a plethora of new studies and publications describing other TAAs in human tumors that, however, were found to be mostly differentiation proteins expressed even in the normal tissue from which the tumor derived. A possible classification of the human TAAs is shown in Table 2.1.

The TAAs recognized by T cells of the first three subgroups have been characterized (Table 2.1), and their T cell peptide epitope sequence has been defined. Therefore, during the last 10 years or so, such TAAs have lent themselves to clinical use in vaccination trials owing to the possibility to synthesize them in purified form and relevant quantities. This availability was not matched by that of unique, mutated TAAs discovered if not in recent years and used in early animal studies [6, 7]. This circumstance thus led to a discrepancy between self-TAAs and mutated TAAs, since the latter were not available as purified peptides and consequently not usable if not as cellular extracts. Therefore many protocols were carried out with molecularly characterized self-TAAs including the differentiation proteins, the C/T, and the universal ones (*see* Table 2.1). The advantage of the availability of molecularly defined TAAs, however, was counteracted by their weak immunogenicity since several forms of tolerance to these self-antigens along with tumor immunosuppressive mechanisms (see below) prevented the occurrence of a strong, clinically meaningful reaction [8, 9]. However, an attempt to define a prioritization in the use of TAAs has been made resulting in a useful classification in terms of advantages and disadvantages of the 75 TAAs considered in this publication [10].

2.2 Formulations Used in Cancer Vaccines

Cancer vaccines have been used under several different formulations without unequivocally showing the superiority of one formulation over the others. By taking the past and successful experience gathered from antiviral vaccines, immunological adjuvants were used as local compounds enabling the injected vaccine to recruit inflammatory cells at the site of injection before vaccine degradation by subcutaneous enzymes.

This allows the TAA to persist locally and thus helps antigen-presenting cells to present such antigens to the immune system usually to the lymph nodes driving the region of vaccine injection.

In early trials, cellular vaccines were mostly used in combination with different adjuvants (e.g., BCG, KLH, Montanide) since they were easily obtainable from autologous or allogeneic cancer cells usually after *in vitro* stabilization of a cancer cell line. This choice was based on the unproved hypothesis that randomly selected neoplastic cells were representative of the antigenic profile of patient's tumor mass. These cell vaccines usually failed to show therapeutic activity in appropriate phase III trials [11].

Subsequently, cancer cells were genetically modified to express and release gene products (e.g., Chemokines as IL-2, IL-4, IL-7, IL-12, GM-CSF) able to help the *in vivo* TAA recognition by T cells and their expansion [12]. However, even after such manipulation, no clear and reproducible increase of the clinical response was obtained in phase II studies [12]. The only vaccine that reached the phase III trial in prostate cancer patients (Vital-2 G-Vax, Cell Genesis) was also disappointing since the study had to be discontinued due to more deaths occurring in the vaccinated arm compared to the placebo arm (see Medical News Today, August, 31, 2008). The reason for this imbalance in death was not identified; nonetheless it is known that very high doses of GM-CSF, released by the cell vaccine, may impair rather than increase patients' antitumor

immune response [13]. However, a successful phase III trial was performed during the last few years leading to its approval by the FDA (Provenge) (see below).

2.2.1 Peptide-/DC-Based Vaccination Against Cancer

As soon as TAA peptides recognized by T cells became available and taking into consideration the ineffectiveness of different B cell-defined TAA to generate widespread tumor cytotoxic antibody response, several phase I–II clinical studies were initiated to assess safety and immune and clinical response in cancer patients, particularly in melanoma-bearing subjects since this neoplasm is considered to be immunogenic [14]. The formulation of these peptide-based cancer vaccines was, however, quite different going from peptide admixed with immunological adjuvants like Freund's incomplete adjuvant-like vaccines (e.g., Montanide) to peptide loaded onto autologous dendritic cells. Moreover, short peptides (8–10 aa) were the first to be used in the clinic, whereas long peptides (13–18aa) were used later on since long peptides were described to be more immunogenic as compared to short ones even within the HLA class I restriction [15].

No major safety problem was found in vaccination protocols based on the use of one or multiple peptides selected *in vitro* for their ability to interact with MHC-specific molecules forming a molecular complex recognizable by patient's T lymphocytes through their TCR. In fact, peptides deriving from C/T or differentiation TAAs (e.g., MAGE-1/3, MART-1, gp100, CEA, PSA) were widely used in different human tumors, and while TAAs-specific T cell response could be generated in 20–80 % of subjects, the clinical outcome remained limited in terms of tumor response (5–20 %) revealing that T cell response induced by the vaccine was not sufficiently strong and/or durable to generate an effective clinical response in the majority of patients [16]. A summary of these results is provided in Table 2.2 which deals with metastatic melanoma; however, it is also representative of other human neoplasms (e.g., colorectal cancer, NSCLC, H/N, prostate cancer, and glioblastoma).

Table 2.2 Results of first generation (1998–2006) of self-peptide-based vaccination of metastatic melanoma patients (phase I–II studies)

Type of peptide TAA	No. of patients	Clinical response (CR+PR) (mean %)	Immune response (%)
Lineage related (e.g., Melan-A)	159	14	20–65
Cancer/testis (e.g., MAGE)	92	17	30–50
DC peptides	124	16	56
DC lysates	106	18	46

Of note: Slingluff et al. [37] reported 100 % immune response and survival benefit in melanoma patients vaccinated with 12 peptides

2.3 Factors That May Impair the Immune Response Against Tumors

A major problem that came out from the many early studies was the lack or weakness of immune response in mice and patients receiving self-TAAs as vaccines usually combined with immunological, nonspecific adjuvants (e.g., Montanide). A large number of studies were therefore conducted in the last two decades pioneered by the observation of Ferrone's and Garrido's groups [17, 18] on the downregulation of class I and II HLA on most tumor cells of a variety of human solid tumors like melanoma, colorectal cancer, etc. This alteration will remove crucial molecules necessary for the TAA presentation to the patient's immune system. Table 2.3 lists the many different mechanisms of tumor escape that have been described in the last few years and that could have prevented an efficient immune response specifically targeting the appropriate TAAs expressed by cancer cells [19]. A new possibility has been reported in melanoma by different authors describing that BRAF-MAPK

signaling is generating cancer-immune evasion [20, 21] though the reverse (i.e., immunostimulation) has been more recently found [22, 23]. In the last few years, the main focus was on the role of myeloid-derived suppressor cells (MDSC) [24, 25] and Tregs [26] as main mechanisms of immune escape by tumor cells. A recent adjunct to the plethora of escape mechanisms is the inflammasome component Nirp3 underlining the complex relationship between inflammation and cancer [27] that impairs vaccine-induced immunity. However, one should consider that several mechanisms may be activated by tumor cells either simultaneously or one after the other according to modifications that occur in the tumor microenvironment and in different tissues in which tumor cells are growing even during different types of therapy [28]. A recent additional mechanism of tumor escape has been described that includes tumor cell secretion of sterol metabolites (LXR ligands), which inhibit the expression of CCR7 on the cell surface of dendritic cells (DCs), thereby disrupting DC migration to the lymph nodes and dampening the antitumor immune priming event [29]. Presently, the major research efforts are directed to find compounds that may help in restoring the full anticancer potential of the immune system [19, 30].

Table 2.3 Factors that interfere with the T cell-mediated antitumor response

Tumor (immunosubversion)	Immune system
Lack of or downregulation of HLA	Immune anergy or ignorance
Dysfunction of antigen presentation	Lack of tissue homing molecules
Release of immunosuppressive factors (IL-10, TGF-beta, VEGF)	T cell receptor dysfunction
Tumor counterattack (Fas/FasL)	Inactivation of T cells within the tumor environment (granzyme B)
IDO, SPARC, Galectin3	Expression of FoxP3, CTLA4, Treg cells
Endoplasmic reticulum stress	MDSC
NFAT1, exosomes	Epithelial/mesenchymal transition
Acidic microenvironment	Tie+ monocytes dysfunction

2.4 New More Successful Clinical Studies of Vaccination in Cancer Patients

During the last few years, however, new knowledge has been gained on the mechanisms of antitumor immunization and tumor immune escape in cancer patients enrolled in earlier vaccination protocols. Such new information has allowed to overcome some of the hurdles that were identified earlier. Thus several phase II and even phase III vaccination protocols came to their conclusion or to an advanced ad interim evaluation stage and provided clinical evidence of benefit for patients with melanoma and other tumors (Table 2.4). Of note, a phase III protocol of vaccination for metastatic prostate cancer patients with autologous dendritic cells loaded with a hybrid molecule made of GM-CSF and PAP (Provenge) [31] was approved by the FDA.

Of great interest also the phase III clinical study carried out by Schwartzentruber and colleagues in 2012 in which the combination of gp100 (210M) class I peptide admixed with Montanide (ISA-51) and high-dose IL-2 *vs.* IL-2

Table 2.4 Evidence for clinical activity of cancer vaccines

Vaccine	Tumor	Phase	No. patients	Stage	Statistics
MAGE 3	NSCLC	II R	182	IB-II	Trend
IDM-2101	NSCLC	II	63	IIIB.IV	NA
II.2+/-gp 100	*Melanoma*	*III*	185	IV	$P<0.002$
Provenge DC	Prostate cancer	III	341/171	HR	$P<0.03$
E75/Her2/neu	Breast cancer	IIR	101/75	IV	$P<0.04$
DC/NHL	NHL	II	18		3CR, 3PR, 8SD
BiovaxID	Follicular lymphoma	*III*	76/41		$P<0.04$
IMA901	RCC	II R	96	Advanced	$P<0.02$

alone showed a statistically relevant benefit for the arm of vaccinated melanoma patients [32]. In fact, the protocol involved 186 stage IV or stage III unresectable, HLA*A0201 patients from 21 centers who received the treatment with a primary endpoint being clinical response (Table 2.4).

In a different randomized phase II study of renal cell cancer patients, vaccination with multiple peptides (IMA901) purified from tumor cells induced an immune response associated with increased survival. Of note, the immune response was improved by the administration of a single immunomodulating dose of cyclophosphamide that downregulated Tregs [33].

2.5 Combination Trials

The availability of new molecular targeted therapeutic agents (e.g., vemurafenib, dabrafenib) and checkpoint blockade antibodies (e.g., ipilimumab, anti-PD-1), particularly for metastatic melanoma [34], has changed the clinical outcome of these subjects and opened the possibility of further increasing the frequency of clinical response both in terms of tumor shrinkage and survival by combining these agents with vaccination [35]. In fact, antibodies modulating the different immune response checkpoints are known to keep the immune response activated, but the appropriate addition of tumor-specific vaccines may focus the immune response on targets and less on off-target tumor molecules, thus potentially increasing the therapeutic effect. A detailed list of published preclinical studies has been recently prepared and published [36]. New clinical studies are being initiated by different research groups to increase the efficacy of immunomodulating antibodies.

2.6 Concluding Remarks

During the last few years, we have seen a clear change from pessimism to optimism in the potential clinical efficacy of cancer vaccines. This change was based on both the discovery of new molecular mechanism of antitumor immunity and tumor immune escape. The first cancer vaccines have been approved by the regulatory agencies in the USA and Europe, but the pipeline of over 200 vaccines will eventually provide new vaccination agents to be added to the now limited list. The combination of vaccination, checkpoint antibodies, and molecular targeting by new kinase inhibitory agents holds great promise and will certainly further improve the response rate of metastatic patients bearing different solid tumors.

Acknowledgment The authors' work was supported by the Italian Association for Cancer Research (Milano) and by the Alliance Against Cancer (Rome).

References

1. Prehn RT, Main JM. Immunity to methylcholanthrene-induced sarcomas. J Natl Cancer Inst. 1957;18:769–78.
2. Klein G, Sjogren HO, Klein E. Demonstration of resistance against methylcholanthrene-induced sarcomas in the primary autochthonous host. Cancer Res. 1960;20:1561–72.
3. van der Bruggen P, Traversari C, Chomez P, et al. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science. 1991; 254:1643–7.
4. Novellino L, Castelli C, Parmiani G. A listing of human tumor antigens recognized by T cells: March 2004 update. Cancer Immunol Immunother. 2005;54: 187–207.
5. Caballero OI, Chen YT. Cancer/testis (CT) antigens: potential targets for immunotherapy. Cancer Sci. 2009;100:2014–21.
6. Parmiani G, De Filippo A, Novellino L, Castelli C. Unique human tumor antigens: immunobiology and clinical use. J Immunol. 2007;178:1975–9.
7. Castle JC, Kreiter S, Diekmann J, et al. Exploiting the mutanome for tumor vaccination. Cancer Res. 2012; 72:1081–91.
8. Blankestein T, Coulie PG, Gilboa E, Jaffee E. The determinants of tumour immunogenicity. Nat Rev Cancer. 2012;12:307–13.
9. Mortarini R, Piris A, Maurichi A, et al. Lack of terminally differentiated tumor-specific CD8+ T cells at tumor site in spite of antitumor immunity to self-antigens in human metastatic melanoma. Cancer Res. 2003;63:2535–45.
10. Cheever MA, Allison JP, Ferris AS, et al. The prioritization of cancer antigens: a National Cancer Institute pilot project for the acceleration of translational research. Clin Cancer Res. 2009;15:5323–37.
11. Finke LH, Wentworth K, Blumenstein B, et al. Lessons from randomized phase III studies with active cancer immunotherapy-Outcome from the 2006 meeting of the cancer vaccine consortium (CVC). Vaccine. 2007;25 Suppl 2:B97–109.

12. Parmiani G, Russo V, Maccalli C, Pilla L. Autologous vs. allogeneic cell-based vaccines? A review. Cancer J. 2011;17:331–6.
13. Parmiani G, Castelli C, Pilla L, et al. Opposite immune functions of GM-CSF administered as vaccine adjuvant in cancer patients. Ann Oncol. 2007;18:226–32.
14. Parmiani G, Castelli C, Dalerba P, et al. Cancer immunotherapy with peptide-based vaccines: what have we achieved? Where are we going? J Natl Cancer Inst. 2002;94:805–18.
15. Bijker MS, van den Eeden SJ, Franken KL, et al. Superior induction of anti-tumor CTL immunity by extended peptide vaccines involves prolonged, DC-focused antigen presentation. Eur J Immunol. 2008; 38:1033–42.
16. Pilla L, Rivoltini L, Patuzzo R, et al. Multipeptide vaccination in cancer patients. Exp Opin Biol Ther. 2009;9:1043–55.
17. Marincola FM, Jaffee E, Hicklin DJ, Ferrone S. Escape of human solid tumors from T-cell recognition: molecular mechanisms and functional significance. Adv Immunol. 2000;74:181–273.
18. Garrido F, Cabrera T, Aptsiauri N. "Hard" and "soft" lesions underlying the HLA class I alterations in cancer cells: implications for immunotherapy. Int J Cancer. 2010;127:249–56.
19. Rivoltini L, Canese P, Huber V, et al. Escape strategies and reasons for failure in the interaction between tumour cells and the immune system: how can we tilt the balance towards immune-mediated cancer control? Exp Opin Biol Ther. 2005;5:463–76.
20. Sumimoto H, Imabayashi F, Iwata T, Kawakami Y. The BRAF-MAPK signalling pathway is essential for cancer-immune evasion in human melanoma cells. J Exp Med. 2006;203:1651–6.
21. Khalili J, Liu S, Rodriguez-Cruz T, et al. Oncogenic BRAF (V600E) promotes stromal cell-mediated immunosuppression via induction of interleukin-1 in melanoma. Clin Cancer Res. 2012;18:5329–40.
22. Bachandran VD, Cavnar MJ, Zeng S, et al. Imatinib potentiates antitumor T cell responses in gastrointestinal stromal tumors through the inhibition of IDO. Nat Med. 2011;7:1094–101.
23. Wilmott JS, Long GV, Howle JR, et al. Selective BRAF inhibitors induced market T-cell infiltration into human metastatic melanoma. Clin Cancer Res. 2011;18:1386–94.
24. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol. 2009;9:162–74.
25. Ostrand-Rosenberg S. Myeloid-derived suppressor cells: more mechanisms for inhibiting antitumor activity. Cancer Immunol Immunother. 2010;59:1593–600.
26. Ahmadzadeh M, Felipe-Silva F, Heemskerk B, et al. FOXP3 expression accurately defines the population of intratumoral regulatory T cells that selectively accumulate in metastatic melanoma lesions. Blood. 2008;112:4953–60.
27. van Deventer HW, Burgents JE, Wu QP, et al. The inflammasome component Nirp3 impairs antitumor vaccines by enhancing the accumulation of tumor-associated myeloid-derived suppressor cells. Cancer Res. 2010;70:10161–8.
28. Camisaschi C, Filipazzi P, Tazzari M, et al. Effects of cyclophosphamide and IL-2 on regulatory CD4+T cell frequency and function in melanoma patients vaccinated with HLA-class I peptides: impact on the antigen-specific T cell response. Cancer Immunol Immunother. 2013;62:897–908.
29. Villablanca EJ, Raccosta L, Fontana R, et al. Tumor-mediated liver X receptor-alpha activation inhibits CC chemokine receptor -7 expression on dendritic cells and dampens antitumor response. Nat Med. 2010;16:98–106.
30. Vieweg J, Su Z, Dahm P, Kusmartsev S. Reversal of tumor-induced immune suppression. Clin Cancer Res. 2007;13:727–32.
31. Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration resistant prostate cancer. N Engl J Med. 2010;363:411–22.
32. Schwartzentruber DJ, Lawson DH, Richards JM, et al. Gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. N Engl J Med. 2011;364:2119–27.
33. Walter S, Weinschenk T, Stenzl A, et al. Multipeptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival. Nat Med. 2012;18:1254–61.
34. Riley JL. Combination checkpoint blockade-Taking melanoma immunotherapy to the next level. N Engl J Med. 2013;369:187–9.
35. Vanneman M, Dranoff G. Combining immunotherapy and targeted therapies in cancer treatments. Nat Rev Cancer. 2012;12:237–51.
36. Grosso JF, Jure-Kunkel MN. CTLA-4 blockade in tumor models: an overview of preclinical and translational research. Cancer Immun. 2013;13:5–19.
37. Slingluff CL, Petroni GR, Chianese-Bullock KA, et al. Immunologic and clinical outcomes of a phase II trial of two multipeptide vaccines for melanoma in the adjuvant setting. Clin Cancer Res. 2007;13:6386–95.

Immunotherapy for Pediatric Solid Tumors

3

Lisa M. Kopp and Emmanuel Katsanis

Contents

L.M. Kopp, DO (✉) • E. Katsanis, MD
Department of Pediatrics, University of Arizona, 1501 N. Campbell Avenue, Tucson, AZ 85724, USA
e-mail: lkopp@peds.arizona.edu; katsanis@peds.arizona.edu

3.1 Introduction

Over 12,000 children and adolescents are diagnosed each year with cancer in the United States [1]. Cancer remains the leading cause of non-accidental death in the pediatric age group. While the incidence of childhood cancer has increased over the last 40 years, the overall cure rates have significantly improved [1, 2]. There have been improvements in the outcome of children with solid tumors; however, these have lagged behind those seen in hematologic malignancies.

3.2 Solid Tumors

Pediatric solid tumors include those located within the central nervous system (CNS), neural tumors, and those outside the CNS, nonneural tumors. In this chapter the focus will be on nonneural solid tumors including bone and soft tissue sarcomas, neuroblastoma, Wilms tumor, hepatoblastoma, and systemic germ cell tumors. Multi-agent chemotherapy, radiation, and surgery have greatly improved survival in pediatric and young adult patients with localized solid tumors. Unfortunately the same is not true for patients with metastatic or recurrent disease, which may benefit from novel alternate therapies such as immunotherapy [1, 3].

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_3, © Springer-Verlag Berlin Heidelberg 2015

3.2.1 Sarcomas

The most common pediatric and adolescent sarcomas include osteosarcoma, Ewing's sarcoma, and rhabdomyosarcoma.

3.2.1.1 Osteosarcoma

Osteosarcoma (OS) is the most common malignant bone tumor in children and adolescents. The cell of origin is thought to be mesenchymal stem cells with an osteoid component [4]. The incidence of OS is approximately 4.5 cases per million per year in the population, and it occurs primarily in adolescents with more than half of cases found in those less than 25 years of age [4, 5]. It is more common in blacks with a slightly increased incidence in males [5]. Although the majority of cases of osteosarcoma are of unknown etiology, there is a higher incidence in individuals with the RB1 mutation, found also in retinoblastoma, TP53 mutation which is associated with Li–Fraumeni syndrome, and RECQ4 mutation found in patients with Rothmund–Thomson syndrome [6]. Patients typically present with pain at the primary tumor site. The most common sites for OS are the long bones of the distal femur, proximal tibia, and proximal humerus. Approximately 20 % of patients will have metastatic disease at diagnosis usually involving bones and lungs [7]. On physical examination a soft tissue mass may or may not be evident. Diagnosis is made through a biopsy of the primary tumor by a skilled orthopedic surgeon. There is no standard staging system utilized in pediatric oncology for OS, and the disease is classified as localized or metastatic. Multimodal therapy consists of 10 weeks of neoadjuvant chemotherapy followed by local control with tumor resection and 20 weeks of adjuvant chemotherapy [4, 7]. Radiation therapy has not been found to be as effective and is only recommended when tumors are unresectable at doses of 60–68 Gy [7]. In children and young adults with localized disease, the event-free and overall survival is around 60–70 %. Unfortunately for patients with metastatic disease, the prognosis is poor ranging from 25 to 40 %. Patients with isolated lung metastasis have a slightly higher overall survival [5].

3.2.1.2 Ewing's Sarcoma

Ewing's sarcoma is the second most common bone tumor in children and adolescents [8]. The source of the Ewing's sarcoma cell is still unknown; however, it is thought to derive from neuroectodermal cells with either neuronal or epithelial origins [9]. The most common cytogenetic translocation found in Ewing's sarcoma is the balanced translocation of t(11;22), which at the molecular level involves the fusion of the Ewing's sarcoma breakpoint region 1 (EWSR1) in 22q12 with the Friend leukemia virus integration 1 (FLI1) gene in 11q24. This fusion is found in 85 % of Ewing's sarcoma, yet multiple alternative gene fusions have recently been found between these two genes [10]. The overall incidence of Ewing's sarcoma is almost three cases per million in the population per year, and as with osteosarcoma, Ewing's sarcoma occurs primarily in adolescents [8]. The greatest incidence is found in whites and males [8]. Patients initially present with complaints of pain at the primary site. The pelvis, femur, and rib are the most common locations, yet one quarter of tumors arise in the soft tissue rather than bone. As in OS the most common locations for metastasis are the lungs and other bones; however, the disease can also be found in the bone marrow. Metastatic disease is found in 25 % of patients at diagnosis. As with OS there is not typically a staging system used and disease is classified as localized or metastatic. Multimodal therapy includes chemotherapy and local control with either radiation or surgery. There have been no prospective studies comparing radiation versus surgery; however, no advantage has been shown utilizing one modality over the other. In the United States, surgery is the modality of choice [4, 7, 11]. Patients with localized disease have survival rates between 60 and 70 % with improved prognosis in those with smaller primary tumors. Those with metastatic disease have a much poorer outcome with 20–30 % survival. Bone metastasis confers an inferior prognosis than lung metastasis [4, 8, 11].

3.2.1.3 Soft Tissue Sarcomas

The soft tissue sarcomas (STS) are the most common extracranial solid tumors found in children

and young adults accounting for more than 7 % of cancer cases [12]. They are comprised of a diverse group of malignant connective tissue tumors including rhabdomyosarcoma (RMS) and non-rhabdomyosarcoma soft tissue sarcomas (NRSTS). Rhabdomyosarcoma comprises almost half of the STS, and the overall incidence of RMS is 4.5 cases per million in the population per year [12, 13]. RMS is derived from immature skeletal muscle, and the two largest subgroups are embryonal (ERMS) and alveolar (ARMS) rhabdomyosarcoma. These groups are classified according to their histologic and biologic features. ERMS is associated with allelic loss of chromosome 11, found more often in younger children, and is associated with a better prognosis. ARMS has two commonly seen gene translocations: t(2;13) and t(1;13), and it is distributed equally throughout childhood and adolescence [13]. RMS has been associated with genetic conditions including neurofibromatosis type I, Rubinstein–Taybi syndrome, Beckwith–Wiedemann syndrome, Costello syndrome, Noonan syndrome, Gorlin basal cell nevus syndrome, and Li–Fraumeni syndrome [14]. RMS is very heterogeneous and can occur in places not even associated with skeletal muscle such as the mouth and bladder. Forty percent of RMS cases are found in the head and neck, 20 % arise in genitourinary sites, 20 % from the extremities, and 20 % from other sites [14]. The most common site for metastasis is the lungs, followed by the bone and rarely the bone marrow. The prognosis for RMS is determined by multiple factors including primary site, stage and group, pathology, and age. The Intergroup Rhabdomyosarcoma Study (IRS) Group formed a grouping system to assess the extent of tumor. In the United States, the IRS grouping classification is used along with a modified TNM staging system and histology to assign the patient to a risk group: low, intermediate, or high. Treatment includes multi-agent chemotherapy and local control with surgery and radiation. A complete resection of the primary tumor is recommended; however, this is not possible in the majority of cases as tumors often arise in sites where resection would cause unacceptable loss of function or cosmetic disfigurement. For all tumors located in the extremity and for paratesticular tumors in children >10 years of age, a regional lymph node biopsy is also recommended, and a sentinel lymph node biopsy may also be helpful [14–16]. All patients with RMS Group II–IV and Group I alveolar receive radiation to the primary site. Chemotherapy is minimal for low-risk disease and is intensified for intermediate- and high-risk disease. As expected, patients with low-risk disease have the most favorable outcome with failure-free survival (FFS) of 90 %, and those with intermediate-risk disease have a 70 % FFS. Despite intensive therapy, those patients with high-risk disease have a poor FFS of 20 % [17].

The NRSTS include fibrosarcomas, liposarcomas, leiomyosarcomas, angiosarcomas, malignant hemangiopericytoma, synovial sarcoma, chondrosarcomas, and malignant peripheral nerve sheath tumors. Due to the individual rarity of these tumors, they are commonly grouped together with the highest incidence of NRSTS occurring in infants and young adults [12]. These tumors have a similar histology to adults STS, however do not necessarily behave the same as in adults, and many of the individual NRSTS have characteristic cytogenetic abnormalities. Tumors <5 cm that are fully resected have the best prognosis and no further treatment is needed. The survival for these patients is around 85 %. However, patients with NRSTS that are >5 cm and those that are unresectable have a survival of only 50 %, and patients with metastatic disease have a survival of only 10 %. Chemotherapy and radiation therapy have been used with some of the NRSTS; however, their efficacy is still unknown as these tumors do not appear to be very sensitive to these modalities [15].

3.2.2 Neuroblastoma

Neuroblastoma is the second most common extracranial solid tumor and the most common tumor found in infancy. The incidence is 4.9 cases per million per year [18]. It is an embryonal tumor of the autonomic nervous system and can arise anywhere in tissues of the sympathetic

nervous system. The majority of cases are sporadic; however, familial cases are associated with mutation in the tyrosine kinase domain of the anaplastic lymphoma kinase (ALK) oncogene and loss of function mutations in the gene PHOX2B [19, 20]. Clinical presentation is dependent on tumor location and/or metastatic spread. The most common location of primary tumors is the abdomen. Approximately 50 % of patients will present with metastatic disease. Regions of metastasis include the bone, lymph nodes (local and noncontiguous), liver, and bone marrow. Children with metastatic tumors, unlike those with localized tumor, typically have a large tumor burden and are very ill at presentation [21, 22]. Staging is done according to the International Neuroblastoma Staging System (INSS) based on tumor resection. The results of the tumor biology along with the patient's age and disease stage allow for classification of the patient's risk status. The current risk stratification will be revised as the International Neuroblastoma Risk Group (INGR) has defined an updated classification system that will be used internationally and in the United States in upcoming protocols [22, 23]. Currently, patients with low-risk disease only require surgery for treatment and have a survival of >98 %. Patients with intermediate-risk disease require surgery and moderate chemotherapy, yet the survival rate remains excellent, 90–95 %. In patients with high-risk disease, survival is very poor, 40–50 %, despite intense treatment. Generally high-risk patients receive three phases of treatment: induction, consolidation, and maintenance as described by Maris in a recent review [21]. Induction consists of chemotherapy and surgical resection, followed by consolidation with myeloablative chemotherapy with stem cell rescue and radiation of the primary tumor site. Maintenance consists of isotretinoin and immunotherapy (anti-GD2) to eliminate minimal residual disease. The disialoganglioside GD2 is uniformly expressed on all neuroblastoma cells. Anti-GD2 antibodies including 3F8 and ch14.18 have shown an improvement in overall survival in clinical trials [24, 25]. The Children's Oncology Group completed a randomized phase III study with ch14.18, and results revealed an improvement in overall survival from 46 to 66 % with the antibody in combination with IL-2 and GM-CSF. This will be discussed in more detail in the upcoming sections on immunotherapy treatment [21, 25].

3.2.3 Wilms Tumor

Wilms tumor, also known as nephroblastoma, is the most common malignant renal tumor of childhood comprising 6 % of childhood cancers. The incidence rate is eight cases per million per year in children younger than 15 years of age in North America. It occurs slightly more often in females and blacks [26]. Wilms tumor is composed of blastemal, stromal, and epithelial cells. Tumors that contain anaplastic cells are classified as unfavorable as these are more aggressive tumors with a worse prognosis [27]. As with neuroblastoma, a small percentage of cases of Wilms tumors are associated with congenital syndromes including WAGR (Wilms tumor, aniridia, genitourinary malformation, mental retardation) syndrome, Denys–Drash syndrome, and Beckwith–Wiedemann syndrome [28]. There have been specific genetic alterations found to be associated with the tumorigenesis of Wilms tumor. The tumor suppressor gene WT1 was the first gene identified in the development of Wilms tumor. Loss of heterozygosity of chromosomes 16q and 1p has also been found in more aggressive Wilms tumors [29, 30]. Clinically infants and children will usually present with a painless abdominal mass and may also have hypertension and/or hematuria. Staging differs between North America and Europe; in North America staging is determined based on histology, genetics, and upfront surgical resection, whereas in Europe staging is determined after 4–6 weeks of upfront chemotherapy. Treatment includes surgery, chemotherapy, and radiation therapy. Patients with bilateral (stage V) disease will have neoadjuvant chemotherapy in order to decrease tumor size and preserve as much normal tumor tissue as possible. Patients with favorable histology tumors have an excellent prognosis, even with metastatic disease. Children with favorable histology and

stage I, II, and III disease had a 4-year survival of >90 %, whereas those with stage IV and V disease had survival >79 % in the latest Children's Oncology Group (COG) study [31]. Those patients with stage I and II disease only receive chemotherapy, whereas patients with stage III and IV disease receive intensified chemotherapy and radiation therapy. Children with anaplastic tumors and/or unfavorable genetics receive more intense chemotherapy and radiation therapy [31, 32]. Patients with focal anaplasia have a better prognosis than those with diffuse anaplasia; however, even with diffuse anaplasia, survival is >70 % for stage I, II, and III disease. Yet, children with stage IV disease with focal or diffuse anaplasia have a very poor prognosis of <30 % [31].

3.2.4 Hepatoblastoma

Primary liver tumors in children are rare and account for only 1 % of tumors in the pediatric age group. Hepatoblastoma is the most common liver tumor with the majority of cases presenting in children less than 4 years of age [33, 34]. The highest incidence is in infants and then declines rapidly with increasing age [33]. Hepatoblastoma occurs more often in premature infants and in males [33, 35]. It is an embryonal tumor with five histologic types, with pure fetal type, having the most favorable prognosis, and small cell undifferentiated type, having the most unfavorable prognosis. Multiple genetic syndromes and familial cancer predisposition conditions including Beckwith–Wiedemann syndrome, Li–Fraumeni syndrome, hemihypertrophy, and familial adenomatous polyposis are associated with hepatoblastoma [34]. Changes in imprinting at the 11–15 loci as well as acquired chromosomal changes are involved in the pathogenesis of hepatoblastoma [36, 37]. Infants and children will usually present with a painless abdominal mass. Serum alpha-fetoprotein (AFP) is elevated in more than 90 % of cases. Tumors that are AFP negative are thought to be more aggressive and have a worse prognosis. It is important to take the child's age into consideration when interpreting AFP as it is commonly elevated in neonates up to 1 year of age. As in Wilms tumor, staging in North America and Europe differs. In North America staging depends on the extent of surgical resection at diagnosis, whereas the European system only utilizes the pretreatment extent of disease and designates patients as standard or high risk. In North America, most institutions follow COG protocols, and children are also separated into risk groups based on postoperative resection and biologic features of the tumors and are divided into low, intermediate, and high risk. Treatment includes surgery and chemotherapy [34, 35, 38]. Complete surgical resection is crucial for survival, and children with complete resection or pure fetal histology usually do not require further treatment. For tumors that are initially unresectable, patients are given up to four cycles of chemotherapy including cisplatin. Tumors are then resected by partial hepatectomy or children receive a liver transplantation followed by an additional two cycles of chemotherapy [34, 35]. Those children with lung metastasis should also have resection of residual metastasis. In the most recent European and North American trials, the prognosis was >90 % for stage I and II low-risk disease. Intermediate-risk disease prognosis is around 70 %, and unfortunately survival is less than 30 % in patients with metastatic high-risk disease [35].

3.2.5 Systemic Germ Cell Tumors

Germ cell tumors (GCT) arise from primordial cells involved in gametogenesis and arise at multiple sites in the body with a variety of histological subtypes including embryonal carcinoma, yolk sac tumors, choriocarcinoma, and teratomas (mature and immature) [39]. Malignant germ cell tumors, arising outside of the central nervous system, account for 2–4 % of all pediatric and adolescent cancers. The annual incidence is eight cases per million people in those less than 20 years of age [40]. In children and adolescents <14 years, there is a higher incidence in females; however, males have a higher incidence in patients >14 years. Cryptorchidism and

syndromes that involve abnormal testicular development, Klinefelter's syndrome and XY dysgenesis, have been found to have an increased risk of testicular GCT. In pubertal and postpubertal adolescents and adults, isochromosome p12 is present in the majority of tumors tested; however, in prepubertal children this is rarely present indicating a clear cytogenetic difference between these groups of patients [39, 41]. Clinically patients will present with symptoms related to the tumor site. For example, testicular or ovarian tumors will usually have symptoms of pain, constipation, and/or urinary retention. In infants and children, the most common sites for GCT are the sacrococcyx and the ovary; the testicle is the next most common location [42, 43]. The most common sites of metastasis include the lungs, liver, and local lymph nodes; bone metastases are very rare. Alpha-fetoprotein and beta subunit of human chorionic gonadotropin (βHCG) are elevated in the majority of malignant GCTs. Lactate dehydrogenase (LDH) may also be elevated; therefore, LDH, AFP, and βHCG should be obtained at diagnosis and prior to surgery. As discussed previously, AFP may also be elevated in hepatic tumors in infants, so it is important to consider this in tumor evaluation [39]. As the adult GCT staging system has not been found to accurately correlate with pediatrics, it is not used in pediatric GCTs. In North America the COG staging system is generally used. All GCTs require surgery and initial complete resection upfront is recommended if possible. If the tumor is a mature teratoma, no additional treatment is needed. If a malignant tumor cannot be fully resected, chemotherapy is given and a second look surgery may be indicated. Chemotherapy treatment following COG studies is based on tumor risk groups and tumor location. Low-risk tumors include stage I testicular and ovarian GCTs and are closely observed requiring chemotherapy if serum tumor markers (AFP, βHCG) do not decline. Intermediate-risk GCTs include testicular tumors stages II–IV, ovarian tumors stages II–III, and extragonadal malignant tumors stages I–II. Stage IV ovarian tumors and stages III–IV extragonadal tumors are classified as high risk [44]. Intermediate- and high-risk tumors require platinum-based chemotherapy, and the most recent trials are attempting to decrease the number of cycles in intermediate-risk patients and intensifying treatment for high-risk groups [39, 43–46]. Radiation is not utilized in the initial treatment of malignant GCTs, as the majority of these tumors are very sensitive to chemotherapy, specifically the platinums. The prognosis is excellent for testicular, ovarian, and sacrococcyx GCTs: stages I–III >85 % and stage IV >80 % [45, 46]. As mediastinal GCTs are rare, the data is limited, but these tumors have a worse overall prognosis, and in one COG (intergroup CCG/POG) study, the overall survival was 71 % [45, 47].

3.3 Immune Therapy and Pediatric Solid Tumors

3.3.1 Monoclonal Antibodies or Inhibitor Targeting

3.3.1.1 Gangliosides

From all pediatric solid tumors, neuroblastoma stands out as the one in which immunotherapy has been most widely applied and has recently become part of standard therapy. Antibody therapy in combination with cytokines has improved outcomes in patients with high-risk neuroblastoma.

Disialoganglioside (GD2) is a carbohydrate antigen whose true function is not known, although it is expressed normally on the tissues of the central nervous system, peripheral nerves, and skin melanocytes. GD2 is also expressed consistently on neuroblastoma making it an ideal target in these patients. Melanoma, some bone and soft tissue sarcomas, lung tumors, and brain tumors have also been found to express GD2 [48]. Murine and mouse–human chimeric and humanized versions of anti-GD2 antibodies have been studied in neuroblastoma clinical trials including 3-F8, 14G2a, ch14.18, and hu14.18 [49]. The first generation of anti-GD2 monoclonal antibodies (mAbs) included 3F8, 14G2a, and ch14.18. Reversible pain (secondary to binding of the antibody to peripheral nerve fibers), fever,

tachycardia, and urticaria were the most common toxicities found in initial clinical trials of 3F8 and 14G2a antibodies. 3F8 is a murine IgG3 monoclonal anti-GD2. Phase I and II studies revealed moderate responses to the antibody in patients with relapsed/recurrent neuroblastoma. In a later phase II study, 3F8 was combined with granulocyte colony macrophage stimulating factor (GM-CSF) in order to stimulate Antibody-dependent cell-mediated cytotoxicity (ADCC) mediated by monocytes and granulocytes. Patients tolerated this combination without significant toxicity, and those with evidence of neuroblastoma involving the bone marrow appeared to benefit the most [48, 50]. Another murine anti-GD2 mAb, 14G2a, was also utilized in a phase I study; however, it was combined with interleukin 2 (IL-2) to augment NK-mediated ADCC, and responses were also noted in some patients [51, 52]. The mAb ch14.18 is a chimeric mouse–human antibody consisting of the variable regions of murine IgG3 anti-GD2 mAb 14.18 and the constant regions of human IgG-κ [48, 52]. This antibody has a longer half-life than 14G2a and showed efficacy in an initial pilot study followed by a phase II trial in children with recurrent/refractory neuroblastoma. Both studies included IL-2 and GM-CSF to enhance ADCC [25, 52]. This was followed by a COG phase III study in high-risk neuroblastoma. In this trial patients were randomized to receive immunotherapy with ch14.18 antibody combined with alternating IL-2 and GM-CSF added to the standard therapy. Patients randomized to receive immunotherapy had significantly improved rates of event-free survival (66 ± 5 % *vs.* 46 ± 5 % at 2 years, $P = 0.01$) and overall survival compared to those receiving the standard therapy alone [25]. These results were so compelling that the COG Neuroblastoma Committee decided to offer ch14.18 to prior trial participants who had been randomly assigned to the no immunotherapy arm [53].

Second-generation GD2 mAbs have been developed and are currently in early clinical trials. Hu14.18–IL-2 is a fusion protein of a humanized second-generation anti-GD2 antibody (Hu14.18) and IL-2. Phase I and II studies of patients with refractory/relapsed neuroblastoma have been completed, and toxicities were similar to those of ch14.18. The phase II study revealed a response rate around 21 % for patients with non-bulky disease [52, 54]. Another second-generation mAb undergoing clinical investigation is a humanized ch14.18 mAb with a mutation to alanine at lysine 322 (Hu 14.18K332A) in order to limit complement fixing and thus the pain associated with the anti-GD2 [48]. GD2 antibodies have recently been used in combination with chimeric antigen receptors (CARs) to target neuroblastoma cells [55, 56]. Genetically engineered T cells with CARs are designed to recognize GD2 and have been used in phase I clinical trials with evidence of activity and persistence [56, 57]. T cells and CARs will be discussed in more detail later in this chapter.

GD2, GD3, and GM3 may also be potential targets for sarcomas. GD2 and GD3 are both expressed variably in sarcomas [58, 59]. Unlike GD2, GD3 is a disialoganglioside that is not expressed on normal tissues. GD3 expression is found in melanomas, soft tissue sarcomas, and tumors of neuroectodermal origin [57, 60]. Similar to GD3, N-glycosylated ganglioside NeuGc-GM3, GM3, has also been found to be expressed primarily in neoplasms and not in normal tissues. GM3 has been used as a target in breast cancer and has been recently found on the surface of Wilms tumor and Ewing's sarcoma [57, 60–62].

3.3.1.2 Her2/Neu

Her2/Neu is the epidermal growth factor receptor 2 oncogene that has been found to be amplified in pediatric medulloblastoma, Wilms tumor, and osteosarcoma. Trastuzumab is the mAb targeting Her2/Neu that has been successful in the treatment of breast cancer [57, 63]. However, its use in pediatric solid tumors has limited data. Trastuzumab was found to be safe when combined with chemotherapy in a phase II trial of newly diagnosed patients with metastatic osteosarcoma. Forty-one patients with Her2/Neu-positive tumors received trastuzumab, yet survival was not significantly increased, and further studies will need to be done to determine its therapeutic benefit in pediatric solid tumors [64].

3.3.1.3 RANK-L

The cytokine RANK-L is a TNF family member expressed on the surface of osteoblasts and is released by activated T cells. RANK-L has been found to be critical to osteoclast formation, function, and survival. Dysregulation in bone remodeling has been found to be key in the pathophysiology of bone metastasis, and RANK-L plays an essential role in this process [65]. Denosumab is a humanized mAb that binds RANK-L ligand and has been used in phase II and III clinical trials in multiple myeloma (MM) and metastatic breast and prostate cancers [65–68]. Denosumab is thought to be an ideal antibody to use in osteosarcoma because of its direct effects on bone tumor pathophysiology. In one study expression of RANK-L was found in 75 % of osteosarcoma samples, and it was related to poor response to neoadjuvant chemotherapy [69]. Denosumab will be utilized in an upcoming COG phase II clinical trial in patients with recurrent osteosarcoma (personal communication).

3.3.1.4 Fibroblast Growth Factor Receptor 4

Fibroblast growth factors and their receptors are an integral part of normal cell development. They are important in regulating cell proliferations, survival, migration, and differentiation. However, deregulation of these growth factors is found in many cancers, and the current thinking is that they act as an oncogene, promoting cancer progression [70]. There are currently MM trials utilizing antibodies to fibroblast growth factor receptor 3 [57]. Recently fibroblastic growth factor receptor 4 (FGFR 4) was found to be overexpressed in pediatric rhabdomyosarcomas with little expression in normal myocytes. Rhabdomyosarcoma tumors that were highly expressing FGFR4 were associated with advanced-stage tumors and poor survival [71, 72]. Therefore, this may be a promising target for immunotherapy of rhabdomyosarcoma.

3.3.1.5 VEGF

It is well known that the growth and metastasis of solid tumors are dependent on angiogenesis. Vascular endothelial growth factor (VEGF) is overexpressed in many types of cancer, making it a useful target for tumor vascular inhibition [73]. Bevacizumab is a humanized antibody against VEGF-A [74]. This antibody does not have direct immune effects but disrupts angiogenesis. It has been used in clinical trials targeting adult cancers such as renal cell carcinoma, breast cancer, and colorectal cancer [73]. In pediatric solid tumors, it has been used compassionately in patients with refractory tumors. In these small groups of patients, some responses have been reported in patients with Ewing's sarcoma, NRSTS, RMS, and neuroblastoma [73, 75]. A phase I clinical trial of patients 1–22 years of age with refractory solid tumors through COG has also been completed. In the phase I trial, there were no dose-limiting toxicities, including no hemorrhage or thrombosis [76]. However, as there were no objective responses, further studies will need to be done to evaluate its clinical efficacy. As with other targeted therapies, bevacizumab may work better in combination with other agents.

Inhibiting VEGF in Ewing's sarcoma mouse models *in vivo* resulted in decreased lytic bone activity and specifically a decrease in RANK-L [77, 78]. Therefore, a combination of VEGF and RANK-L inhibition may be an option for future clinical trials in Ewing's sarcoma and osteosarcoma.

3.3.1.6 Insulin-Like Growth Factor 1 Receptors

Insulin-like growth factor 1 receptor (IGF-1R) has been found to be important in the growth of solid tumors, specifically sarcomas. In the past it was difficult to target IGF-1R because of its similarity to the insulin receptor leading to toxicities occurring without specific inhibition. Recently there has been development of humanized mAbs that target IGR-1R without major toxicities [79]. As with bevacizumab there is not a direct immune effect, rather targeting the pathway. The Pediatric Preclinical Testing Program evaluated the human antibody SCH 717454 in solid tumor xenograft models. It was found to have broad antitumor activity in pediatric solid tumor models including Ewing's sarcoma, osteosarcoma, rhabdomyosarcoma, and neuroblastoma [80]. Cixutumumab (IMC-A12), a fully human IgG1 mAb against

IGF-1R, was used in a phase I/II trial in pediatric patients with refractory solid tumors through COG. The drug was well tolerated; however, there was a limited single-agent activity [81]. It is thought that IGF-1R mAbs will work best in combination with other targeting agents such as mTOR inhibitors as they have been shown to increase the IGF-1R serine/threonine kinase AKT. The combination of mTOR inhibition and IGF-1R AKT inhibition leads to a more effective killing of RMS cell lines [79, 82]. In a recent multi-institutional study which included 20 patients aged 14–41 with refractory Ewing's sarcoma and desmoplastic small-round-cell tumor, cixutumumab was combined with the mTOR inhibitor temsirolimus. This combination was well tolerated, and there was one complete response in these heavily treated patients. Also, five of the patients with Ewing's sarcoma had at least a 20 % decrease in their tumor size [83]. Additional phase III studies will need to be completed in pediatric sarcoma patients.

3.3.1.7 TRAIL

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of the TNF superfamily that has the ability to activate death receptors inducing tumor cells to undergo apoptosis. Many tumor cells express the TRAIL receptors, TRAIL-R1 and TRAIL-R2, and these receptors initiate apoptosis via TRAIL. This mechanism seems to be selective for tumors [84, 85]. Lexatumumab (HGS-ETR2) is a human mAb that binds to and activates TRAIL-R2. It has been tested in a phase I study in adults with refractory/relapsed solid tumors with evidence of stabilizing disease in some patients [85]. Mapatumumab is a human mAb, which targets TRAIL-R1, and a phase I trial was recently reported where it was used in combination with paclitaxel and carboplatin in adults with refractory solid malignancies. In this trial 5 patients had partial response and 12 patients had stable disease [86]. It has been found that osteosarcoma and Ewing's sarcoma cell lines which express TRAIL death receptors are sensitive to TRAIL-mediated apoptosis [87]. Hopefully these agents will be tested in pediatric solid tumors in the near future.

3.3.1.8 Anaplastic Lymphoma Kinase

The anaplastic lymphoma kinase (ALK) gene is a receptor kinase in the insulin receptor superfamily and is expressed during neuronal development, then downregulated after birth. ALK's expression is thought to increase tumor growth and is found in a variety of tumors including lung cancer, anaplastic large cell lymphoma, neuroblastoma, neuroectodermal tumors, glioblastoma, rhabdomyosarcoma, and melanoma. ALK is expressed in 8–10 % of neuroblastomas, and germline ALK mutations are found in the majority of familial cases [52, 57, 88]. Crizotinib is the first FDA-approved ALK inhibitor. It is an ATP-competitive 2,4-pyrimidinediamine derivative that binds to the inactive form of ALK [89, 90]. Crizotinib has been used successfully in early non-small cell lung cancer patients and is currently being tested in an ongoing COG phase I/II trial in pediatric patients with neuroblastoma and other solid tumors [52, 91].

3.3.2 Adoptive T-Cell Therapy and Chimeric Antigen Receptors

Utilizing chimeric antigen receptors (CARs) that allow cytotoxic T lymphocytes (CTLs) to target cancer cells has been challenging on several levels. The MHC molecules on tumors such as neuroblastoma are usually downregulated, and epitopes for targeting are largely unknown for many pediatric tumors. Neuroblastoma has been the first pediatric solid tumor in which CAR T cells have been tested in clinical trials. A phase I trial in neuroblastoma patients with recurrent/refractory disease received Epstein–Barr virus (EBV) CTLs that were genetically modified to recognize GD2. Three of the 11 patients with active disease at infusion had a complete response, and no significant toxicity was observed. Long-term follow-up was done, and low-level persistence of the GD2–CAR T cells was found and associated with longer survival [55, 56]. Also of note, patients who received the GD2–CAR T cells did not experience significant pain as has been observed in

anti-GD2 mAb therapy [56]. CAR therapy has also been combined with Her2/Neu to target solid tumors. At Baylor College of Medicine, there is an ongoing phase I clinical trial in advanced pediatric sarcomas utilizing Her2/Neu–CAR T cells. Patients with refractory sarcomas or metastatic osteosarcoma that express Her2/Neu receive escalating doses of Her2–CD28 T cells (NCT00902044). There has been a significant focus on utilizing CAR therapy in pediatric lymphomas and leukemia. Multiple phase I trials are currently open, and there has been recently published success in refractory acute lymphoblastic leukemia utilizing CAR–CD19 T cells [92, 93]. Further potential targets for CAR-based therapy are currently under investigation for pediatric solid tumors [92].

3.3.3 Tumor Vaccines

Cancer vaccines are constructed to induce immunologic memory to specific antigens with the hope of sustained tumor killing primarily by CTL. There have been countless adult cancer vaccine trials with varying success. Producing effective therapeutic vaccines is considerably more challenging than developing preventive vaccines. There is a consensus that cancer vaccines will have the best response in patients with minimal residual disease devoid of major immunosuppressive effect from T regulatory or myeloid suppressive cells. However, there is currently no standard for "optimal" tumor vaccines, with various sources studied that have included peptides, whole proteins, cell lysates or irradiated tumor cells, heat shock or chaperone protein-based vaccines, genetically modified tumor cells, or DNA/RNA vaccines. Vaccines using antigens derived from total tumor cell lysates, tumor-derived chaperone proteins, or apoptotic or necrotic tumor cells allow for a wider array of antigens reducing the emergence of tumor escape variants and do not require identification of specific antigens [49, 94]. In addition to the antigen component of the vaccine, an adjuvant component is also important. Adjuvants have ranged from attenuated bacterial products; emulsions such as Montanide or liposomal adjuvants; tensioactive agents such as saponins, alum, and other minerals; to cytokines such as GM-CSF [49, 95].

Antigen-pulsed dendritic cells (DCs) have been utilized in many vaccine trials as they play a central role in the initiation and regulation of tumor-specific immune responses able to activate effector T and B lymphocytes as well as promote NK cell activation [94]. The largest dendritic cell vaccine trial in pediatric patients published to date included 30 patients with recurrent or metastatic Ewing's sarcoma or alveolar rhabdomyosarcoma. Patients had to have confirmed t(11;22) or t(2;13) translocations. The patients received cytoreductive chemotherapy and then were treated with DCs pulsed with tumor-specific peptides derived from tumor-specific breakpoints and E7, a peptide known to bind HLA-A2. All patients generated influenza-specific immune responses and 39 % of patients to the tumor-specific breakpoints, which was used to assess patient immunocompetence. There was increased survival in the group that received tumor vaccine, but this study was not randomized. There were no significant toxicities reported [96]. Smaller studies have also been published utilizing vaccines in pediatric solid tumors. A pilot study conducted at the NIH used a cancer vaccine in five pediatric patients (three neuroblastoma, one Ewing's sarcoma, one synovial sarcoma) with relapsed/refractory solid tumors post-chemotherapy, radiation, and autologous peripheral blood stem cell transplant. Patients received autologous DCs pulsed with tumor-specific synthetic peptides or tumor lysates. A delayed-type hypersensitivity (DTH) test for tumor response was detected in all five patients. There was no significant toxicity, and one of the neuroblastoma patients had stable disease for 27 months, and even more impressive the Ewing's sarcoma patient had a complete response for 77 months [97]. A phase I trial using active IL-12-secreting type 1 DCs was completed in pediatric and adolescent patients with a variety of solid tumors with refractory or metastatic disease including Wilms tumor, adrenal cortical carcinoma, desmoplastic small-round-cell tumor, fibrosarcoma, hepatocellular carcinoma,

osteosarcoma, Ewing's sarcoma, and renal cell carcinoma. Fourteen patients received subcutaneous injections and eight patients received intranodal vaccine injections. The majority of patients did have a positive DTH test. No serious toxicities occurred, and all patients given the vaccine intranodally were alive at the end of the trial as opposed to about half of the subcutaneously treated patients. However, the follow-up period was short (2–13 months). The majority of patients did not have measurable tumor responses except for one patient with lung metastasis that did achieve a decrease in size in some lung metastases. The remaining patients demonstrated stabilization of disease [98]. Another DC vaccine in refractory pediatric solid tumors including sarcomas, neuroblastomas, and renal tumors was conducted in 15 patients. Once again there were no serious adverse advents, and DTH response was found in seven of the ten patients that completed the immunization series. Regression of multiple metastatic sites was found in one patient, and five patients had stable disease with a 16–30-month follow-up [99].

Multiple vaccine studies have been completed in neuroblastoma patients. A phase I vaccine study in 11 neuroblastoma patients was conducted using DCs pulsed with tumor RNA after standard chemotherapy, surgery, radiation, and high-dose chemotherapy with stem cell rescue. The vaccine was found to be safe with no measurable toxicity. Of the three patients evaluated for tumor-specific response, two demonstrated a response. One of these patients remained alive with stable disease 14 months after diagnosis [100]. Rousseau et al. initially conducted a phase I study in relapsed advanced neuroblastoma patients using an allogeneic neuroblastoma tumor cell vaccine combining lymphoactin with IL-2. Lymphoactin encourages lymphocyte chemotaxis and works synergistically with IL-2. The only adverse event was reversible panniculitis and bone pain. There were immune responses found in the majority of patients as well as complete remission in two patients and partial response in one [101]. The group followed this with another phase I trial in seven patients with recurrent neuroblastoma utilizing a tumor vaccine consisting of autologous instead of allogeneic neuroblastoma cells that were genetically modified to secrete IL-2 and lymphoactin. Toxicity was limited to grade I and II localized reactions at the injection site, pain and fever. Tumor-specific immune responses were measured in six patients and were found in five. Two of the seven patients had stable disease throughout the study [102]. The group of investigators then completed a phase I/II study in high-risk neuroblastoma patients utilizing autologous neuroblastoma cells genetically modified to secrete IL-2. Thirteen patients with small tumor burden were enrolled consisting of those who had achieved a complete response, very good partial response, or partial response to their initial therapy. There were no serious toxicities, and median event-free survival was 22 months for patients in first remission with four patients alive and three of them without disease recurrence [103]. This is proof of principle that vaccines may have an improved response in the setting of minimal disease.

The Wilms Tumor gene 1 (WT1) is expressed on many pediatric solid malignancies including Wilms tumor, neuroblastoma, and rhabdomyosarcoma. It is expressed more intensely in alveolar than embryonal subtype of rhabdomyosarcoma [104]. WT1 was ranked as the most promising tumor antigen by the NCI in 2009 [105]. It has been used as an antigen in multiple trials in patients with leukemia. A phase I/II trial was completed using the WT1 peptide vaccine in five pediatric and young adult patients with rhabdomyosarcoma, osteosarcoma, liposarcoma, synovial sarcoma, and acute lymphoblastic leukemia. All patients had relapsed WT1 overexpressing tumors. The only adverse effect was injection site erythema. WT1-specific CTLs were found in three of the four solid tumors. The patient with rhabdomyosarcoma had a complete response, and the patient with liposarcoma had stable disease [106]. This may prove to be a useful target for pediatric solid tumors, particularly alveolar rhabdomyosarcoma [104].

Cancer/testis antigens (CTAs) are a group of antigens expressed on many tumor types including pediatric sarcomas (osteosarcoma, RMS,

NRMS, Ewing's sarcoma) and neuroblastoma. CTAs comprise 70 families with over 140 antigens [57, 107]. Their biologic function is not fully understood, but because of their immunogenicity, they are being studied as T cell targets for vaccine and adoptive cellular therapy. However, not all antigens are immunogenic in all patients, and expression of the antigens may vary between patients. Their expression was found to be the highest in osteosarcoma and RMS and may serve as targets for future cancer vaccines [95, 107].

3.3.4 Cytokines

3.3.4.1 Interferons

Interferons are cytokines that induce antitumor effects by a variety of mechanisms including antiangiogenic and direct antitumor activity [108]. Interferons also participate in activation of the innate immune system including macrophages and NK cells. They are involved in activating DCs and consequently contribute to initiation of adaptive immune responses mediated by cytotoxic T and B cells [109, 110]. In the Karolinska Hospital, the largest treatment center for sarcomas in Sweden, alpha interferon was used to treat osteosarcoma patients with localized disease at varying doses over a 14-year period. A retrospective review was completed recently reviewing the results of 89 consecutive patients. Alpha interferon was used as a single agent after upfront tumor resection, and patients were treated for 2–5 years. From 1971 to 1984, 70 patients were treated with 3 MIU daily for 1 month followed by three times weekly for 17 months. From 1985 to 1990, 19 patients were treated with 3 MIU daily for 3–5 years. The overall survival during this time period was 43 % with little known toxicity, suggesting alpha interferon had some effect [111]. The German/Austrian cooperative group study COSS-80 randomized 158 patients with localized osteosarcoma to interferon beta after upfront standard chemotherapy. Patients received 100,000 U/kg twice weekly for 2 weeks followed by daily injections for 4 weeks and then returned to twice weekly injections for the final 16 weeks. They did not find a significant increase in disease-free survival; however, this was a lower dose of a different interferon, and it was administered for a shorter period of time compared to the Scandinavian experience [108, 112]. Pegylated interferon has been developed since the Scandinavian and COSS trials were completed. The pegylation allows for delayed clearance of the interferon, so it may be administered at a higher dose once weekly [113]. The common side effects from interferons have included fatigue, fever, rigors, diarrhea, and myalgia. The question of whether interferon alpha is effective in osteosarcoma will hopefully be answered by the recently closed European and American Osteosarcoma Study Group trial. This phase III trial randomized patients with localized osteosarcoma and a good histological response after neoadjuvant chemotherapy to postoperative chemotherapy (methotrexate, cisplatin, and doxorubicin) with or without pegylated interferon alpha 2b for 75 weeks with a dose escalation to 1 mcg/kg (NCT00134030).

Gamma interferon (INF-γ) has been shown to enhance TRAIL against Ewing's sarcoma in preclinical models [113]. This combination of INF-γ and the TRAIL receptor agonist mAb lexatumumab is currently being tested in a phase I trial of pediatric patients with refractory solid tumors (NCT00428272) [49]. Gamma interferon has also been found to enhance T-cell trafficking of neuroblastoma cells. Initially patient-derived neuroblastoma cells and patient-derived T cells were injected into immunodeficient mice, and the addition of INF-γ for 18 days was found to upregulate MHC class I expression and significantly enhanced infiltration of T cells into the tumor. Next, a pilot study was completed (NCI-90-C0210) in five pediatric patients with high-risk neuroblastoma. Tumors were sampled before and after administration of 5 days of subcutaneous INF-γ. None of the patients' tumor samples expressed MHC class I prior to INF-γ injection, and two out of the five patients did show upregulation of neuroblastoma cell MHC I after treatment which correlated with infiltrating T cells [114]. These encouraging limited preliminary results suggest that this agent may have some utility in neuroblastoma.

3.3.4.2 Interleukin 2

Interleukin 2 (IL-2) has been shown to be efficacious in renal cell carcinoma and melanoma, albeit with significant side effects, including capillary leak syndrome [115]. As a single agent, IL-2 has been given following autologous stem cell transplantation to refractory pediatric patients with solid tumors. In these reports, it was found to increase T and NK cells; however, this did not lead to increase in survival [116–119]. A Pediatric Oncology Group phase I trial was conducted in 14 pediatric patients with refractory solid tumors including neuroblastoma, sarcomas, and renal tumors as well as 2 leukemia patients. There were no objective tumor responses despite an apparent increase in NK cells and an increased ability to kill NK-resistant Daudi tumor cells *in vitro* [120]. IL-2 is currently being used effectively in combination with GM-CSF with the ganglioside mAbs to enhance ADCC as well as part of a tumor vaccine in combination with lymphoactin in neuroblastoma patients as discussed previously [25, 101–103].

3.3.4.3 GM-CSF

Granulocyte–macrophage colony-stimulating factor is a cytokine that regulates the innate and adaptive immune systems. It is known to stimulate proliferation and differentiation of hematopoietic progenitor cells as well as increasing the activity of neutrophils, monocytes, macrophages, and DCs [121, 122]. Using localized GM-CSF is thought to cause immunostimulatory effects and achieve enhanced tumor killing. Two phase I studies have been completed in metastatic solid tumors with aerosolized GM-CSF as a single agent specifically targeting pulmonary metastasis. At the Mayo Clinic, a phase I study was completed in seven young adults with relapsed solid tumors including NRSTS, Ewing's sarcoma, osteosarcoma, renal cell carcinoma, and melanoma. There were no significant changes in pulmonary function tests and no toxicities were seen. No increases in leukocyte counts were found in the patients' peripheral blood counts. A 13-year-old patient with Ewing's sarcoma had a CR with 12 months of follow-up, and at that time his lung metastases were removed and were not found to have any viable tumor. Stable disease was found in patients with osteosarcoma, NRSTS, and melanoma for more than 6 months [123].

COG also completed a phase I study of aerosolized GM-CSF in 43 pediatric patients with osteosarcoma with first pulmonary recurrence. Patients had a thoracotomy following two cycles of GM-CSF in order to evaluate the effects. Nodules were tested after the second cycle of GM-CSF for immunostimulatory effects, specifically presence of DC recruitment or upregulation of Fas/FasL, but this was not identified. There was no increase in overall survival; however, it is not known whether the dose was adequate or the drug reached its target [124]. As with other immune therapies, inhaled GM-CSF may work better in combination with other immunostimulatory agents, and more testing will be needed. GM-CSF is also currently being utilized in combination with IL-2 and the ganglioside mAbs in neuroblastoma patients, as discussed previously, to enhance ADCC [25].

3.3.5 Activation of Innate Immunity

3.3.5.1 NK Cells

Natural killer (NK) cells play a crucial role in the innate immune system through killing viruses and tumors. They differentiate infected or malignant cells from normal "self" cells by a complex balance between receptor and ligand interactions. However, NK cells are thought to also translate signals to adaptive immunity via DCs. Interferon-primed DCs activate NK cells thorough IL-15. These primed NK cells then promote DC maturation, which induces a T-cell response [125, 126]. NK cell-mediated lysis correlates with the surface expression of activating and inhibitory receptors on NK cells and of the corresponding ligands on tumor cells [127, 128]. The inhibitory Killer-cell immunoglobulin-like receptor (KIR) on NK cells bind to class I HLA molecules. NK cells regulated by KIR interactions can mediate cytotoxicity against HLA class I mismatched targets. In patients with leukemia, it has been shown that alloreactive HLA haploidentical NK cells in the stem cell transplant setting lead to enhanced engraftment and reduced graft versus host disease (GVHD) and prevent leukemic relapse [129].

Infusion of human haploidentical NK cells without hematopoietic transplantation in patients with acute myeloid leukemia (AML) has been studied by Miller et al. demonstrating an association between KIR ligand mismatch and induction of remission in poor-prognosis AML patients [130]. There are preclinical investigations using NK cell therapy in pediatric solid tumors showing that pediatric solid tumors' cell lines (Ewing's sarcoma, rhabdomyosarcoma, neuroblastoma, and osteosarcoma) are sensitive to NK cell cytotoxicity [131–134]. As the amount of cytotoxicity is proportional to the NK cell to target ratio, activation and expansion of NK cells has been described in order to increase killing effectiveness [132, 135]. Perez-Martinez et al. completed a pilot study of six patients with refractory solid tumors. Patients received a reduced-intensity conditioning regimen with a haploidentical transplant with T-cell-depleted graphs followed by adoptive transfer of NK cells. All patients demonstrated an initial clinical response. Three patients are alive >20 months post-transplant, two patients died of progressive disease, and one patient died of severe GVHD. Donor NK cell KIR ligands post-transplant were studied in order to evaluate response to therapy. The authors concluded that as the graphs were T cell depleted, NK cells were thought to play a role in graft versus tumor (GVT) effect [136, 137]. Currently, for pediatric patients with refractory solid tumors, there are multiple phase I and pilot studies open utilizing NK cells. A phase I/II study for pediatric patients with refractory leukemia and solid tumors is currently open at the University of Wisconsin, Madison. The patient and their parents each have KIR typing completed, and the parent with the greatest KIR mismatch undergoes mobilization followed by T-cell depletion. The patients receive reduced-intensity conditioning followed by infusion of the NK cell product (NCT00582816). Another phase I study is open at the National Cancer Institute for pediatric patients with recurrent bone or soft tissue sarcomas and neuroblastomas. Patients receive a conditioning regimen followed by a T-cell-depleted and NK cell-depleted allogeneic peripheral blood stem cell transplant. The donor NK cells are activated and expanded *ex vivo* and infused on days +7 and +49 (NCT01287104). St. Jude Children's Research Hospital has a pilot study using expanded donor NK cell infusion in patients with refractory Ewing's sarcoma and RMS. Family members are screened for a haploidentical match. After donor NK cells are collected, they are activated and expanded *ex vivo*. Patients receive a conditioning regimen with cyclophosphamide, fludarabine, and IL-2 followed by haploidentical donor-derived NK cell infusion (NCT00640796). There is early optimism that this immunotherapeutic approach may have an impact against these tumors.

3.3.5.2 Muramyl Tripeptide Phosphatidylethanolamine

Muramyl tripeptide phosphatidylethanolamine (MTP-PE) is a synthetic analog of a component of bacterial cell walls. MTP-PE is incorporated into liposomes that allow targeted delivery to monocytes and macrophages. Monocytes and macrophages phagocytize the liposome-encapsulated MTP-PE leading to upregulation of IL-1α, IL-1β, IL-6, IL-8, TNF-α, as well as monocyte chemotactic and activating factor genes. These activated macrophages kill tumor cells but not normal cells *in vitro* [138, 139]. Addition of chemotherapy has not decreased the effectiveness of MTP-PE [140, 141]. A randomized phase III trial was performed by the Children's Cancer Group and Pediatric Oncology Group in pediatric patients with osteosarcoma and tested MTP-PE using a 2 × 2 design. MTP-PE was combined with cisplatin, doxorubicin, and high-dose methotrexate with or without ifosfamide. The analysis of this study was complex; however, an updated report in 2008 indicated there was a statistically significant improvement in overall survival for patients in the MTP-PE arm [142]. MTP-PE has not been yet approved by the FDA but is available for compassionate use.

3.3.6 Allogeneic Stem Cell Transplant

There has been limited success improving overall survival in pediatric patients with metastatic solid

tumors with high-dose chemotherapy and autologous stem cell rescue with the exception of neuroblastoma [143]. Performing an allogeneic stem cell transplant in solid tumors has shown a potential GVT benefit in smaller studies and case reports [126, 136, 137, 144–146]. These case reports have illustrated that patients with minimal disease prior to transplant developed antitumor responses associated with GVHD suggestive of GVT effects. In both Ewing's sarcoma and RMS, the cancer/testis antigens from the MAGE and XAGE families are expressed, and as discussed previously, the CTAs are thought to be excellent targets for cytotoxic T lymphocyte. It is believed that these pathways may be targeted by the GVT effect of the allogeneic T cells [107].

Shook et al. utilized matched allogeneic transplant with a reduced-intensity conditioning (RIC) regimen in 24 pediatric patients with refractory solid tumors. The solid tumors included neuroblastoma, Wilms tumor, rhabdoid renal tumor, RMS, Ewing's sarcoma, Hodgkin lymphoma, non-hodgkin lymphoma, and hepatoblastoma. All patients were heavily pretreated, and only three patients were in CR prior to transplant. The patients received a 6/6 HLA-matched sibling donor or a matched unrelated donor after a RIC regimen consisting of fludarabine 30 mg/m^2 and 2 Gy of total body irradiation. All patients tolerated the conditioning well. Four patients with disease prior to transplantation achieved a CR, and three patients with a CR prior to transplant remained disease-free for the follow-up period of 3, 6, and 74 months post-transplant. Acute GVHD occurred in 15 patients, and 6 patients developed high-grade acute GVHD in the matched unrelated donor group versus 2 children transplanted with sibling donors. However, there was no statistical difference in the groups and those who developed GVHD; moreover, there was no difference in survival in those who developed GVHD versus those who did not [146]. In this trial as in other studies, tumor responses were attributed to a GVT effect since they had RIC pre-transplant; however, future larger trials are needed to confirm that HSCT can be used as a platform for more effective immunotherapy in pediatric solid tumors.

3.4 Challenges with Immune Therapy in Pediatrics

There are limitless opportunities as an overexpressed or abnormally expressed protein can serve as a target for cancer vaccines, antibody therapy, as well as NK cell, dendritic cell, and T-cell therapy. The challenge remains in choosing which targets will have the highest yield. Orentas et al. recently published their method for identifying tumor antigen candidates for pediatric solid tumors at the NCI [71]. Through analyzing gene expression profiles and linking these results to the current annotation data base, they were able to identify multiple potential immunotherapy targets for 11 pediatric solid tumors [71]. This is very exciting as one of the major challenges with pediatric solid tumor has been the ability to identify targets. These are only preliminary targets that will need to be incorporated into CARs and antibodies; nonetheless, they may constitute a significant advancement in solid tumor immunotherapy. Another challenge of immune therapy that is not limited to pediatric solid tumors has been the evaluation of tumor response. Response criteria were initially standardized with the development of the World Health Organization (WHO) guidelines, Response Evaluation Criteria in Solid Tumors (RECIST) [147]. The RECIST criteria are sufficient to evaluate the response of tumor shrinkage seen with cytotoxic chemotherapy, however not with immunotherapy. Therefore, the immune-related response criteria (irRC) were published after reviewing the melanoma trial incorporating ipilimumab, a fully human mAb which blocks CTL antigen 4 in 2009 [148]. Results of the ipilimumab trial revealed four distinct patterns: (1) shrinkage in baseline lesions, without new lesions, (2) durable stable disease (in some patients followed by slow, steady decline in total tumor burden), (3) response after an increase in total tumor burden, and (4) response in the presence of new lesions. All of these patterns were associated with increased survival [149]. The irRC criteria still have the same thresholds of response as the WHO criteria but are modified to take immune response into account [148]. The irRC criteria will likely be incorporated into future pediatric immunotherapy trials.

3.5 Concluding Remarks

Pediatric cancer encompasses only 1 % of newly diagnosed cancers in the United States. Pediatric nonneural solid tumors comprise about 20 % of pediatric malignancies [1]. Pediatric solid tumors that are metastatic or recurrent have a very poor survival which has not significantly changed over the past 50 years. Despite the small representation of pediatric solid tumors in oncology, the numerous pediatric clinical trials reviewed herein are evidence of the optimism that immunotherapy will have a distinct role in the treatment of pediatric solid tumors. One of the main reasons for the relatively numerous pediatric phase I trials for such a small population of patients is the acknowledgment of the value of these clinical trials by pediatric oncologists. The collaborative efforts of the Children's Oncology Group as well as European Pediatric Groups have been the driving force behind many of these trials. The undeniable success of the anti-GD2 antibody with IL-2 and GM-CSF in metastatic neuroblastoma is due to the ability of COG to organize meaningful large-scale immunotherapy clinical trials [25]. This phase III trial proved that immunotherapy is not only feasible but can improve the overall survival in pediatric patients with solid tumors that carry a poor prognosis. North American–European collaborations on solid tumors have been proven feasible with the German–Austrian–Swiss Cooperative Osteosarcoma Study Group, the European Osteosarcoma Intergroup, and the Scandinavian Sarcoma Group for the most recent osteosarcoma phase III clinical trial. Future collaborative clinical trials in other solid tumors will likely incorporate immunotherapeutic strategies to approved regimens for a greater therapeutic impact. Based on promising results with the different immunologic agents summarized in this chapter, there is a rapidly growing interest in applying immunotherapy in pediatric cancers.

References

1. American Cancer Society. Cancer facts & figures 2012. Atlanta: The Society; 2012.
2. Smith MA, Seibel NL, Altekruse SF, Ries LA, Melbert DL, O'Leary M, et al. Outcomes for children and adolescents with cancer: challenges for the twenty-first century. J Clin Oncol. 2010;28(15):2625–34.
3. Widemann BC, Adamson PC. Fundamentals of cancer chemotherapy, Cancer in Children and Adolescents, vol. 1. Boston: Jones and Bartlett; 2010.
4. Arndt CA, Rose PS, Folpe AL, Laack NN. Common musculoskeletal tumors of childhood and adolescence. Mayo Clin Proc. 2012;87(5):475–87.
5. Mirabello L, Troisi RJ, Savage SA. Osteosarcoma incidence and survival rates from 1973 to 2004: data from the surveillance, epidemiology, and end results program. Cancer. 2009;115(7):1531–43.
6. Gorlick R. Current concepts on the molecular biology of osteosarcoma. Cancer Treat Res. 2009;152:467–78.
7. Bielack SS, Carrle D, Hardes J, Schuck A, Paulussen M. Bone tumors in adolescents and young adults. Curr Treat Options Oncol. 2008;9(1):67–80.
8. Esiashvili N, Goodman M, Marcus Jr RB. Changes in incidence and survival of Ewing sarcoma patients over the past 3 decades: surveillance epidemiology and end results data. J Pediatr Hematol Oncol. 2008;30(6):425–30.
9. Buckley JD, Pendergrass TW, Buckley CM, Pritchard DJ, Nesbit ME, Provisor AJ, et al. Epidemiology of osteosarcoma and Ewing's sarcoma in childhood: a study of 305 cases by the Children's Cancer Group. Cancer. 1998;83(7):1440–8.
10. Blau O. Molecular investigation of Ewing sarcoma: about detecting translocations. EMBO Mol Med. 2012;4(6):449–50.
11. Balamuth NJ, Womer RB. Ewing's sarcoma. Lancet Oncol. 2010;11(2):184–92.
12. Ferrari A, Sultan I, Huang TT, Rodriguez-Galindo C, Shehadeh A, Meazza C, et al. Soft tissue sarcoma across the age spectrum: a population-based study from the surveillance epidemiology and end results database. Pediatr Blood Cancer. 2011;57(6):943–9.
13. Ognjanovic S, Linabery AM, Charbonneau B, Ross JA. Trends in childhood rhabdomyosarcoma incidence and survival in the United States, 1975–2005. Cancer. 2009;115(18):4218–26.
14. Paulino AC, Okcu MF. Rhabdomyosarcoma. Curr Probl Cancer. 2008;32(1):7–34.
15. Meyer WH, Cripe T. Stevens MCG. Soft tissue sarcomas. 1st ed. Cancer in children and adolescents. Jones and Bartlett. 2010; Sudbury, MA.
16. Leaphart C, Rodeberg D. Pediatric surgical oncology: management of rhabdomyosarcoma. Surg Oncol. 2007;16(3):173–85.
17. Malempati S, Hawkins DS. Rhabdomyosarcoma: review of the Children's Oncology Group (COG) Soft-Tissue Sarcoma Committee experience and rationale for current COG studies. Pediatr Blood Cancer. 2012;59(1):5–10.

18. Navalkele P, O'Dorisio MS, O'Dorisio TM, Zamba GK, Lynch CF. Incidence, survival, and prevalence of neuroendocrine tumors versus neuroblastoma in children and young adults: nine standard SEER registries, 1975–2006. Pediatr Blood Cancer. 2011;56(1):50–7.
19. Mosse YP, Laudenslager M, Khazi D, Carlisle AJ, Winter CL, Rappaport E, et al. Germline PHOX2B mutation in hereditary neuroblastoma. Am J Hum Genet. 2004;75(4):727–30.
20. Chen Y, Takita J, Choi YL, Kato M, Ohira M, Sanada M, et al. Oncogenic mutations of ALK kinase in neuroblastoma. Nature. 2008;455(7215):971–4.
21. Maris JM. Recent advances in neuroblastoma. N Engl J Med. 2010;362(23):2202–11.
22. Maris JM, Hogarty MD, Bagatell R, Cohn SL.Neuroblastoma.Lancet.2007;369(9579):2106–20.
23. Cohn SL, Pearson AD, London WB, Monclair T, Ambros PF, Brodeur GM, et al. The International Neuroblastoma Risk Group (INRG) classification system: an INRG task force report. J Clin Oncol. 2009;27(2):289–97.
24. Cheung NK, Kushner BH, Yeh SD, Larson SM. 3F8 monoclonal antibody treatment of patients with stage 4 neuroblastoma: a phase II study. Int J Oncol. 1998;12(6):1299–306.
25. Yu AL, Gilman AL, Ozkaynak MF, London WB, Kreissman SG, Chen HX, et al. Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. N Engl J Med. 2010;363(14):1324–34.
26. Kaste SC, Dome JS, Babyn PS, Graf NM, Grundy P, Godzinski J, et al. Wilms tumour: prognostic factors, staging, therapy and late effects. Pediatr Radiol. 2008;38(1):2–17.
27. Bonadio JF, Storer B, Norkool P, Farewell VT, Beckwith JB, D'Angio GJ. Anaplastic Wilms' tumor: clinical and pathologic studies. J Clin Oncol. 1985;3(4):513–20.
28. Scott RH, Walker L, Olsen OE, Levitt G, Kenney I, Maher E, et al. Surveillance for Wilms tumour in at-risk children: pragmatic recommendations for best practice. Arch Dis Child. 2006;91(12):995–9.
29. Dome JS, Coppes MJ. Recent advances in Wilms tumor genetics. Curr Opin Pediatr. 2002;14(1):5–11.
30. Pritchard-Jones K, Vujanic G. Multiple pathways to Wilms tumor: how much is genetic? Pediatr Blood Cancer. 2006;47(3):232–4.
31. Grundy P, Dome JS, Kalapurakal J, Perlman EJ, Ritchey M. Renal tumors. 1st ed. Cancer in children and adolescents. Jones and Bartlett. 2010; Sudbury, MA.
32. Buckley KS. Pediatric genitourinary tumors. Curr Opin Oncol. 2012;24(3):291–6.
33. Multerys M, Goodman M, Smith MA, et al. Hepatic tumors. Cancer incidence, survival among children, adolescents: United States SEER program 1975–1995. Bethesda: SEER Program. National Cancer Institute; 1999.
34. Meyers RL. Tumors of the liver in children. Surg Oncol. 2007;16(3):195–203.
35. Litten JB, Tomlinson GE. Liver tumors in children. Oncologist. 2008;13(7):812–20.
36. Rainier S, Dobry CJ, Feinberg AP. Loss of imprinting in hepatoblastoma. Cancer Res. 1995;55(9):1836–8.
37. Tomlinson GE, Douglass EC, Pollock BH, Finegold MJ, Schneider NR. Cytogenetic evaluation of a large series of hepatoblastomas: numerical abnormalities with recurring aberrations involving 1q12-q21. Genes Chromosomes Cancer. 2005;44(2):177–84.
38. Hadzic N, Finegold MJ. Liver neoplasia in children. Clin Liver Dis. 2011;15(2):443–62, vii–x.
39. Giller RH. Pediatric systemic germ cell tumors. 1st ed. Cancer in children and adolescents. Jones and Bartlett. 2010; Sudbury, MA.
40. United States Cancer Statistics. In: Surveillance, Epidemiology End Results (SEER). 2008. http://seer.cancer.gov
41. Bussey KJ, Lawce HJ, Olson SB, Arthur DC, Kalousek DK, Krailo M, et al. Chromosome abnormalities of eighty-one pediatric germ cell tumors: sex-, age-, site-, and histopathology-related differences–a Children's Cancer Group study. Genes Chromosomes Cancer. 1999;25(2):134–46.
42. Malogolowkin MH, Mahour GH, Krailo M, Ortega JA. Germ cell tumors in infancy and childhood: a 45-year experience. Pediatr Pathol. 1990;10(1–2):231–41.
43. Gobel U, Schneider DT, Calaminus G, Haas RJ, Schmidt P, Harms D. Germ-cell tumors in childhood and adolescence. GPOH MAKEI and the MAHO study groups. Ann Oncol. 2000;11(3):263–71.
44. Horton Z, Schlatter M, Schultz S. Pediatric germ cell tumors. Surg Oncol. 2007;16(3):205–13.
45. Cushing B, Giller R, Cullen JW, Marina NM, Lauer SJ, Olson TA, et al. Randomized comparison of combination chemotherapy with etoposide, bleomycin, and either high-dose or standard-dose cisplatin in children and adolescents with high-risk malignant germ cell tumors: a pediatric intergroup study – Pediatric Oncology Group 9049 and Children's Cancer Group 888. J Clin Oncol. 2004;22(13):2691–700.
46. Rogers PC, Olson TA, Cullen JW, Billmire DF, Marina N, Rescorla F, et al. Treatment of children and adolescents with stage II testicular and stages I and II ovarian malignant germ cell tumors: a Pediatric Intergroup Study–Pediatric Oncology Group 9048 and Children's Cancer Group 8891. J Clin Oncol. 2004;22(17):3563–9.
47. Billmire D, Vinocur C, Rescorla F, Colombani P, Cushing B, Hawkins E, et al. Malignant mediastinal germ cell tumors: an intergroup study. J Pediatr Surg. 2001;36(1):18–24.
48. Navid F, Santana VM, Barfield RC. Anti-GD2 antibody therapy for GD2-expressing tumors. Curr Cancer Drug Targets. 2010;10(2):200–9.
49. Capitini CM, Mackall CL, Wayne AS. Immune-based therapeutics for pediatric cancer. Expert Opin Biol Ther. 2010;10(2):163–78.
50. Kushner BH, Kramer K, Cheung NK. Phase II trial of the anti-G(D2) monoclonal antibody 3F8 and granulocyte-macrophage colony-stimulating factor for neuroblastoma. J Clin Oncol. 2001;19(22):4189–94.

51. Frost JD, Hank JA, Reaman GH, Frierdich S, Seeger RC, Gan J, et al. A phase I/IB trial of murine monoclonal anti-GD2 antibody 14.G2a plus interleukin-2 in children with refractory neuroblastoma: a report of the Children's Cancer Group. Cancer. 1997;80(2):317–33.
52. Matthay KK, George RE, Yu AL. Promising therapeutic targets in neuroblastoma. Clin Cancer Res. 2012;18(10):2740–53.
53. Unguru Y, Joffe S, Fernandez CV, Yu AL. Ethical issues for control-arm patients after revelation of benefits of experimental therapy: a framework modeled in neuroblastoma. J Clin Oncol. 2013; 31(5):641–6.
54. Shusterman S, London WB, Gillies SD, Hank JA, Voss SD, Seeger RC, et al. Antitumor activity of hu14.18-IL2 in patients with relapsed/refractory neuroblastoma: a Children's Oncology Group (COG) phase II study. J Clin Oncol. 2010;28(33):4969–75.
55. Pule MA, Savoldo B, Myers GD, Rossig C, Russell HV, Dotti G, et al. Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. Nat Med. 2008;14(11):1264–70.
56. Louis CU, Savoldo B, Dotti G, Pule M, Yvon E, Myers GD, et al. Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. Blood. 2011;118(23):6050–6.
57. Orentas RJ, Lee DW, Mackall C. Immunotherapy targets in pediatric cancer. Front Oncol. 2012;2:3.
58. Heiner JP, Miraldi F, Kallick S, Makley J, Neely J, Smith-Mensah WH, et al. Localization of GD2-specific monoclonal antibody 3F8 in human osteosarcoma. Cancer Res. 1987;47(20):5377–81.
59. Bernstein ML. Targeted therapy in pediatric and adolescent oncology. Cancer. 2011;117(10 Suppl):2268–74.
60. Pedrazzoli P, Secondino S, Perfetti V, Comoli P, Montagna D. Immunotherapeutic intervention against sarcomas. J Cancer. 2011;2:350–6.
61. Mulens V, de la Torre A, Marinello P, Rodriguez R, Cardoso J, Diaz R et al. Immunogenicity and safety of a NeuGcGM3 based cancer vaccine: results from a controlled study in metastatic breast cancer patients. Hum Vaccin. 2010;6(9):736–44.
62. Scursoni AM, Galluzzo L, Camarero S, Pozzo N, Gabri MR, de Acosta CM, et al. Detection and characterization of N-glycosylated gangliosides in Wilms tumor by immunohistochemistry. Pediatr Dev Pathol. 2010;13(1):18–23.
63. Gonzalez-Angulo AM, Hortobagyi GN, Esteva FJ. Adjuvant therapy with trastuzumab for HER-2/neu-positive breast cancer. Oncologist. 2006;11(8):857–67.
64. Ebb D, Meyers P, Grier H, Bernstein M, Gorlick R, Lipshultz SE, et al. Phase II trial of trastuzumab in combination with cytotoxic chemotherapy for treatment of metastatic osteosarcoma with human epidermal growth factor receptor 2 overexpression: a report from the children's oncology group. J Clin Oncol. 2012;30(20):2545–51.
65. Castellano D, Sepulveda JM, Garcia-Escobar I, Rodriguez-Antolin A, Sundlov A, Cortes-Funes H. The role of RANK-ligand inhibition in cancer: the story of denosumab. Oncologist. 2011;16(2):136–45.
66. Fizazi K, Lipton A, Mariette X, Body JJ, Rahim Y, Gralow JR, et al. Randomized phase II trial of denosumab in patients with bone metastases from prostate cancer, breast cancer, or other neoplasms after intravenous bisphosphonates. J Clin Oncol. 2009;27(10):1564–71.
67. Smith MR, Egerdie B, Hernandez Toriz N, Feldman R, Tammela TL, Saad F, et al. Denosumab in men receiving androgen-deprivation therapy for prostate cancer. N Engl J Med. 2009;361(8):745–55.
68. Stopeck AT, Lipton A, Body JJ, Steger GG, Tonkin K, de Boer RH, et al. Denosumab compared with zoledronic acid for the treatment of bone metastases in patients with advanced breast cancer: a randomized, double-blind study. J Clin Oncol. 2010;28(35):5132–9.
69. Lee JA, Jung JS, Kim DH, Lim JS, Kim MS, Kong CB, et al. RANKL expression is related to treatment outcome of patients with localized, high-grade osteosarcoma. Pediatr Blood Cancer. 2011;56(5):738–43.
70. Wesche J, Haglund K, Haugsten EM. Fibroblast growth factors and their receptors in cancer. Biochem J. 2011;437(2):199–213.
71. Orentas RJ, Yang JJ, Wen X, Wei JS, Mackall CL, Khan J. Identification of cell surface proteins as potential immunotherapy targets in 12 pediatric cancers. Front Oncol. 2012;2:194.
72. Taylor JG, Cheuk AT, Tsang PS, Chung JY, Song YK, Desai K, et al. Identification of FGFR4-activating mutations in human rhabdomyosarcomas that promote metastasis in xenotransplanted models. J Clin Invest. 2009;119(11):3395–407.
73. Benesch M, Windelberg M, Sauseng W, Witt V, Fleischhack G, Lackner H, et al. Compassionate use of bevacizumab (Avastin) in children and young adults with refractory or recurrent solid tumors. Ann Oncol. 2008;19(4):807–13.
74. Ferrara N, Hillan KJ, Gerber HP, Novotny W. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. Nat Rev Drug Discov. 2004;3(5):391–400.
75. Hingorani P, Eshun F, White-Collins A, Watanabe M. Gemcitabine, docetaxel, and bevacizumab in relapsed and refractory pediatric sarcomas. J Pediatr Hematol Oncol. 2012;34(7):524–7.
76. Glade Bender JL, Adamson PC, Reid JM, Xu L, Baruchel S, Shaked Y, et al. Phase I trial and pharmacokinetic study of bevacizumab in pediatric patients with refractory solid tumors: a Children's Oncology Group Study. J Clin Oncol. 2008;26(3): 399–405.
77. Guan H, Zhou Z, Wang H, Jia SF, Liu W, Kleinerman ES. A small interfering RNA targeting vascular endothelial growth factor inhibits Ewing's sarcoma

growth in a xenograft mouse model. Clin Cancer Res. 2005;11(7):2662–9.
78. Guan H, Zhou Z, Cao Y, Duan X, Kleinerman ES. VEGF165 promotes the osteolytic bone destruction of ewing's sarcoma tumors by upregulating RANKL. Oncol Res. 2009;18(2–3):117–25.
79. Kolb EA, Gorlick R. Development of IGF-IR inhibitors in pediatric sarcomas. Curr Oncol Rep. 2009;11(4):307–13.
80. Kolb EA, Gorlick R, Houghton PJ, Morton CL, Lock R, Carol H, et al. Initial testing (stage 1) of a monoclonal antibody (SCH 717454) against the IGF-1 receptor by the pediatric preclinical testing program. Pediatr Blood Cancer. 2008;50(6):1190–7.
81. Malempati S, Weigel B, Ingle AM, Ahern CH, Carroll JM, Roberts CT, et al. Phase I/II trial and pharmacokinetic study of cixutumumab in pediatric patients with refractory solid tumors and Ewing sarcoma: a report from the Children's Oncology Group. J Clin Oncol. 2012;30(3):256–62.
82. Wan X, Harkavy B, Shen N, Grohar P, Helman LJ. Rapamycin induces feedback activation of Akt signaling through an IGF-1R-dependent mechanism. Oncogene. 2007;26(13):1932–40.
83. Naing A, LoRusso P, Fu S, Hong DS, Anderson P, Benjamin RS, et al. Insulin growth factor-receptor (IGF-1R) antibody cixutumumab combined with the mTOR inhibitor temsirolimus in patients with refractory Ewing's sarcoma family tumors. Clin Cancer Res. 2012;18(9):2625–31.
84. Daniels RA, Turley H, Kimberley FC, Liu XS, Mongkolsapaya J, Ch'En P, et al. Expression of TRAIL and TRAIL receptors in normal and malignant tissues. Cell Res. 2005;15(6):430–8.
85. Wakelee HA, Patnaik A, Sikic BI, Mita M, Fox NL, Miceli R, et al. Phase I and pharmacokinetic study of lexatumumab (HGS-ETR2) given every 2 weeks in patients with advanced solid tumors. Ann Oncol. 2010;21(2):376–81.
86. Leong S, Cohen RB, Gustafson DL, Langer CJ, Camidge DR, Padavic K, et al. Mapatumumab, an antibody targeting TRAIL-R1, in combination with paclitaxel and carboplatin in patients with advanced solid malignancies: results of a phase I and pharmacokinetic study. J Clin Oncol. 2009;27(26):4413–21.
87. Picarda G, Lamoureux F, Geffroy L, Delepine P, Montier T, Laud K, et al. Preclinical evidence that use of TRAIL in Ewing's sarcoma and osteosarcoma therapy inhibits tumor growth, prevents osteolysis, and increases animal survival. Clin Cancer Res. 2010;16(8):2363–74.
88. Webb TR, Slavish J, George RE, Look AT, Xue L, Jiang Q, et al. Anaplastic lymphoma kinase: role in cancer pathogenesis and small-molecule inhibitor development for therapy. Expert Rev Anticancer Ther. 2009;9(3):331–56.
89. Christensen JG, Zou HY, Arango ME, Li Q, Lee JH, McDonnell SR, et al. Cytoreductive antitumor activity of PF-2341066, a novel inhibitor of anaplastic lymphoma kinase and c-Met, in experimental models of anaplastic large-cell lymphoma. Mol Cancer Ther. 2007;6(12 Pt 1):3314–22.
90. Rodig SJ, Shapiro GI. Crizotinib, a small-molecule dual inhibitor of the c-Met and ALK receptor tyrosine kinases. Curr Opin Investig Drugs. 2010;11(12):1477–90.
91. Park JR, Bagatell R, Hogarty M. Children's Oncology Group's 2013 blueprint for research: neuroblastoma. Pediatr Blood Cancer. 2013;60(6):1044–7.
92. Lee DW, Barrett DM, Mackall C, Orentas R, Grupp SA. The future is now: chimeric antigen receptors as new targeted therapies for childhood cancer. Clin Cancer Res. 2012;18(10):2780–90.
93. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med. 2013;368(16):1509–18.
94. Janikashvili N, Larmonier N, Katsanis E. Personalized dendritic cell-based tumor immunotherapy. Immunotherapy. 2010;2(1):57–68.
95. Pollack SM, Loggers ET, Rodler ET, Yee C, Jones RL. Immune-based therapies for sarcoma. Sarcoma. 2011;2011:438940.
96. Mackall CL, Rhee EH, Read EJ, Khuu HM, Leitman SF, Bernstein D, et al. A pilot study of consolidative immunotherapy in patients with high-risk pediatric sarcomas. Clin Cancer Res. 2008;14(15):4850–8.
97. Suminoe A, Matsuzaki A, Hattori H, Koga Y, Hara T. Immunotherapy with autologous dendritic cells and tumor antigens for children with refractory malignant solid tumors. Pediatr Transplant. 2009;13(6):746–53.
98. Dohnal AM, Witt V, Hugel H, Holter W, Gadner H, Felzmann T. Phase I study of tumor Ag-loaded IL-12 secreting semi-mature DC for the treatment of pediatric cancer. Cytotherapy. 2007;9(8):755–70.
99. Geiger JD, Hutchinson RJ, Hohenkirk LF, McKenna EA, Yanik GA, Levine JE, et al. Vaccination of pediatric solid tumor patients with tumor lysate-pulsed dendritic cells can expand specific T cells and mediate tumor regression. Cancer Res. 2001;61(23):8513–9.
100. Caruso DA, Orme LM, Amor GM, Neale AM, Radcliff FJ, Downie P, et al. Results of a phase I study utilizing monocyte-derived dendritic cells pulsed with tumor RNA in children with stage 4 neuroblastoma. Cancer. 2005;103(6):1280–91.
101. Rousseau RF, Haight AE, Hirschmann-Jax C, Yvon ES, Rill DR, Mei Z, et al. Local and systemic effects of an allogeneic tumor cell vaccine combining transgenic human lymphotactin with interleukin-2 in patients with advanced or refractory neuroblastoma. Blood. 2003;101(5):1718–26.
102. Russell HV, Strother D, Mei Z, Rill D, Popek E, Biagi E, et al. Phase I trial of vaccination with autologous neuroblastoma tumor cells genetically modified to secrete IL-2 and lymphotactin. J Immunother. 2007;30(2):227–33.
103. Russell HV, Strother D, Mei Z, Rill D, Popek E, Biagi E, et al. A phase 1/2 study of autologous neuroblastoma tumor cells genetically modified to

secrete IL-2 in patients with high-risk neuroblastoma. J Immunother. 2008;31(9):812–9.
104. Oue T, Uehara S, Yamanaka H, Takama Y, Oji Y, Fukuzawa M. Expression of Wilms tumor 1 gene in a variety of pediatric tumors. J Pediatr Surg. 2011;46(12):2233–8.
105. Cheever MA, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT, et al. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. Clin Cancer Res. 2009;15(17):5323–37.
106. Hashii Y, Sato E, Ohta H, Oka Y, Sugiyama H, Ozono K. WT1 peptide immunotherapy for cancer in children and young adults. Pediatr Blood Cancer. 2010;55(2):352–5.
107. Jacobs JF, Brasseur F, Hulsbergen-van de Kaa CA, van de Rakt MW, Figdor CG, Adema GJ, et al. Cancer-germline gene expression in pediatric solid tumors using quantitative real-time PCR. Int J Cancer. 2007;120(1):67–74.
108. Whelan J, Patterson D, Perisoglou M, Bielack S, Marina N, Smeland S, et al. The role of interferons in the treatment of osteosarcoma. Pediatr Blood Cancer. 2010;54(3):350–4.
109. Dunn GP, Koebel CM, Schreiber RD. Interferons, immunity and cancer immunoediting. Nat Rev Immunol. 2006;6(11):836–48.
110. Khawli LA, Hu P, Epstein AL. Cytokine, chemokine, and co-stimulatory fusion proteins for the immunotherapy of solid tumors. Handb Exp Pharmacol. 2008;181:291–328.
111. Muller CR, Smeland S, Bauer HC, Saeter G, Strander H. Interferon-alpha as the only adjuvant treatment in high-grade osteosarcoma: long term results of the Karolinska Hospital series. Acta Oncol. 2005;44(5):475–80.
112. Winkler K, Beron G, Kotz R, Salzer-Kuntschik M, Beck J, Beck W, et al. Neoadjuvant chemotherapy for osteogenic sarcoma: results of a Cooperative German/Austrian study. J Clin Oncol. 1984;2(6):617–24.
113. Merchant MS, Yang X, Melchionda F, Romero M, Klein R, Thiele CJ, et al. Interferon gamma enhances the effectiveness of tumor necrosis factor-related apoptosis-inducing ligand receptor agonists in a xenograft model of Ewing's sarcoma. Cancer Res. 2004;64(22):8349–56.
114. Reid GS, Shan X, Coughlin CM, Lassoued W, Pawel BR, Wexler LH, et al. Interferon-gamma-dependent infiltration of human T cells into neuroblastoma tumors in vivo. Clin Cancer Res. 2009;15(21):6602–8.
115. Khawli LA, Hu P, Epstein AL. Cytokine, chemokine, and co-stimulatory fusion proteins for the immunotherapy of solid tumors. In: Chernajovsky Y, Nissim A, editors. Therapeutic antibodies. Handbook of experimental pharmacology 181. Berlin/Heidelberg: Springer; 2008.
116. Vlk V, Eckschlager T, Kavan P, Kabickova E, Koutecky J, Sobota V, et al. Clinical ineffectiveness of IL-2 and/or IFN alpha administration after autologous PBSC transplantation in pediatric oncological patients. Pediatr Hematol Oncol. 2000;17(1):31–44.
117. Kalwak K, Ussowicz M, Gorczynska E, Turkiewicz D, Toporski J, Dobaczewski G, et al. Immunologic effects of intermediate-dose IL-2 i.v. after autologous hematopoietic cell transplantation in pediatric solid tumors. J Interferon Cytokine Res. 2003;23(4):173–81.
118. Schwinger W, Klass V, Benesch M, Lackner H, Dornbusch HJ, Sovinz P, et al. Feasibility of high-dose interleukin-2 in heavily pretreated pediatric cancer patients. Ann Oncol. 2005;16(7):1199–206.
119. Bonig H, Laws HJ, Wundes A, Verheyen J, Hannen M, Kim YM, et al. In vivo cytokine responses to interleukin-2 immunotherapy after autologous stem cell transplantation in children with solid tumors. Bone Marrow Transplant. 2000;26(1):91–6.
120. Truitt RL, Piaskowski V, Kirchner P, McOlash L, Camitta BM, Casper JT. Immunological evaluation of pediatric cancer patients receiving recombinant interleukin-2 in a phase I trial. J Immunother (1991). 1992;11(4):274–85.
121. Armitage JO. Emerging applications of recombinant human granulocyte-macrophage colony-stimulating factor. Blood. 1998;92(12):4491–508.
122. Hercus TR, Broughton SE, Ekert PG, Ramshaw HS, Perugini M, Grimbaldeston M, et al. The GM-CSF receptor family: mechanism of activation and implications for disease. Growth Factors. 2012;30(2):63–75.
123. Anderson PM, Markovic SN, Sloan JA, Clawson ML, Wylam M, Arndt CA, et al. Aerosol granulocyte macrophage-colony stimulating factor: a low toxicity, lung-specific biological therapy in patients with lung metastases. Clin Cancer Res. 1999;5(9):2316–23.
124. Arndt CA, Koshkina NV, Inwards CY, Hawkins DS, Krailo MD, Villaluna D, et al. Inhaled granulocyte-macrophage colony stimulating factor for first pulmonary recurrence of osteosarcoma: effects on disease-free survival and immunomodulation. a report from the Children's Oncology Group. Clin Cancer Res. 2010;16(15):4024–30.
125. Degli-Esposti MA, Smyth MJ. Close encounters of different kinds: dendritic cells and NK cells take centre stage. Nat Rev Immunol. 2005;5(2):112–24.
126. Lucas KG, Schwartz C, Kaplan J. Allogeneic stem cell transplantation in a patient with relapsed Ewing sarcoma. Pediatr Blood Cancer. 2008;51(1):142–4.
127. Farag SS, Fehniger TA, Ruggeri L, Velardi A, Caligiuri MA. Natural killer cell receptors: new biology and insights into the graft-versus-leukemia effect. Blood. 2002;100(6):1935–47.
128. Lanier LL. NK cell recognition. Annu Rev Immunol. 2005;23:225–74.
129. Raulet DH, Held W. Natural killer cell receptors: the offs and ons of NK cell recognition. Cell. 1995;82(5):697–700.
130. Miller JS, Soignier Y, Panoskaltsis-Mortari A, McNearney SA, Yun GH, Fautsch SK, et al.

Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. Blood. 2005;105(8):3051–7.
131. Ahn YO, Weigel B, Verneris MR. Killing the killer: natural killer cells to treat Ewing's sarcoma. Clin Cancer Res. 2010;16(15):3819–21.
132. Cho D, Shook DR, Shimasaki N, Chang YH, Fujisaki H, Campana D. Cytotoxicity of activated natural killer cells against pediatric solid tumors. Clin Cancer Res. 2010;16(15):3901–9.
133. Honorati MC, Neri S, Cattini L, Facchini A. IL-17 enhances the susceptibility of U-2 OS osteosarcoma cells to NK cell lysis. Clin Exp Immunol. 2003;133(3):344–9.
134. Castriconi R, Dondero A, Cilli M, Ognio E, Pezzolo A, De Giovanni B, et al. Human NK cell infusions prolong survival of metastatic human neuroblastoma-bearing NOD/scid mice. Cancer Immunol Immunother. 2007;56(11):1733–42.
135. Denman CJ, Senyukov VV, Somanchi SS, Phatarpekar PV, Kopp LM, Johnson JL, et al. Membrane-bound IL-21 promotes sustained ex vivo proliferation of human natural killer cells. PLoS One. 2012;7(1):e30264.
136. Perez-Martinez A, Leung W, Munoz E, Iyengar R, Ramirez M, Vicario JL, et al. KIR-HLA receptor-ligand mismatch associated with a graft-versus-tumor effect in haploidentical stem cell transplantation for pediatric metastatic solid tumors. Pediatr Blood Cancer. 2009;53(1):120–4.
137. Perez-Martinez A, de Prada Vicente I, Fernandez L, Gonzalez-Vicent M, Valentin J, Martin R, et al. Natural killer cells can exert a graft-vs-tumor effect in haploidentical stem cell transplantation for pediatric solid tumors. Exp Hematol. 2012;40(11):882–91 e1.
138. Asano T, Kleinerman ES. Liposome-encapsulated MTP-PE: a novel biologic agent for cancer therapy. J Immunother Emphasis Tumor Immunol. 1993;14(4):286–92.
139. Kleinerman ES, Jia SF, Griffin J, Seibel NL, Benjamin RS, Jaffe N. Phase II study of liposomal muramyl tripeptide in osteosarcoma: the cytokine cascade and monocyte activation following administration. J Clin Oncol. 1992;10(8):1310–6.
140. Anderson P. Liposomal muramyl tripeptide phosphatidylethanolamine: ifosfamide-containing chemotherapy in osteosarcoma. Future Oncol. 2006;2(3):333–43.
141. Kleinerman ES, Meyers PA, Raymond AK, Gano JB, Jia SF, Jaffe N. Combination therapy with ifosfamide and liposome-encapsulated muramyl tripeptide: tolerability, toxicity, and immune stimulation. J Immunother Emphasis Tumor Immunol. 1995;17(3):181–93.
142. Meyers PA, Schwartz CL, Krailo MD, Healey JH, Bernstein ML, Betcher D, et al. Osteosarcoma: the addition of muramyl tripeptide to chemotherapy improves overall survival–a report from the Children's Oncology Group. J Clin Oncol. 2008;26(4):633–8.
143. Matthay KK, Villablanca JG, Seeger RC, Stram DO, Harris RE, Ramsay NK, et al. Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis-retinoic acid. Children's Cancer Group. N Engl J Med. 1999;341(16):1165–73.
144. Ohta H, Hashii Y, Yoshida H, Kusuki S, Tokimasa S, Yoneda A, et al. Allogeneic hematopoietic stem cell transplantation against recurrent rhabdomyosarcoma. J Pediatr Hematol Oncol. 2011;33(1):e35–8.
145. Lang P, Pfeiffer M, Muller I, Schumm M, Ebinger M, Koscielniak E, et al. Haploidentical stem cell transplantation in patients with pediatric solid tumors: preliminary results of a pilot study and analysis of graft versus tumor effects. Klin Padiatr. 2006;218(6):321–6.
146. Shook DR, Triplett BM, Srinivasan A, Hartford C, Dallas MH, Pillai A, et al. Successful allogeneic hematopoietic cell engraftment after a minimal conditioning regimen in children with relapsed or refractory solid tumors. Biol Blood Marrow Transplant. 2013;19(2):291–7.
147. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer. 1981;47(1):207–14.
148. Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbe C, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res. 2009;15(23):7412–20.
149. Hoos A. Evolution of end points for cancer immunotherapy trials. Ann Oncol. 2012;23 Suppl 8:viii47–52.

Immunotherapeutic Strategies for Multiple Myeloma

4

Michal Bar-Natan, Kenneth C. Anderson, and David E. Avigan

Contents

4.1 Introduction

Multiple myeloma (MM) is a common hematologic malignancy with approximately 20,000 new cases diagnosed each year. Disease progression is characterized by the clonal expansion of malignant plasma cells associated with the clinical sequelae of anemia, lytic bone lesions, renal dysfunction, and compromised immunity. The advent of biologic-based therapies such as lenalidomide and bortezomib has resulted in improved patient outcomes in the last 5–10 years. However, curative outcomes remain elusive. There has been an increased appreciation of the critical role host immunity plays in the evolution of disease and the potential therapeutic efficacy of immune-based therapies. These treatment approaches hold the potential promise of selective targeting of the malignant clone, disruption of stromal-plasma cell interactions, and generation of sustained anti-tumor immunity and durable response. However, the development of clinically efficacious immunotherapy is dependent on achieving greater understanding of the complex interactions between the immunologic milieu and disease.

M. Bar-Natan, MD
Department of Hematology/Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA

D.E. Avigan, MD (✉)
Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA
e-mail: davigan@bidmc.harvard.edu

K.C. Anderson, MD
Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02215, USA

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_4,

4.2 Immune Therapy for Myeloma: Overcoming Tumor-Associated Immune Suppression

Patients with myeloma exhibit prominent deficiencies in humoral immunity due to the dominance of the malignant plasma cell clone and the suppression of the normal B-cell repertoire [1]. A hallmark of the disease is the increased risk for viral and bacterial infections due to encapsulated organisms that are dependent on opsonization for systemic clearance [2]. Interactions between myeloma cells and stromal elements create an immunosuppressive microenvironment through the release of cytokines and soluble factors [3]. Myeloid suppressor cells and plasmacytoid dendritic cells (DCs) further contribute to the immunosuppressive milieu. Inhibition of antigen-presenting cell function may also contribute to tumor-associated tolerance and the loss of protective immunity. A variety of soluble factors such as vascular endothelial growth factor (VEGF) and indoleamine block the maturation and activation of antigen-presenting cells resulting in an increase of DCs with an inhibitory phenotype at the tumor site [4, 5].

The evolution of disease from monoclonal gammopathy of undetermined significance (MGUS) to MM is characterized by progressive deficiencies in T-cell immunity. There is a loss of complexity of the T-cell repertoire, including the absence of clones targeting defined myeloma-associated antigens such as SOX2 [6]. There is concomitant loss of effector cell function, expansion of regulatory T cells, and T-cell polarization toward an inhibitory phenotype in the tumor bed. Other T-cell subpopulations, such as Vγ9Vδ2 T cells, also demonstrate impaired activation. Loss of myeloma-specific immunity disrupts the homeostatic equilibrium, thereby allowing for the unrestrained growth of the malignant plasma cell clone.

The activation of immune effector cells and the targeting of tumor cells are modulated by the checkpoint inhibitor pathways mediated by CTL-4 and PD-1/PD-L1. In the non-disease setting, these negative costimulatory molecules maintain the normal equilibrium of host immunity by supporting the prevention of autoreactivity through the establishment of peripheral tolerance. In contrast, tumor cells upregulate these pathways as a means of preventing T-cell activation and blocking the killing of malignant cells by effector cells. PD-L1 expression has been demonstrated in human myeloma cell lines as well as primary cells. PD-1 is expressed by circulating and bone marrow-derived T cells. Of note, the percentage of T cells expressing PD-1 is increased in patients with bulk disease and after immune stimulation, potentially muting the induction of tumor-specific immunity [7–9].

Natural killer (NK) cells constitute a key cellular subset of the innate immune system with the potential to target malignant cells. NK cell reactivity is mediated through the expression of an array of inhibitory and activating receptors. Once activated, NK cells lyse target cells through secretion of cytotoxic granules such as perforin or granzyme B or via death receptors including Fas, and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)-related pathways [10–12]. Studies have shown that NK cell function is preserved in the setting of MGUS and newly diagnosed MM; yet, with progression of the disease to advanced stages, there is a decline in the NK cell activity against MM [13–15]. Myeloma cells secrete TGF-β, IL-6, IL-10, and PGE2 which suppress NK cell function. The monoclonal immunoglobulin produced by MM may also directly impair NK cells. MM is associated with increased levels of soluble IL-2 receptor and IL-15 and displays the IL-15R, leading to an autocrine feedback loop that impairs NK cell activation, proliferation, and function [16–18]. Furthermore, the balance between activating and inhibitory NK cell receptors and ligands is changed. There is upregulation of inhibitory ligands on MM cells such as MHC class I and PD-L1 [7, 19, 20], while concurrently there is reduction in activating ligand expression. Moreover, soluble forms of activating ligand are secreted, and activating receptors such as DNAM-1, NKG2D, NKp30, and CD16 have reduced levels [21–25].

4.3 Antibody-Mediated Strategies

The efficacy of antibody therapy is potentially dependent on several mechanisms involving accessory cell populations. Antibody-mediated binding of tumor cells may trigger Antibody-dependent cell-mediated cytotoxicity (ADCC)

via immune effector populations that express Fcγ receptors, such as NK cells, neutrophils, mononuclear phagocytes, and DCs. After activation of Fc receptors, cytotoxicity is mediated through at least two different mechanisms: one involving the release of perforin and granzyme from effector cells, and the other involving death ligands Fas ligand and TRAIL [26]. Alternatively, cell lysis may be accomplished by the antibody-mediated activation of the classical complement cascade at the tumor site (CDC).

Release of tumor antigens via cell lysis may further amplify the antitumor immune response via cross-presentation of tumor-derived peptides via MHC class I molecules, resulting in activation of $CD8^+$ cytotoxic T lymphocyte [27]. DCs are capable of presenting peptides from engulfed apoptotic cells on MHC class I molecule to elicit antigen-specific $CD8^+$ T-cell responses [28]. ADCC mediated by monoclonal antibody (mAb) might trigger cross-presentation by DCs and promote adaptive immune responses, as DCs can engulf the resultant apoptotic tumor cells and subsequently present tumor antigens (Ags) on MHC class I and II molecules. In addition, cross-presentation can be mediated by phagocytosis of dying Ab-coated tumor cells through FcγRs [29]. As such, efficacy of humoral therapy is impacted by the underlying immune competence of the patient and may be associated with a secondary cellular immune response.

Antibody-based therapy has been pursued in an effort to selectively target myeloma cells while minimizing toxicity to normal tissues. Antibody therapies have focused on cell surface markers expressed by plasma cells such as CD38, CD138, and the tumor adhesion molecule CS1.

4.3.1 CS1

CS1, a cell surface glycoprotein (CD2 subset 1, CRACC, SLAMF7, CD319, or 19A24) member of the immunoglobulin gene superfamily, is highly expressed in CD138-purified primary tumor cells from the majority of MM patients (over 97 %), with low levels of circulating CS1 detectable in MM patient sera but not in healthy donors [30]. CS1 is believed to participate in promoting and supporting MM cell adhesion to bone marrow stromal cells [30].

Elotuzumab (HuLuc63) is a humanized anti-CS1 mAb which binds with high affinity to MM cells and significantly inhibits their adhesion to bone marrow stromal cells. This inhibition could result in inhibition of the stimulatory effects of bone marrow stromal cells on myeloma cell growth and survival [30]. Elotuzumab was also found to induce death of myeloma cells through ADCC [30, 31]. *In vivo* xenograft studies have shown that elotuzumab induces inhibition of MM tumor growth in mouse models [30, 31]. Elotuzumab triggered autologous ADCC against primary MM cells, and pretreatment with conventional or novel anti-MM drugs, especially lenalidomide, markedly enhanced elotuzumab-induced MM cell lysis [30].

Elotuzumab demonstrated minimal single-agent activity in phase I studies of patients with relapsed/refractory disease [32]. However, preclinical data suggested that elotuzumab demonstrates synergy with other biologic agents such as bortezomib and lenalidomide [30, 33]. In a phase I study of elotuzumab and bortezomib, the overall response rate (ORR) was 48 %, and responses were achieved in 67 % of bortezomib-refractory patients. The median time to tumor progression (TTP) was 9.46 months [34]. In a phase Ib combination study with lenalidomide and dexamethasone, the ORR was 82 % for all treated patients ($n=28$), 95 % for lenalidomide-naive patients ($n=22$), and 83 % among patients who had been refractory to their most recent treatment ($n=12$) [35]. In a phase II study of the same combination, the ORR was 84 % for all patients ($n=71$), with median progression-free survival (PFS) of 26.9 months after a median follow-up of 18.1 months [36]. Adverse events attributed to elotuzumab were tolerable and included infusional reactions [32, 35, 36]. Elotuzumab is also being investigated as a therapeutic strategy to delay progression from smoldering myeloma to clinically active disease.

4.3.2 CD38

Malignant plasma cells strongly express CD38, making this a target of interest in the development of therapeutic antibodies in MM. Daratumumab is a human mAb that has been shown to effectively kill myeloma cells *in vitro* and in murine models

[37]. In preclinical studies, it has been demonstrated that lenalidomide potently enhances the efficacy of this antibody [38]. In a phase I dose-escalation study in relapsed or refractory MM (RRMM) patients, 11/29 (38 %) had some reduction in paraprotein, including 5 (17 %) with more than 50 % reduction. Also a marked reduction in bone marrow (BM) plasma cells was seen in the higher doses [39]. Daratumumab is currently being evaluated in clinical trials in patients with relapsed/refractory MM in combination with dexamethasone and lenalidomide or bortezomib (trials NCT01615029 and NCT01620879, respectively).

Two other anti-CD38 Abs, SAR650984 and MOR03087, are also currently being evaluated in patients with RRMM (NCT01749969, NCT01084252, NCT01421186).

4.3.3 Interleukin-6 (IL-6)

Investigators have examined the efficacy of antibody therapy targeting the interaction of myeloma cells with critical aspects of the BM microenvironment including IL-6, insulin-like growth factor-1 (IGF-1), VEGF, and B-cell-activating factor (BAFF) [40, 41]. IL-6 is a pleiotropic cytokine that has been shown to play a crucial role in growth and survival of MM cells within the BM milieu. IL-6 is predominantly produced and secreted by BM stromal cells (BMSCs), mediating MM cell growth, preventing apoptotic cell death, promoting myeloma cell survival, and conferring drug resistance. IL-6 activates Ras/MEK/ERK cascade, JAK2/signal transducer and activator of transcription (STAT)-3 cascade, and PI3K/Akt cascade [42]. Siltuximab (CNTO 328) is a chimeric mAb targeting IL-6. Treatment of IL-6-dependent MM cell lines with siltuximab resulted in inhibited proliferation in a dose-dependent manner, both in the presence and absence of BMSCs [43]. In phase I studies minimal clinical activity was observed following single-agent therapy with siltuximab in patients with relapsed or relapsed and refractory disease [44, 45]. Preclinical studies demonstrated synergy between siltuximab and bortezomib [43], but in a randomized phase II study, combination therapy with bortezomib and siltuximab did not demonstrate enhanced response or survival as compared to bortezomib alone [46].

4.3.4 PD-1/PD-L1

The PD-1/PD-L1 pathway is upregulated in MM and provides a critical inhibitory signal that disrupts immune activation and promotes immune tolerance toward the myeloma cell. CT-011, a humanized anti-PD-1 mAb, enhances human NK cell function against autologous primary MM cells, through increased NK cell trafficking; enhanced immune complex formation between patient-derived NK cells and PD-L1-bearing, primary autologous MM tumor cells, and increased NK cytotoxicity [20]. Lenalidomide downregulates PD-L1 expression on myeloma cells and therefore synergized with CT-011 in the activation of NK cells and subsequent cytotoxicity against myeloma cells [47]. PD-1 blockade also enhances myeloma-specific T-cell immunity *in vitro* and *in vivo* [7, 8]. Preclinical studies demonstrated enhanced T-cell responses to autologous DC/myeloma fusion vaccines as manifested by polarization toward a Th1 phenotype, suppression of regulatory T cells, and increased cytotoxic T lymphocyte (CTL) response [9]. CT-011 prevented the vaccine-mediated increase in T-cell expression of PD-1 [9].

In a phase I clinical trial, CT-011 was administrated to patients with advanced hematological malignancies including MM. CT-011 was safe and well tolerated, with clinical benefit seen in 33 % of patients ($n=15$) including one complete remission (CR). Treatment with CT-011 was accompanied with an elevated percentage of peripheral blood $CD4^+$ T cells [48]. In an ongoing phase II clinical trial, the safety of CT-011 alone, and in combination with a DC/myeloma fusion vaccine, is being evaluated following autologous stem cell transplantation (ASCT) (NCT01067287). Preliminary results have demonstrated that CT-011 has been well tolerated and that posttransplant administration of CT-011 was associated with the expansion of myeloma-specific T cells which persisted at 6 months following completion of therapy [49].

For additional Abs that are being evaluated in MM, see Table 4.1.

Table 4.1 mAb in clinical evaluation for multiple myeloma

Mechanism	Ag	Short description of function	Ab name	Results of earlier trials	Ongoing clinical trials
mAbs targeting proteins involved in myeloma cell adhesion	CS1	See text	Elotuzumab		
	Syndecan-1 (CD138)	Cell surface heparan sulfate proteoglycan. Receptor for the extracellular matrix molecules collagen and fibronectin, also binds via its heparan sulfate chains to various soluble extracellular molecules, including hepatocyte growth factor and fibroblast growth factor [26, 127]	nBT062		A phase I/IIa multidose-escalation study of BT062 in combination with lenalidomide and dexamethasone in subjects with relapsed or relapsed/refractory multiple myeloma (NCT01638936)
			nBT062-SPBD-DM4	In a phase I study of BT062 conjugated to DM4 in RRMM, 13 of 32 patients (40 %) achieved stable disease [128]	A phase I/IIa multidose-escalation study to evaluate maximum tolerated dose (MTD), pharmacokinetics (PK), safety and efficacy of BT062 in subjects with relapsed or relapsed/refractory multiple myeloma (NCT01001442)
	CD56	Membrane glycoprotein from the immunoglobulin superfamily expressed on muscle cells and neurons. It appears to mediate cell adhesion, migration, invasion, and anti-apoptosis	Lorvotuzumab (IMGN901)	In a phase I lorvotuzumab monotherapy study in heavily pretreated MM patients OR was 18 % ($n=28$) with 2 PR and 3 MR [129]	
				In a phase I combination study of lorvotuzumab with lenalidomide and dexamethasone in RRMM, the ORR was 59 % ($n=32$) with 1 sCR, 1 CR, 8 VGPR, and 9 PR. Higher doses complicated by increased incidence of peripheral neuropathy [130]	NCT00991562 mentioned to the left
	CD38	See text	Daratumumab		
	Intercellular adhesion molecule-1 (ICAM-1) (CD54)	ICAM-1 mediates adhesion of myeloma cells to bone marrow stromal cells and thereby contributes to cell adhesion-mediated drug resistance	BI-505		A phase I dose-escalation study to determine the safety, pharmacokinetics, and pharmacodynamics of BI-505, in patients with relapsed/refractory multiple myeloma (NCT01025206)
					A single-arm, open-label, phase II clinical trial evaluating disease response following treatment with BI-505, in patients with smoldering multiple myeloma (NCT01838369)

(continued)

Table 4.1 (continued)

Mechanism	Ag	Short description of function	Ab name	Results of earlier trials	Ongoing clinical trials
mAbs neutralizing growth factors or inhibiting growth-promoting receptors	IL-6 and IL-6 receptor	See text	Siltuximab; CNTO 328		
			mAb 1339; hPM1; tocilizumab		
	Insulin-like growth factor-1 receptor (IGF-1)	IGF-1 is an important growth factor for myeloma cells. It acts synergistically with IL-6 on myeloma cell growth and survival. IGF-1 also protects myeloma cells against anticancer drugs including dexamethasone and bortezomib [41, 131, 132]. IGF-1R is aberrantly expressed on MM cells in about 75 % of the cases and is associated with disease severity [132, 133]	AVE1642	In a phase I study of the anti-(IGF-1R)mAb, AVE1642, as single agent and in combination with bortezomib in patients with relapsed MM, in the monotherapy arm, the drug was well tolerated with no MTD reached (1 DLT of hyperglycemia). Of 15 patients, 1 patient had MR and 7 SD. In the combination arm, out of 11 patients only 2 (18 %) had objective response (1 CR and 1 PR) which was considered insufficient [134]	
			Figitumumab (CP-751,871)	In a phase I study of figitumumab in RRMM, no DLT were identified. Monotherapy did not result in objective responses. Dexamethasone could be added when less than PR was achieved. Of the 27 patients with the combination therapy 6 achieved PR and 3 MR [135]	
			IMC-A12		
	Vascular endothelial growth factor (VEGF)	Key cytokine that promotes angiogenesis in a variety of tumor types including in the bone marrow and therefore contributing to myeloma growth and survival. VEGF also functions as a growth factor for myeloma cells, stimulates IL-6 production in stromal cells, triggers myeloma cell migration, upregulates antiapoptotic proteins, and inhibits maturation of dendritic cells [26, 41]	Bevacizumab	In a phase II randomized trial of bevacizumab versus bevacizumab and thalidomide for RRMM, in the bevacizumab alone arm, 1 patient achieved SD ($n=6$) with median EFS of only 49d for the cohort. For the combination arm, 2 PR and 3 SD ($n=6$), similar results as single-agent thalidomide [136]	Bevacizumab, lenalidomide, and dexamethasone in treating patients with relapsed or refractory stage II or stage III multiple myeloma (NCT00410605)
				In a randomized phase II study of bevacizumab and bortezomib versus bortezomib in RRMM (AMBER), the ORR was similar between the two arms, 51 % in the combination arm ($n=49$) and 43.4 % in the bortezomib monotherapy arm ($n=53$). The median response duration was 6.9 m and 6 m, respectively [137]	

				In a phase II trial of bevacizumab, lenalidomide, and dexamethasone in RRMM, the addition of bevacizumab did not translate to either increased response rate or prolongation of PFS (ORR 64 %, $n=36$) with some increased gastrointestinal and cardiac toxicity [138]	A phase II trial of bevacizumab combined with lenalidomide and dexamethasone (BEV/REV/DEX) in relapsed or refractory multiple myeloma (NCT00410605)
				A phase II study of bevacizumab and bortezomib in patients with RRMM (NCT00464178)	
	B-cell-activating factor (BAFF)	BAFF is a member of the tumor necrosis factor superfamily. It is produced in the bone marrow microenvironment by monocytes, osteoclasts, and neutrophils [139]. BAFF triggers activation of NF-κB, PI3K, and MAPK pathways, resulting in survival and dexamethasone resistance of myeloma cells [140]. BAFF also increases adhesion of MM cells to BMSCs [141]	Tabalumab (LY2127399)		A multicenter, randomized, double-blind, placebo-controlled phase II study of tabalumab in combination with bortezomib and dexamethasone in patients with previously treated multiple myeloma (NCT01602224)
					A phase I study of LY2127399 in combination with bortezomib and dexamethasone in Japanese patients with relapsed or refractory multiple myeloma NCT01556438
					A phase I safety study of LY2127399 in combination with bortezomib in patients with relapsed or refractory multiple myeloma (NCT00689507)
	CD74	CD74 is a transmembrane protein that forms the invariant portion of HLA-DR. CD74 also functions as a receptor for macrophage migration-inhibitory factor [142]. Its activation leads to proliferation and survival of cells through activation of signaling cascades including the NF-kB pathway [143]	Milatuzumab (hLL1, IMMU-115),	In a phase I dose-escalating trial of milatuzumab in RRMM, 2 DLT (infusion reaction, unexplained anemia) and 5 SAEs (bacterial meningitis, confusion/hypercalcemia, fever, cord compression, epistaxis/thrombocytopenia) were reported. No OR observed. 4 of 21 patients had disease stabilization for at least 3 months [144]	
			IMMU-110-doxorubicin conjugated to milatuzumab		A phase I/II study of hLL1-DOX (milatuzumab-doxorubicin antibody-drug conjugate) in patients with multiple myeloma (NCT01101594)

(continued)

Table 4.1 (continued)

Mechanism	Ag	Short description of function	Ab name	Results of earlier trials	Ongoing clinical trials
	CD40	CD40 is a member of the tumor necrosis factor (TNF) receptor superfamily and is highly expressed on MM cells and on BMSCs. CD40 activation induces an array of biological effects, including MM cell proliferation and migration. In addition, CD40 stimulation is important for myeloma cells adhesion to BMSCs, leading to augmented production of IL-6 and VEGF in these cells [145–148]	Dacetuzumab (SGN-40)	In a phase I multidose study of dacetuzumab in advanced MM patients, the maximum tolerated dose was 12 mg/kg/week. 9 of 44 patients (20 %) had a best clinical response of stable disease [149]	
				In a phase Ib, dose-escalation study of dacetuzumab, lenalidomide, and low-dose dexamethasone, the combination was well tolerated. 13 of 33 evaluable patients (39 %) achieved an objective response (1 CR, 12 PR); other responses were 4 MR, 10 SD [150]	
			XmAbCD40		
			Lucatumumab HCD122; CHIR-12.12	In an open-label, multicenter, phase I study of lucatumumab in RRMM, the MTD was 4.5 mg/kg. 12 of 28 patients (43 %) had SD and 1 PR [151]	
mAbs-activating death receptors	TRAIL-R1 and TRAIL-R2	TNF-related apoptosis-inducing ligand (TRAIL) is a member of the death receptor ligand family, a subclass of the tumor necrosis factor family. Its binding induces formation of a death-inducing signaling complex, ultimately leading to caspase activation and initiation of apoptosis. The physiological function of TRAIL is reported to be in immune surveillance and immune-mediated tumor suppression [152]	Mapatumumab, HGS-ETR1	In a phase II trial of mapatumumab in combination with bortezomib in patients with bortezomib naïve RRMM, the addition of mapatumumab to bortezomib did not increase ORR, PFS, or duration of response compared to bortezomib alone. ORR was 51 % for bortezomib alone versus 42 % in the combination arm ($n = 104$) [153]	
			Lexatumumab, HGS-ETR2		

mAbs improving the antitumor immune response	Killer-cell immunoglobulin-like receptors (KIR)	KIRs are transmembrane glycoproteins expressed by natural killer cells and subsets of T cells and function as key regulators of NK cell activity [154]	1–7F9/ IPH2101	In a phase I trial of the anti-KIR antibody IPH2101 in patients with RRMM, the drug was safe and tolerable. No objective responses were observed. 11 of 32 patients (34 %) achieved a best response of stable disease [117]	Multicenter phase II study on the antitumor activity, safety, and pharmacology of two dose regimens of IPH2101, in patients with smoldering multiple myeloma (KIRMONO) (NCT01222286)
				In interim results of a phase I trial of the anti-inhibitory KIR antibody, IPH2101, and lenalidomide in MM of 13 patients, 1 achieved unconfirmed CR, 3 PR, 2 MR, and 1 SD [155]	Multicenter phase I study on the safety, antitumor activity, and pharmacology of IPH2101, a human monoclonal anti-KIR, combined with lenalidomide in patients with multiple myeloma experiencing a first or second relapse (NCT01217203)
					Randomized phase II study evaluating the antitumor activity, safety, and pharmacology of two dose regimens of IPH2101, in patients with multiple myeloma in stable partial response after a first-line therapy (NCT00999830)
					A phase II trial of IPH2101 (anti-KIR) in smoldering multiple myeloma (NCT01248455)
	PD-L1	See text	CT-011		
	CD200	D200 is a highly conserved type I transmembrane glycoprotein that is expressed on many cell types. The expression of the receptor for CD200 (CD200R1) is restricted to myeloid-derived antigen-presenting cells and certain populations of T cells. CD200 imparts an immunoregulatory signal through CD200R, leading to the suppression of T-cell-mediated immune responses. CD200 is expressed on MM cells of the majority of newly diagnosed MM patients. And its expression predicts poor prognosis for patients receiving ASCT [156]	Samalizumab (ALXN6000)	In a phase I/II study, single-agent samalizumab was evaluated in patients with myeloma or chronic lymphocytic leukemia ($n=26$; 3MM). Treatment was well tolerated with antitumor activity evident in some patients receiving multiple cycles, which was associated with reduction in regulatory T cells and increase in activated T cells [157]	

(continued)

Table 4.1 (continued)

Mechanism	Ag	Short description of function	Ab name	Results of earlier trials	Ongoing clinical trials
mAbs targeting mediators of bone disease	RANKL	Skeletal effects of MM believed to be mediated through myeloma-derived cytokines which have been demonstrated to induce RANKL production. RANKL promotes the proliferation, differentiation, survival, and fusion of osteoclastic precursor cells, activates osteoclastic precursor cells toward mature osteoclasts, and inhibits apoptosis of mature osteoclasts. RANKL exerts its biologic effects through binding to and activating the specific receptor, RANK, a transmembrane member of the TNFR superfamily [158]	Denosumab	In a phase II trial of denosumab in the treatment of relapsed or plateau-phase MM, denosumab effectively inhibited the RANKL pathway as evidenced by suppressed levels of the bone turnover marker, serum C-terminal telopeptide of type 1 collagen. However, no patient had an objective response [159]	A randomized, double-blind, multicenter study of denosumab compared with zoledronic acid (Zometa®) in the treatment of bone disease in subjects with newly diagnosed multiple myeloma (NCT01345019)
				In a phase III trial, denosumab was noninferior (trending to superiority) to zoledronic acid (2A) in preventing or delaying time to first on-study skeletal-related events (SRE) in patients with advanced cancer metastatic to bone or myeloma (number of myeloma patients, 180 of 1,776 included in the study) [160]	An open-label, single-arm, extension study to evaluate the long-term safety of denosumab in the treatment of bone metastases in subjects with advanced cancer or multiple myeloma (NCT00950911)
	Dickkopf-1	Dickkopf-related protein 1 (DKK1), a soluble Wnt-signaling inhibitor, is produced by myeloma cells. It inhibits osteoblast differentiation, and its expression is correlated with the presence of lytic bone lesions in patients with MM [161, 162]	BHQ880	In a phase I/II study of BHQ880 in combination with ZA in patients with RRMM with prior SRE, the combination treatment was well tolerated [163]	A single-arm, open-label, phase II clinical trial evaluating disease response following treatment with intravenous BHQ880, in previously untreated patients with high-risk, smoldering multiple myeloma (NCT01302886)
				In a phase II study in previously untreated patients with smoldering MM at risk for progression, BHQ880 given as monotherapy was well tolerated and resulted in the first evidence of anabolic bone activity using a novel imaging modality (increases in bone strength at 6 months, in 4 of 5 patients evaluated using quantitative computed tomography with finite element analysis) [164]	

4.4 Cellular Immunotherapy for Multiple Myeloma

4.4.1 Allogeneic Transplantation

The unique potential efficacy of cellular immunotherapy for myeloma is highlighted by the observation that allogeneic transplantation induces durable remissions in a subset of patients due to the graft-versus-myeloma effect [50–53]. A summary of early data of myeloablative transplantation from the European Bone Marrow Transplant Registry demonstrated that 28 % of patients remained in remission 7 years posttransplant, suggesting that durable responses were potentially achievable [54]. However, the median survival was only 10 months due to extremely high treatment-related mortality, raising a difficult choice for physicians and patients as to the applicability of this strategy. In a more recent report of 158 patients undergoing autologous or allogeneic transplantation based on donor availability, the event-free survival (EFS) following allogeneic transplantation was 33 and 31 % at 5 and 10 years, respectively, consistent with the presence of a subgroup that appear to have sustained disease response [55]. The role of the graft-versus-myeloma effect in preventing disease recurrence was further supported by a retrospective report from the European registry, in which patients with limited or extensive chronic graft-versus-host (cGVHD) disease demonstrated markedly improved 3-year survival (84 and 58 %, respectively) as compared to those without cGVHD (29 %) [56].

Donor lymphocyte infusion (DLI) as a treatment for posttransplant relapse has been shown to induce disease response, achievement of molecular remission, reconstitution of TCR Vβ repertoire, and long-term disease control in a subset of patients [57–60]. However, DLI therapy is complicated by GVHD due to the lack of myeloma specificity of the alloreactive lymphocytes [61]. Efforts to limit toxicity through the use of reduced intensity conditioning regimens have resulted in a decrease in treatment-related mortality but a concomitant increase in the risk of relapse. As such, immune-based targeting of myeloma cells by alloreactive lymphocytes may carry the unique potential for curative outcomes; nonetheless, the lack of specificity and toxicity significantly limits its use.

Investigators have examined strategies to induce autologous cellular immune responses that selectively target myeloma-associated antigens while minimizing toxicity to normal tissue.

One such strategy is the use of cancer vaccines to foster the expansion of tumor-specific lymphocytes. Myeloma-associated antigens that have been explored as targets for immunotherapy include the idiotype protein, MUC1, WT1, PRAME, CYP1B1, and HSP96 [62–69]. Vaccine strategies have included the introduction of myeloma-specific antigens in the context of immune adjuvants and the loading of individual or whole-cell-derived antigens onto antigen-presenting cells such as DCs.

4.4.2 DC-Based Vaccines as a Platform for Antigen Presentation

DCs represent a diverse network of antigen-presenting cells that play a prominent role in mediating immune responsiveness [70]. Circulating DC populations have been identified as myeloid and plasmacytoid in origin with the capacity to elicit Th1 and Th2 responses, respectively. Plasmacytoid DCs have been shown to contribute to the stromal environment in myeloma and may contribute to tumor-mediated tolerance [4]. Myeloma antigens administered in the context of immune adjuvants may recruit and activate native DC populations that subsequently internalize and present tumor antigens [71–73]. However, functional deficiencies have been demonstrated in DCs derived from myeloma patients which may impact their ability to elicit immunologic responses [5]. Alternatively, myeloid DCs with strong expression of costimulatory molecules and stimulatory cytokines may be generated *ex vivo* through cytokine stimulation of precursor populations [74]. DCs generated *ex vivo* and loaded with myeloma-associated antigens may act as a platform for cancer vaccines

[75]. Strategies to introduce tumor antigens include pulsing with peptides, proteins, or lysates [76], electroporation with tumor-derived RNA or DNA [8, 77, 78], loading of tumor-derived apoptotic bodies [79], transduction with viral vectors expressing tumor antigens potentially enhanced by costimulatory molecules [80–83], and the use of whole-cell fusion between DCs and myeloma cells [84–86].

4.4.3 Myeloma Vaccines: Single-Antigen Approaches

The idiotype protein represents a truly tumor-specific antigen created by the unique immunoglobulin gene arrangement intrinsic to the malignant clone [87]. Vaccination with the idiotype protein in conjunction with granulocyte-macrophage colony-stimulating factor (GM-CSF) or IL-12 was associated with antigen-specific T-cell responses. Prolonged disease-free progression was observed in patients exhibiting an immunologic response [88]. Responses have also been observed following vaccination with antigen-presenting cells pulsed with M protein or with DCs loaded with idiotype and exposed to CD40L to induce maturation [89–93]. Vaccination with idiotype-pulsed antigen-presenting cells posttransplant was associated with improved progression-free survival as compared to a historical control cohort.

A peptide-based vaccine for WT1 administered with immune adjuvant has been shown to elicit immunologic response in patients with hematological malignancies and a decrease in measures of disease [94, 95]. In a recent study, WT1-specific immunity following allogeneic transplantation for myeloma was associated with long-term disease control. Peptide-based vaccine for MUC1 is currently being explored in patients with myeloma (NCT01232712). Expression of several cancer-testis antigens has been demonstrated and has been shown to be targeted by donor-derived humoral responses following allogeneic transplantation, confirming their potential immunogenicity. The cancer-testis antigen, NY-ESO, demonstrates increased expression by plasma cells in the setting of advanced disease, creating an appealing target for immune-based therapy [96]. Repetitive stimulation with DCs pulsed with an NY-ESO-derived peptide elicits a strong CTL response *in vitro*, demonstrating an activated phenotype capable of lysing primary myeloma cells [97]. Recent studies have identified a series of antigens recognized by T cells in patients following syngeneic transplantation.

Several other peptides which are highly expressed on myeloma cells and are important in the pathogenesis of the disease have been identified as potential immunogenic targets. Heteroclitic XBP1 (X-box-binding protein 1) (unspliced 184–192 and spliced 367–375), CD138 (syndecan-1)260–268, and CS1239-247 were shown each alone and in a cocktail combination of the four to generate specific CTLs enriched for effector and activated T cells, Ag-specific cytotoxicity against MM cell lines, as well as increased degranulation, proliferation, and INF-γ secretion [98–101].

4.4.4 Myeloma Vaccines: Whole-Cell Approaches

The use of whole-cell-derived antigens for vaccination may elicit a broad polyclonal response that is better able to target the heterogeneity of the myeloma cell population [86]. Consistent with this hypothesis, a murine model demonstrated the emergence of idiotype-negative variants following idiotype-based vaccinations, while whole myeloma cell-based vaccines did not induce resistance [102]. DCs pulsed with tumor lysates have been shown to induce myeloma-associated immunity, although the clinical efficacy was uncertain [76].

The authors have developed a vaccine model in which patient-derived myeloma cells are fused with autologous DCs, creating a hybridoma which expresses a broad array of myeloma antigens in the context of enhanced costimulation [86]. In a murine model, DC/MM fusions were shown to be protective against lethal challenge with syngeneic myeloma cells, and therapeutic efficacy was further enhanced by coadministration

of IL-12 [103]. In preclinical human studies, fusion of DCs and MM cells elicited the expansion of activated T cells that potently lysed autologous myeloma cells *in vitro*.

A phase I clinical trial was completed in which successive cohorts of patients with advanced myeloma underwent vaccination with escalating doses of autologous DC/MM fusions [104]. Patients had undergone a median of four prior treatment regimens. Myeloma cells were derived from bone marrow aspirates, and DCs were generated from adherent mononuclear cells cultured with GM-CSF and IL-4 and matured with TNF-α. Patients underwent serial vaccination in conjunction with GM-CSF. Vaccine-associated toxicity consisted of transient grade 1–2 vaccine site reactions most commonly, while clinically significant autoimmunity was not observed. Biopsy of the vaccine bed demonstrated a dense infiltrate of $CD8^+$ T cells consistent with T-cell expansion occurring at the site of vaccination. Vaccination resulted in the expansion of myeloma-specific T cells in the majority of patients as manifested by the percent of $CD4^+$ and/or $CD8^+$ T cells expressing IFN-γ following *ex vivo* exposure to autologous tumor lysate. On SEREX analysis, humoral responses against novel proteins were noted after vaccination. These findings were consistent with the induction of myeloma-specific immunity in patients with advanced disease. Of note, 66 % of patients demonstrated a period of disease stability ranging from several months to greater than 2 years after vaccination.

The authors have completed a phase II clinical trial in which patients underwent vaccination with DC/MM fusions in conjunction with autologous stem cell transplantation. It was postulated that vaccine response would be augmented following transplant-mediated cytoreduction and in the context of lymphopoietic reconstitution with the associated depletion of regulatory T cells. It was demonstrated that the posttransplant period was associated with the expansion of myeloma-reactive T cells which were further boosted by vaccination with DC/MM fusions. Vaccination was associated with the conversion of partial to complete responses greater than 100 days posttransplant in a subset of patients. A clinical trial is now underway examining the efficacy of PD-1 blockade in conjunction with the DC/MM fusion vaccine following autologous transplantation, and a national cooperative group study for the assessment of DC/MM fusion vaccine with lenalidomide versus lenalidomide maintenance alone is being planned.

4.4.5 NK Cell Therapy

Augmentation of NK cell-mediated immunity has been explored as therapy for MM. Preclinical models have demonstrated that thalidomide and lenalidomide increase the production of IL-2 by T cells, which stimulates NK cell activation and function against MM [105]. Lenalidomide has also been shown to increase CD16 and LFA-1 expression on NK cells, which facilitates an ADCC response against MM [106]. Lenalidomide also modulates the balance of NK cell-activating and inhibitory ligand expression on MM cells. It decreases expression of PD-L1 and enhances expression of ULBP-1(NKG2D ligand) on MM cells, which both result in improved NK cell immune response, as well as recognition and lysis of MM tumor targets [20, 107]. Bortezomib decreases MM expression of MHC class I and enhances the sensitivity of myeloma to NK cell-mediated lysis [108].

The importance of NK cell-mediated immunity in modulating disease outcome was highlighted by the observation that levels of autologous NK cells reinfused with autologous transplantation correlates with absolute lymphocyte recovery after ASCT for MM and non-hodgkin lymphoma [109]. Lymphocyte subset analyses revealed that an absolute NK cell count of 80/μL or more on day +15 post-SCT correlated significantly with improved progression-free survival [110]. In patients with MM undergoing allogeneic SCT, killer-cell immunoglobulin-like receptor (KIR)-ligand mismatch predicting for NK activation was protective against relapse [111]. In addition, the infusion of T-cell-depleted, haploidentical, KIR-mismatched NK cells, followed by delayed

autograft stem cell rescue, has been shown to induce a near-complete/complete response rate of nearly 50 % [112]. Improved disease-free and overall survival was observed in myeloma patients who received grafts from donors with KIR haplotype B, which is associated with more activating receptor genes than KIR haplotype A [113]. Lenalidomide therapy for patients with progressive MM following allogeneic SCT has been associated with an overall response rate of 66 %, and immunomonitoring data show that lenalidomide augments NK cell expression of the activating receptor NKp44 [114]. Moreover, in a recent phase I/II study of lenalidomide given early after allogeneic SCT for MM, lenalidomide treatment resulted in an increase of activating receptors NKp30 and NKp44 on NK cells, as well as an increase in NK cell-mediated cytotoxicity directed against myeloma associated with an increase in the rate of complete remission [115].

IPH2101 is a fully human mAb which cross-reacts with KIR2DL1, KIR2DL2, and KIR2DL3 receptors and prevents their inhibitory signaling, thereby enhancing *in vitro* and *in vivo* NK cell killing of autologous tumor cells [107, 116]. In a phase I trial of IPH2101 in patients with RRMM, the drug was safe and tolerable, but objective responses were not observed [117]. Data suggest that combination of IPH2101 and lenalidomide may exert synergistic effects, as IPH2101 suppresses negative regulatory signals and lenalidomide augments NK cell function and upregulates activating ligands [107].

Other classes of drugs with anti-MM effects may also confer efficacy, at least in part, through recovery or enhancement of the NK cell versus MM effect. For example, histone deacetylase inhibitors increase the tumor surface expression of ligands for the activating NK receptors NKG2D and DNAM-1, thereby facilitating tumor cell recognition by NK cells and augmenting NK cell-mediated lysis of myeloma cells [118, 119].

Ex vivo expansion of NK cells from MM patients using good manufacturing practice (GMP)-compliant components has been demonstrated. NK cells expanded on average 1,600-fold. These expanded NK cells showed significant cytotoxicity against primary autologous MM cells and were able to retain their tolerance against normal cells [120]. Phase I studies utilizing this technology are underway. Another successful method of *ex vivo* NK cell expansion using coculture with K562 cells transfected with 41BBL and membrane-bound interleukin-15 has resulted in 804 and 351 fold expansion from healthy donors and myeloma patients, respectively. These cells killed both allogeneic and autologous primary myeloma cells as well as inhibited myeloma tumor growth in a murine model [121]. Phase II clinical trials have been initiated examining this approach in relapsed high-risk MM (NCT01313897) and asymptomatic MM (NCT01884688).

4.4.6 Engineered T Cells

A promising area of cancer immunotherapy involves the *ex vivo* expansion of activated T cells that target tumor cells. One strategy has been the development of chimeric antigen receptor cells (CARs) in which antibody targeting a cell surface protein on the malignant cell is transduced into the T-cell receptor apparatus, such that selective binding of the tumor is associated with activation of receptor and cell-mediated lysis. An important advance was the cotransduction of a costimulatory molecule such as 41BB to facilitate T-cell expansion and survival. Promising results have been obtained using CARs targeting CD19 in patients with advanced chronic lymphocytic leukemia and acute lymphocytic leukemia with persistence of the engineered cells in the circulation associated with long-term protection [122–124]. Investigators have begun exploring myeloma-specific targets such as CD38, B-cell maturation antigen (BCMA), and CS1. Of note, the choice of antibody epitope appears to have an important effect on T-cell efficacy. The Ag should be expressed by MM cells on their surface, but not by essential cells or organs. It should be expressed by all tumor cells or be essential for their maintenance.

In a recent study, BCMA was found to have restricted expression on plasma cells. Anti-BCMA-CAR-transduced T cells killed MM cell

lines and were able to eradicate tumors in a mouse model. The anti-BCMA-CAR-transduced T cells produced IFN-γ when stimulated with primary MM cells and killed primary MM cells [125].

In another recently published study, NY-ESO-1 was found to be expressed in ~10 % of MM patients. A high-affinity CAR recognizing the immuno-dominant NY-ESO-1157–165 peptide in the context of the HLA-A*02:01 molecule was constructed. These cells (called redirected T cells) had subpopulations of effector and memory cells. They were able to lyse target cells and express IFN-γ. The memory cells showed signs of differentiation upon Ag restimulation and secreted IL-2. Moreover, these redirected T cells were protective against tumor growth in a mouse model [126].

4.5 Concluding Remarks

Potent anti-myeloma immunity has been demonstrated in the allogeneic transplant setting. However, the lack of specificity of alloreactive T cells represents a major limitation of this approach. In the autologous setting, a number of antigens have been identified on malignant plasma cells which may be targeted by both humoral and cell-mediated immunotherapeutic strategies, and encouraging results have been demonstrated both preclinically and in clinical trials. Future directions will focus on: (1) integrating immunotherapeutic approaches in the setting of low disease burden and (2) combining both cellular and humoral immunotherapy with immunomodulatory drugs to enhance autologous anti-MM immunity and improve patient outcome.

References

1. Pilarski LM, Andrews EJ, Mant MJ, Ruether BA. Humoral immune deficiency in multiple myeloma patients due to compromised B-cell function. J Clin Immunol. 1986;6(6):491–501. PubMed PMID: 3023434.
2. Savage DG, Lindenbaum J, Garrett TJ. Biphasic pattern of bacterial infection in multiple myeloma. Ann Intern Med. 1982;96(1):47–50. PubMed PMID: 6976144.
3. Pratt G, Goodyear O, Moss P. Immunodeficiency and immunotherapy in multiple myeloma. Br J Haematol. 2007;138(5):563–79. PubMed PMID: 17686051.
4. Chauhan D, Singh AV, Brahmandam M, Carrasco R, Bandi M, Hideshima T, et al. Functional interaction of plasmacytoid dendritic cells with multiple myeloma cells: a therapeutic target. Cancer Cell. 2009;16(4):309–23. PubMed PMID: 19800576, Epub 2009/10/06.eng.
5. Hayashi T, Hideshima T, Akiyama M, Raje N, Richardson P, Chauhan D, et al. Ex vivo induction of multiple myeloma-specific cytotoxic T lymphocytes. Blood. 2003;102(4):1435–42. PubMed PMID: 12714512, Epub 2003/04/26.eng.
6. Spisek R, Kukreja A, Chen LC, Matthews P, Mazumder A, Vesole D, et al. Frequent and specific immunity to the embryonal stem cell-associated antigen SOX2 in patients with monoclonal gammopathy. J Exp Med. 2007;204(4):831–40. PubMed PMID: 17389240, Pubmed Central PMCID: 2118551.
7. Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proc Natl Acad Sci U S A. 2002;99(19):12293–7. PubMed PMID: 12218188, Epub 2002/09/10.eng.
8. Liu J, Hamrouni A, Wolowiec D, Coiteux V, Kuliczkowski K, Hetuin D, et al. Plasma cells from multiple myeloma patients express B7-H1 (PD-L1) and increase expression after stimulation with IFN-{gamma} and TLR ligands via a MyD88-, TRAF6-, and MEK-dependent pathway. Blood. 2007;110(1):296–304. PubMed PMID: 17363736, Epub 2007/03/17.eng.
9. Rosenblatt J, Glotzbecker B, Mills H, Vasir B, Tzachanis D, Levine JD, et al. PD-1 blockade by CT-011, anti-PD-1 antibody, enhances ex vivo T-cell responses to autologous dendritic cell/myeloma fusion vaccine. J Immunother. 2011;34(5):409–18. PubMed PMID: 21577144, Epub 2011/05/18.eng.
10. Godfrey J, Benson Jr DM. The role of natural killer cells in immunity against multiple myeloma. Leuk Lymphoma. 2012;53(9):1666–76. PubMed PMID: 22423650. Epub 2012/03/20.eng.
11. Bryceson YT, March ME, Ljunggren HG, Long EO. Activation, coactivation, and costimulation of resting human natural killer cells. Immunol Rev. 2006;214:73–91. PubMed PMID: 17100877, Epub 2006/11/15.eng.
12. Smyth MJ, Cretney E, Kelly JM, Westwood JA, Street SE, Yagita H, et al. Activation of NK cell cytotoxicity. Mol Immunol. 2005;42(4):501–10. PubMed PMID: 15607806, Epub 2004/12/21.eng.
13. Jurisic V, Srdic T, Konjevic G, Markovic O, Colovic M. Clinical stage-depending decrease of NK cell activity in multiple myeloma patients. Med Oncol. 2007;24(3):312–7. PubMed PMID: 17873307, Epub 2007/09/18.eng.
14. De Rossi G, De Sanctis G, Bottari V, Tribalto M, Lopez M, Petrucci MT, et al. Surface markers and cytotoxic activities of lymphocytes in monoclonal gammopathy of undetermined significance and untreated multiple myeloma. Increased phytohemagglutinin-induced cellular cytotoxicity and inverted helper/suppressor cell ratio are features common to both diseases.

Cancer Immunol Immunother. 1987;25(2):133–6. PubMed PMID: 3664530, Epub 1987/01/01.eng.
15. Garcia-Sanz R, Gonzalez M, Orfao A, Moro MJ, Hernandez JM, Borrego D, et al. Analysis of natural killer-associated antigens in peripheral blood and bone marrow of multiple myeloma patients and prognostic implications. Br J Haematol. 1996;93(1):81–8. PubMed PMID: 8611480, Epub 1996/04/01.eng.
16. Becknell B, Caligiuri MA. Interleukin-2, interleukin-15, and their roles in human natural killer cells. Adv Immunol. 2005;86:209–39. PubMed PMID: 15705423, Epub 2005/02/12.eng.
17. Tinhofer I, Marschitz I, Henn T, Egle A, Greil R. Expression of functional interleukin-15 receptor and autocrine production of interleukin-15 as mechanisms of tumor propagation in multiple myeloma. Blood. 2000;95(2):610–8. PubMed PMID: 10627470, Epub 2000/01/11.eng.
18. Nielsen H, Nielsen HJ, Tvede N, Klarlund K, Mansa B, Moesgaard F, et al. Immune dysfunction in multiple myeloma. Reduced natural killer cell activity and increased levels of soluble interleukin-2 receptors. APMIS. 1991;99(4):340–6. PubMed PMID: 2036217, Epub 1991/04/01.eng.
19. Carbone E, Neri P, Mesuraca M, Fulciniti MT, Otsuki T, Pende D, et al. HLA class I, NKG2D, and natural cytotoxicity receptors regulate multiple myeloma cell recognition by natural killer cells. Blood. 2005;105(1):251–8. PubMed PMID: 15328155, Epub 2004/08/26.eng.
20. Benson Jr DM, Bakan CE, Mishra A, Hofmeister CC, Efebera Y, Becknell B, et al. The PD-1/PD-L1 axis modulates the natural killer cell versus multiple myeloma effect: a therapeutic target for CT-011, a novel monoclonal anti-PD-1 antibody. Blood. 2010;116(13):2286–94. PubMed PMID: 20460501. Epub 2010/05/13.eng.
21. Fauriat C, Mallet F, Olive D, Costello RT. Impaired activating receptor expression pattern in natural killer cells from patients with multiple myeloma. Leukemia. 2006;20(4):732–3. PubMed PMID: 16437151, Epub 2006/01/27.eng.
22. El-Sherbiny YM, Meade JL, Holmes TD, McGonagle D, Mackie SL, Morgan AW, et al. The requirement for DNAM-1, NKG2D, and NKp46 in the natural killer cell-mediated killing of myeloma cells. Cancer Res. 2007;67(18):8444–9. PubMed PMID: 17875681, Epub 2007/09/19.eng.
23. Soriani A, Zingoni A, Cerboni C, Iannitto ML, Ricciardi MR, Di Gialleonardo V, et al. ATM-ATR-dependent up-regulation of DNAM-1 and NKG2D ligands on multiple myeloma cells by therapeutic agents results in enhanced NK-cell susceptibility and is associated with a senescent phenotype. Blood. 2009;113(15):3503–11. PubMed PMID: 19098271, Epub 2008/12/23.eng.
24. von Lilienfeld-Toal M, Frank S, Leyendecker C, Feyler S, Jarmin S, Morgan R, et al. Reduced immune effector cell NKG2D expression and increased levels of soluble NKG2D ligands in multiple myeloma may not be causally linked. Cancer Immunol Immunother. 2010;59(6):829–39. PubMed PMID: 20024547. Epub 2009/12/22.eng.
25. Costello RT, Boehrer A, Sanchez C, Mercier D, Baier C, Le Treut T, et al. Differential expression of natural killer cell activating receptors in blood versus bone marrow in patients with monoclonal gammopathy. Immunology. 2013;139(3):338–41. PubMed PMID: 23360454. Epub 2013/01/31.eng.
26. van de Donk NW, Kamps S, Mutis T, Lokhorst HM. Monoclonal antibody-based therapy as a new treatment strategy in multiple myeloma. Leukemia. 2012;26(2):199–213. PubMed PMID: 21852787. Epub 2011/08/20.eng.
27. Weiner LM, Surana R, Wang S. Monoclonal antibodies: versatile platforms for cancer immunotherapy. Nat Rev Immunol. 2010;10(5):317–27. PubMed PMID: 20414205. Epub 2010/04/24.eng.
28. Albert ML, Sauter B, Bhardwaj N. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. Nature. 1998;392(6671):86–9. PubMed PMID: 9510252, Epub 1998/03/24.eng.
29. Dhodapkar KM, Krasovsky J, Williamson B, Dhodapkar MV. Antitumor monoclonal antibodies enhance cross-presentation of cellular antigens and the generation of myeloma-specific killer T cells by dendritic cells. J Exp Med. 2002;195(1):125–33. PubMed PMID: 11781371, Epub 2002/01/10.eng.
30. Tai YT, Dillon M, Song W, Leiba M, Li XF, Burger P, et al. Anti-CS1 humanized monoclonal antibody HuLuc63 inhibits myeloma cell adhesion and induces antibody-dependent cellular cytotoxicity in the bone marrow milieu. Blood. 2008;112(4):1329–37. PubMed PMID: 17906076, Epub 2007/10/02.eng.
31. Hsi ED, Steinle R, Balasa B, Szmania S, Draksharapu A, Shum BP, et al. CS1, a potential new therapeutic antibody target for the treatment of multiple myeloma. Clin Cancer Res. 2008;14(9):2775–84. PubMed PMID: 18451245, Epub 2008/05/03.eng.
32. Zonder JA, Mohrbacher AF, Singhal S, van Rhee F, Bensinger WI, Ding H, et al. A phase 1, multicenter, open-label, dose escalation study of elotuzumab in patients with advanced multiple myeloma. Blood. 2012;120(3):552–9. PubMed PMID: 22184404. Epub 2011/12/21.eng.
33. van Rhee F, Szmania SM, Dillon M, van Abbema AM, Li X, Stone MK, et al. Combinatorial efficacy of anti-CS1 monoclonal antibody elotuzumab (HuLuc63) and bortezomib against multiple myeloma. Mol Cancer Ther. 2009;8(9):2616–24. PubMed PMID: 19723891, Epub 2009/09/03.eng.
34. Jakubowiak AJ, Benson DM, Bensinger W, Siegel DS, Zimmerman TM, Mohrbacher A, et al. Phase I trial of anti-CS1 monoclonal antibody elotuzumab in combination with bortezomib in the treatment of relapsed/refractory multiple myeloma. J Clin Oncol. 2012;30(16):1960–5. PubMed PMID: 22291084. Epub 2012/02/01.eng.

35. Lonial S, Vij R, Harousseau JL, Facon T, Moreau P, Mazumder A, et al. Elotuzumab in combination with lenalidomide and low-dose dexamethasone in relapsed or refractory multiple myeloma. J Clin Oncol. 2012;30(16):1953–9.
36. Richardson PG, Jagannath S, Moreau P, Jakubowiak A, Raab MS, Facon T, Vij R, White DJ, Reece D, Benboubker L, Zonder JA, Deng W, Kroog G, Singhal AK, Lonial S, editors. A phase 2 study of elotuzumab (Elo) in combination with lenalidomide and low-dose dexamethasone (Ld) in patients (pts) with relapsed/refractory multiple myeloma (R/R MM): updated results. ASH annual meeting abstracts; 2012; Atlanta: Blood. 2012;120:202.
37. de Weers M, Tai YT, van der Veer MS, Bakker JM, Vink T, Jacobs DC, et al. Daratumumab, a novel therapeutic human CD38 monoclonal antibody, induces killing of multiple myeloma and other hematological tumors. J Immunol. 2011;186(3):1840–8. PubMed PMID: 21187443.
38. van der Veer MS, de Weers M, van Kessel B, Bakker JM, Wittebol S, Parren PW, et al. Towards effective immunotherapy of myeloma: enhanced elimination of myeloma cells by combination of lenalidomide with the human CD38 monoclonal antibody daratumumab. Haematologica. 2011;96(2):284–90. PubMed PMID: 21109694, Pubmed Central PMCID: 3031697.
39. Plesner T, Lokhorst H, Gimsing P, Nahi H, Lisby S, Richardson PG, editors. Daratumumab, a CD38 monoclonal antibody in patients with multiple myeloma – data from a dose-escalation phase I/II study. ASH annual meeting; 2012; Atlanta: Blood. 2012;120:73.
40. Mahindra A, Hideshima T, Anderson KC. Multiple myeloma: biology of the disease. Blood Rev. 2010;24 Suppl 1:S5–11. PubMed PMID: 21126636. Epub 2010/12/04.eng.
41. van de Donk NW, Lokhorst HM, Bloem AC. Growth factors and antiapoptotic signaling pathways in multiple myeloma. Leukemia. 2005;19(12):2177–85. PubMed PMID: 16239913, Epub 2005/10/22.eng.
42. Hideshima T, Podar K, Chauhan D, Anderson KC. Cytokines and signal transduction. Best Pract Res Clin Haematol. 2005;18(4):509–24. PubMed PMID: 16026734.
43. Voorhees PM, Chen Q, Kuhn DJ, Small GW, Hunsucker SA, Strader JS, et al. Inhibition of interleukin-6 signaling with CNTO 328 enhances the activity of bortezomib in preclinical models of multiple myeloma. Clin Cancer Res. 2007;13(21):6469–78. PubMed PMID: 17975159, Epub 2007/11/03.eng.
44. van Zaanen HC, Lokhorst HM, Aarden LA, Rensink HJ, Warnaar SO, van der Lelie J, et al. Chimaeric anti-interleukin 6 monoclonal antibodies in the treatment of advanced multiple myeloma: a phase I dose-escalating study. Br J Haematol. 1998;102(3):783–90. PubMed PMID: 9722307, Epub 1998/08/29.eng.
45. Kurzrock R, Voorhees PM, Casper C, Furman RR, Fayad L, Lonial S, et al. A phase I, open-label study of siltuximab, an anti-IL-6 monoclonal antibody, in patients with B-cell non-Hodgkin lymphoma, multiple myeloma, or Castleman disease. Clin Cancer Res. 2013;19(13):3659–70. PubMed PMID: 23659971. Epub 2013/05/11.eng.
46. Orlowski RZ, Gercheva L, Williams C, Sutherland HJ, Robak T, Masszi T, Goranova-Marinova V, Dimopoulos MA, Cavenagh JD, Spicka I, Maiolino A, Suvorov A, Blade J, Samoilova OS, Van De Velde H, Bandekar R, Kranenburg R, Xie H, Jean-Francois R, editors. Phase II, randomized, double blind, placebo-controlled study comparing siltuximab plus bortezomib versus bortezomib alone in pts with relapsed/refractory multiple myeloma. ASCO annual meeting; 2012; Chicago: Journal of Clinical Oncology. 2012;30:8018
47. Luptakova K, Rosenblatt J, Glotzbecker B, Mills H, Stroopinsky D, Kufe T, et al. Lenalidomide enhances anti-myeloma cellular immunity. Cancer Immunol Immunother. 2013;62(1):39–49. PubMed PMID: 22733396. Epub 2012/06/27. Eng.
48. Berger R, Rotem-Yehudar R, Slama G, Landes S, Kneller A, Leiba M, et al. Phase I safety and pharmacokinetic study of CT-011, a humanized antibody interacting with PD-1, in patients with advanced hematologic malignancies. Clin Cancer Res. 2008;14(10):3044–51. PubMed PMID: 18483370, Epub 2008/05/17. eng.
49. Rosenblatt J, Avivi I, Vasir B, Uhl L, Katz T, Somaiya P, Mills H, Joyce R, Levine JD, Tzachanis D, Boussiotis VA, Luptakova K, Arnason JE, Drummy N, Delaney C, Breault E, Held V, Bisharat L, Giallombardo N, Conway K, Mortellite J, Wagoner J, Schickler M, Rotem-Yehudar R, Richardson PG, Laubach JP, Munshi NC, Anderson KC, Rowe JM, Kufe D, Avigan D, editors. Blockade of PD-1 in combination with dendritic cell/myeloma fusion cell vaccination following autologous stem cell transplantation. ASH annual meeting; 2012; Atlanta: Blood. 2012:120:578.
50. Bird JM, Russell NH, Samson D. Minimal residual disease after bone marrow transplantation for multiple myeloma: evidence for cure in long-term survivors. Bone Marrow Transplant. 1993;12(6):651–4. PubMed PMID: 8136748, Epub 1993/12/01. eng.
51. Seiden MV, Schlossman R, Andersen J, Freeman A, Robertson M, Soiffer R, et al. Monoclonal antibody-purged bone marrow transplantation therapy for multiple myeloma. Leuk Lymphoma. 1995;17(1–2):87–93. PubMed PMID: 7773166, Epub 1995/03/01. eng.
52. Bensinger WI, Buckner CD, Anasetti C, Clift R, Storb R, Barnett T, et al. Allogeneic marrow transplantation for multiple myeloma: an analysis of risk factors on outcome. Blood. 1996;88(7):2787–93. PubMed PMID: 8839877, Epub 1996/10/01. eng.
53. Russell NH, Miflin G, Stainer C, McQuaker JG, Bienz N, Haynes AP, et al. Allogeneic bone marrow transplant for multiple myeloma. Blood. 1997;89(7):2610–1. PubMed PMID: 9116309, Epub 1997/04/01. eng.

54. Gahrton G, Tura S, Ljungman P, Blade J, Brandt L, Cavo M, et al. Prognostic factors in allogeneic bone marrow transplantation for multiple myeloma. J Clin Oncol Off J Am Soc Clin Oncol. 1995;13(6):1312–22. PubMed PMID: 7751876, Epub 1995/06/01. eng.
55. Kuruvilla J, Shepherd JD, Sutherland HJ, Nevill TJ, Nitta J, Le A, et al. Long-term outcome of myeloablative allogeneic stem cell transplantation for multiple myeloma. Biol Blood Marrow Transplant. 2007;13(8):925–31. PubMed PMID: 17640596, Epub 2007/07/21. eng.
56. Crawley C, Lalancette M, Szydlo R, Gilleece M, Peggs K, Mackinnon S, et al. Outcomes for reduced-intensity allogeneic transplantation for multiple myeloma: an analysis of prognostic factors from the Chronic Leukaemia Working Party of the EBMT. Blood. 2005;105(11):4532–9. PubMed PMID: 15731182, Epub 2005/02/26. eng.
57. Bellucci R, Alyea EP, Weller E, Chillemi A, Hochberg E, Wu CJ, et al. Immunologic effects of prophylactic donor lymphocyte infusion after allogeneic marrow transplantation for multiple myeloma. Blood. 2002;99(12):4610–7. PubMed PMID: 12036895, Epub 2002/05/31. eng.
58. Kroger N, Kruger W, Renges H, Zabelina T, Stute N, Jung R, et al. Donor lymphocyte infusion enhances remission status in patients with persistent disease after allografting for multiple myeloma. Br J Haematol. 2001;112(2):421–3. PubMed PMID: 11167841, Epub 2001/02/13. eng.
59. Badros A, Barlogie B, Morris C, Desikan R, Martin SR, Munshi N, et al. High response rate in refractory and poor-risk multiple myeloma after allotransplantation using a nonmyeloablative conditioning regimen and donor lymphocyte infusions. Blood. 2001;97(9):2574–9. PubMed PMID: 11313244, Epub 2001/04/21. eng.
60. Orsini E, Alyea EP, Schlossman R, Canning C, Soiffer RJ, Chillemi A, et al. Changes in T cell receptor repertoire associated with graft-versus-tumor effect and graft-versus-host disease in patients with relapsed multiple myeloma after donor lymphocyte infusion. Bone Marrow Transplant. 2000;25(6):623–32. PubMed PMID: 10734296, Epub 2000/03/29. eng.
61. Lokhorst HM, Wu K, Verdonck LF, Laterveer LL, van de Donk NW, van Oers MH, et al. The occurrence of graft-versus-host disease is the major predictive factor for response to donor lymphocyte infusions in multiple myeloma. Blood. 2004;103(11):4362–4. PubMed PMID: 14976044, Epub 2004/02/21. eng.
62. Qian J, Wang S, Yang J, Xie J, Lin P, Freeman 3rd ME, et al. Targeting heat shock proteins for immunotherapy in multiple myeloma: generation of myeloma-specific CTLs using dendritic cells pulsed with tumor-derived gp96. Clin Cancer Res. 2005;11(24 Pt 1):8808–15. PubMed PMID: 16361569, Epub 2005/12/20. eng.
63. Takahashi T, Makiguchi Y, Hinoda Y, Kakiuchi H, Nakagawa N, Imai K, et al. Expression of MUC1 on myeloma cells and induction of HLA-unrestricted CTL against MUC1 from a multiple myeloma patient. J Immunol. 1994;153(5):2102–9. PubMed PMID: 8051415, Epub 1994/09/01. eng.
64. Brossart P, Schneider A, Dill P, Schammann T, Grunebach F, Wirths S, et al. The epithelial tumor antigen MUC1 is expressed in hematological malignancies and is recognized by MUC1-specific cytotoxic T-lymphocytes. Cancer Res. 2001;61(18):6846–50. PubMed PMID: 11559560.
65. Lim SH, Wang Z, Chiriva-Internati M, Xue Y. Sperm protein 17 is a novel cancer-testis antigen in multiple myeloma. Blood. 2001;97(5):1508–10. PubMed PMID: 11222401, Epub 2001/02/27. eng.
66. Szmania S, Tricot G, van Rhee F. NY-ESO-1 immunotherapy for multiple myeloma. Leuk Lymphoma. 2006;47(10):2037–48. PubMed PMID: 17071474, Epub 2006/10/31. eng.
67. Atanackovic D, Arfsten J, Cao Y, Gnjatic S, Schnieders F, Bartels K, et al. Cancer-testis antigens are commonly expressed in multiple myeloma and induce systemic immunity following allogeneic stem cell transplantation. Blood. 2007;109(3):1103–12. PubMed PMID: 17023585, Epub 2006/10/07. eng.
68. Hundemer M, Schmidt S, Condomines M, Lupu A, Hose D, Moos M, et al. Identification of a new HLA-A2-restricted T-cell epitope within HM1.24 as immunotherapy target for multiple myeloma. Exp Hematol. 2006;34(4):486–96. PubMed PMID: 16569595, Epub 2006/03/30. eng.
69. Pellat-Deceunynck C, Mellerin MP, Labarriere N, Jego G, Moreau-Aubry A, Harousseau JL, et al. The cancer germ-line genes MAGE-1, MAGE-3 and PRAME are commonly expressed by human myeloma cells. Eur J Immunol. 2000;30(3):803–9. PubMed PMID: 10741395, Epub 2000/03/31. eng.
70. Avigan D. Dendritic cells: development, function and potential use for cancer immunotherapy. Blood Rev. 1999;13(1):51–64.
71. Wen YJ, Min R, Tricot G, Barlogie B, Yi Q. Tumor lysate-specific cytotoxic T lymphocytes in multiple myeloma: promising effector cells for immunotherapy. Blood. 2002;99(9):3280–5. PubMed PMID: 11964294, Epub 2002/04/20. eng.
72. Dembic Z, Schenck K, Bogen B. Dendritic cells purified from myeloma are primed with tumor-specific antigen (idiotype) and activate CD4+ T cells. Proc Natl Acad Sci U S A. 2000;97(6):2697–702. PubMed PMID: 10706628, Epub 2000/03/08. eng.
73. O'Neill DW, Adams S, Bhardwaj N. Manipulating dendritic cell biology for the active immunotherapy of cancer. Blood. 2004;104(8):2235–46. PubMed PMID: 15231572, Epub 2004/07/03. eng.
74. Szabolcs P, Moore MA, Young JW. Expansion of immunostimulatory dendritic cells among the myeloid progeny of human CD34+ bone marrow precursors cultured with c-kit ligand, granulocyte-macrophage colony-stimulating factor, and TNF-alpha. J Immunol. 1995;154(11):5851–61.
75. Racanelli V, Leone P, Frassanito MA, Brunetti C, Perosa F, Ferrone S, et al. Alterations in the antigen processing-presenting machinery of transformed plasma cells are associated with reduced recognition by CD8+

T cells and characterize the progression of MGUS to multiple myeloma. Blood. 2011;115(6):1185–93. PubMed PMID: 20008301, Epub 2009/12/17. eng.
76. Lee JJ, Choi BH, Kang HK, Park MS, Park JS, Kim SK, et al. Induction of multiple myeloma-specific cytotoxic T lymphocyte stimulation by dendritic cell pulsing with purified and optimized myeloma cell lysates. Leuk Lymphoma. 2007;48(10):2022–31. PubMed PMID: 17917970, Epub 2007/10/06. eng.
77. Signori E, Iurescia S, Massi E, Fioretti D, Chiarella P, De Robertis M, et al. DNA vaccination strategies for anti-tumour effective gene therapy protocols. Cancer Immunol Immunother. 2010;59(10):1583–91. PubMed PMID: 20390416. Epub 2010/04/15. eng.
78. Fioretti D, Iurescia S, Fazio VM, Rinaldi M. DNA vaccines: developing new strategies against cancer. J Biomed Biotechnol. 2010;2010:174378. PubMed PMID: 20368780, Epub 2010/04/07.eng.
79. Spisek R, Chevallier P, Morineau N, Milpied N, Avet-Loiseau H, Harousseau JL, et al. Induction of leukemia-specific cytotoxic response by cross-presentation of late-apoptotic leukemic blasts by autologous dendritic cells of nonleukemic origin. Cancer Res. 2002;62(10):2861–8. PubMed PMID: 12019165.
80. Gulley JL, Arlen PM, Tsang KY, Yokokawa J, Palena C, Poole DJ, et al. Pilot study of vaccination with recombinant CEA-MUC-1-TRICOM poxviral-based vaccines in patients with metastatic carcinoma. Clin Cancer Res. 2008;14(10):3060–9. PubMed PMID: 18483372, Epub 2008/05/17.eng.
81. Kaufman HL, Kim-Schulze S, Manson K, DeRaffele G, Mitcham J, Seo KS, et al. Poxvirus-based vaccine therapy for patients with advanced pancreatic cancer. J Transl Med. 2007;5:60. PubMed PMID: 18039393, Epub 2007/11/28.eng.
82. Lou E, Marshall J, Aklilu M, Cole D, Chang D, Morse M. A phase II study of active immunotherapy with PANVAC or autologous, cultured dendritic cells infected with PANVAC after complete resection of hepatic metastases of colorectal carcinoma. Clin Colorectal Cancer. 2006;5(5):368–71. PubMed PMID: 16512998, Epub 2006/03/04.eng.
83. Litzinger MT, Foon KA, Tsang KY, Schlom J, Palena C. Comparative analysis of MVA-CD40L and MVA-TRICOM vectors for enhancing the immunogenicity of chronic lymphocytic leukemia (CLL) cells. Leuk Res. 2010;34(10):1351–7. PubMed PMID: 20122733, Epub 2010/02/04.eng.
84. Rosenblatt J, Kufe D, Avigan D. Dendritic cell fusion vaccines for cancer immunotherapy. Expert Opin Biol Ther. 2005;5(5):703–15. PubMed PMID: 15934845, Epub 2005/06/07.eng.
85. Gong J, Chen D, Kashiwaba M, Kufe D. Induction of antitumor activity by immunization with fusions of dendritic and carcinoma cells. Nat Med. 1997;3(5):558–61.
86. Vasir B, Borges V, Wu Z, Grosman D, Rosenblatt J, Irie M, et al. Fusion of dendritic cells with multiple myeloma cells results in maturation and enhanced antigen presentation. Br J Haematol. 2005;129(5):687–700. PubMed PMID: 15916692.
87. Lynch RG, Graff RJ, Sirisinha S, Simms ES, Eisen HN. Myeloma proteins as tumor-specific transplantation antigens. Proc Natl Acad Sci U S A. 1972;69(6):1540–4. PubMed PMID: 4113870, Epub 1972/06/01.eng.
88. Osterborg A, Yi Q, Henriksson L, Fagerberg J, Bergenbrant S, Jeddi-Tehrani M, et al. Idiotype immunization combined with granulocyte-macrophage colony-stimulating factor in myeloma patients induced type I, major histocompatibility complex-restricted, CD8- and CD4-specific T-cell responses. Blood. 1998;91(7):2459–66. PubMed PMID: 9516146.
89. Wen YJ, Ling M, Bailey-Wood R, Lim SH. Idiotypic protein-pulsed adherent peripheral blood mononuclear cell-derived dendritic cells prime immune system in multiple myeloma. Clin Cancer Res. 1998;4(4):957–62. PubMed PMID: 9563890, Epub 1998/05/14.eng.
90. Lim SH, Bailey-Wood R. Idiotypic protein-pulsed dendritic cell vaccination in multiple myeloma. Int J Cancer. 1999;83(2):215–22. PubMed PMID: 10471530, Epub 1999/09/02.eng.
91. Cull G, Durrant L, Stainer C, Haynes A, Russell N. Generation of anti-idiotype immune responses following vaccination with idiotype-protein pulsed dendritic cells in myeloma. Br J Haematol. 1999;107(3):648–55. PubMed PMID: 10583271, Epub 1999/12/03.eng.
92. Titzer S, Christensen O, Manzke O, Tesch H, Wolf J, Emmerich B, et al. Vaccination of multiple myeloma patients with idiotype-pulsed dendritic cells: immunological and clinical aspects. Br J Haematol. 2000;108(4):805–16. PubMed PMID: 10792287, Epub 2000/05/03.eng.
93. Reichardt VL, Okada CY, Liso A, Benike CJ, Stockerl-Goldstein KE, Engleman EG, et al. Idiotype vaccination using dendritic cells after autologous peripheral blood stem cell transplantation for multiple myeloma–a feasibility study. Blood. 1999;93(7):2411–9. PubMed PMID: 10090953.
94. Yasukawa M, Fujiwara H, Ochi T, Suemori K, Narumi H, Azuma T, et al. Clinical efficacy of WT1 peptide vaccination in patients with acute myelogenous leukemia and myelodysplastic syndrome. Am J Hematol. 2009;84(5):314–5. PubMed PMID: 19338044, Epub 2009/04/02.eng.
95. Ochsenreither S, Fusi A, Geikowski A, Stather D, Busse A, Stroux A, et al. Wilms' tumor protein 1 (WT1) peptide vaccination in AML patients: predominant TCR CDR3beta sequence associated with remission in one patient is detectable in other vaccinated patients. Cancer Immunol Immunother. 2012;61(3):313–22. PubMed PMID: 21898091, Epub 2011/09/08.eng.
96. van Rhee F, Szmania SM, Zhan F, Gupta SK, Pomtree M, Lin P, et al. NY-ESO-1 is highly expressed in poor-prognosis multiple myeloma and induces spontaneous humoral and cellular immune responses. Blood. 2005;105(10):3939–44. PubMed PMID: 15671442, Epub 2005/01/27.eng.

97. Batchu RB, Moreno AM, Szmania SM, Bennett G, Spagnoli GC, Ponnazhagan S, et al. Protein transduction of dendritic cells for NY-ESO-1-based immunotherapy of myeloma. Cancer Res. 2005;65(21):10041–9. PubMed PMID: 16267030, Epub 2005/11/04.eng.
98. Bae J, Smith R, Daley J, Mimura N, Tai YT, Anderson KC, et al. Myeloma-specific multiple peptides able to generate cytotoxic T lymphocytes: a potential therapeutic application in multiple myeloma and other plasma cell disorders. Clin Cancer Res. 2012;18(17):4850–60. PubMed PMID: 22753586.
99. Bae J, Song W, Smith R, Daley J, Tai YT, Anderson KC, et al. A novel immunogenic CS1-specific peptide inducing antigen-specific cytotoxic T lymphocytes targeting multiple myeloma. Br J Haematol. 2012;157(6):687–701. PubMed PMID: 22533610.
100. Bae J, Tai YT, Anderson KC, Munshi NC. Novel epitope evoking CD138 antigen-specific cytotoxic T lymphocytes targeting multiple myeloma and other plasma cell disorders. Br J Haematol. 2011;155(3):349–61. PubMed PMID: 21902685.
101. Bae J, Carrasco R, Lee AH, Prabhala R, Tai YT, Anderson KC, et al. Identification of novel myeloma-specific XBP1 peptides able to generate cytotoxic T lymphocytes: a potential therapeutic application in multiple myeloma. Leukemia. 2011;25(10):1610–9. PubMed PMID: 21660045, Pubmed Central PMCID: 3483794.
102. Cohen S, Haimovich J, Hollander N. Dendritic cell-based therapeutic vaccination against myeloma: vaccine formulation determines efficacy against light chain myeloma. J Immunol. 2009;182(3):1667–73. PubMed PMID: 19155516.
103. Gong J, Koido S, Chen D, Tanaka Y, Huang L, Avigan D, et al. Immunization against murine multiple myeloma with fusions of dendritic and plasmacytoma cells is potentiated by interleukin 12. Blood. 2002;99(7):2512–7.
104. Rosenblatt J, Vasir B, Uhl L, Blotta S, Macnamara C, Somaiya P, et al. Vaccination with dendritic cell/tumor fusion cells results in cellular and humoral antitumor immune responses in patients with multiple myeloma. Blood. 2011;117(2):393–402. PubMed PMID: 21030562, Epub 2010/10/30.eng.
105. Hayashi T, Hideshima T, Akiyama M, Podar K, Yasui H, Raje N, et al. Molecular mechanisms whereby immunomodulatory drugs activate natural killer cells: clinical application. Br J Haematol. 2005;128(2):192–203. PubMed PMID: 15638853, Epub 2005/01/11.eng.
106. Tai YT, Li XF, Catley L, Coffey R, Breitkreutz I, Bae J, et al. Immunomodulatory drug lenalidomide (CC-5013, IMiD3) augments anti-CD40 SGN-40-induced cytotoxicity in human multiple myeloma: clinical implications. Cancer Res. 2005;65(24):11712–20. PubMed PMID: 16357183, Epub 2005/12/17.eng.
107. Benson Jr DM, Bakan CE, Zhang S, Collins SM, Liang J, Srivastava S, et al. IPH2101, a novel anti-inhibitory KIR antibody, and lenalidomide combine to enhance the natural killer cell versus multiple myeloma effect. Blood. 2011;118(24):6387–91. PubMed PMID: 22031859. Epub 2011/10/28.eng.
108. Shi J, Tricot GJ, Garg TK, Malaviarachchi PA, Szmania SM, Kellum RE, et al. Bortezomib down-regulates the cell-surface expression of HLA class I and enhances natural killer cell-mediated lysis of myeloma. Blood. 2008;111(3):1309–17. PubMed PMID: 17947507, Epub 2007/10/20.eng.
109. Porrata LF, Gastineau DA, Padley D, Bundy K, Markovic SN. Re-infused autologous graft natural killer cells correlates with absolute lymphocyte count recovery after autologous stem cell transplantation. Leuk Lymphoma. 2003;44(6):997–1000. PubMed PMID: 12854901, Epub 2003/07/12.eng.
110. Siddiqui MA, Svetomir NM, Nevala WK, Uhl CB, Morice WG, Porrata LF, editors. Day 15 natural killer (NK) cell recovery predicts progression free survival after autologous stem cell transplantation in non-hodgkin's lymphoma. ASH annual meeting; 2006; Orlando: Blood. 2006;108:2914.
111. Kroger N, Shaw B, Iacobelli S, Zabelina T, Peggs K, Shimoni A, et al. Comparison between antithymocyte globulin and alemtuzumab and the possible impact of KIR-ligand mismatch after dose-reduced conditioning and unrelated stem cell transplantation in patients with multiple myeloma. Br J Haematol. 2005;129(5):631–43. PubMed PMID: 15916686, Epub 2005/05/27.Eng.
112. Shi J, Tricot G, Szmania S, Rosen N, Garg TK, Malaviarachchi PA, et al. Infusion of haplo-identical killer immunoglobulin-like receptor ligand mismatched NK cells for relapsed myeloma in the setting of autologous stem cell transplantation. Br J Haematol. 2008;143(5):641–53. PubMed PMID: 18950462, Epub 2008/10/28.eng.
113. Kroger N, Zabelina T, Berger J, Duske H, Klyuchnikov E, Binder T, et al. Donor KIR haplotype B improves progression-free and overall survival after allogeneic hematopoietic stem cell transplantation for multiple myeloma. Leukemia. 2011;25(10):1657–61. PubMed PMID: 21647155. Epub 2011/06/08.eng.
114. Lioznov M, El-Cheikh Jr J, Hoffmann F, Hildebrandt Y, Ayuk F, Wolschke C, et al. Lenalidomide as salvage therapy after allo-SCT for multiple myeloma is effective and leads to an increase of activated NK (NKp44(+)) and T (HLA-DR(+)) cells. Bone Marrow Transplant. 2010;45(2):349–53. PubMed PMID: 19584825. Epub 2009/07/09.eng.
115. Wolschke C, Stubig T, Hegenbart U, Schonland S, Heinzelmann M, Hildebrandt Y, et al. Postallograft lenalidomide induces strong NK cell-mediated anti-myeloma activity and risk for T cell-mediated GvHD: Results from a phase I/II dose-finding study. Exp Hematol. 2013;41(2):134–42 e3. PubMed PMID: 23085463. Epub 2012/10/23.eng.
116. Romagne F, Andre P, Spee P, Zahn S, Anfossi N, Gauthier L, et al. Preclinical characterization of

1-7F9, a novel human anti-KIR receptor therapeutic antibody that augments natural killer-mediated killing of tumor cells. Blood. 2009;114(13):2667–77. PubMed PMID: 19553639, Epub 2009/06/26.eng.

117. Benson Jr DM, Hofmeister CC, Padmanabhan S, Suvannasankha A, Jagannath S, Abonour R, et al. A phase 1 trial of the anti-KIR antibody IPH2101 in patients with relapsed/refractory multiple myeloma. Blood. 2012;120(22):4324–33. PubMed PMID: 23033266. Epub 2012/10/04.eng.
118. Schmudde M, Braun A, Pende D, Sonnemann J, Klier U, Beck JF, et al. Histone deacetylase inhibitors sensitize tumour cells for cytotoxic effects of natural killer cells. Cancer Lett. 2008;272(1):110–21. PubMed PMID: 18718708, Epub 2008/08/23.eng.
119. Wu X, Tao Y, Hou J, Meng X, Shi J. Valproic acid upregulates NKG2D ligand expression through an ERK-dependent mechanism and potentially enhances NK cell-mediated lysis of myeloma. Neoplasia. 2012;14(12):1178–89. PubMed PMID: 23308050. Epub 2013/01/12.eng.
120. Alici E, Sutlu T, Bjorkstrand B, Gilljam M, Stellan B, Nahi H, et al. Autologous antitumor activity by NK cells expanded from myeloma patients using GMP-compliant components. Blood. 2008;111(6):3155–62. PubMed PMID: 18192509, Epub 2008/01/15.eng.
121. Garg TK, Szmania SM, Khan JA, Hoering A, Malbrough PA, Moreno-Bost A, et al. Highly activated and expanded natural killer cells for multiple myeloma immunotherapy. Haematologica. 2012;97(9):1348–56. PubMed PMID: 22419581. Epub 2012/03/16.eng.
122. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med. 2011;365(8):725–33. PubMed PMID: 21830940, Pubmed Central PMCID: 3387277.
123. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med. 2013;368(16):1509–18. PubMed PMID: 23527958.
124. Brentjens RJ, Davila ML, Riviere I, Park J, Wang X, Cowell LG, et al. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. Sci Transl Med. 2013;5(177):177ra38. PubMed PMID: 23515080. Pubmed Central PMCID: 3742551.
125. Carpenter RO, Evbuomwan MO, Pittaluga S, Rose JJ, Raffeld M, Yang S, et al. B-cell maturation antigen is a promising target for adoptive T-cell therapy of multiple myeloma. Clin Cancer Res. 2013;19(8):2048–60. PubMed PMID: 23344265. Epub 2013/01/25.eng.
126. Schuberth PC, Jakka G, Jensen SM, Wadle A, Gautschi F, Haley D, et al. Effector memory and central memory NY-ESO-1-specific re-directed T cells for treatment of multiple myeloma. Gene Ther. 2013;20(4):386–95. PubMed PMID: 22739387. Epub 2012/06/29.eng.
127. Dhodapkar MV, Abe E, Theus A, Lacy M, Langford JK, Barlogie B, et al. Syndecan-1 is a multifunctional regulator of myeloma pathobiology: control of tumor cell survival, growth, and bone cell differentiation. Blood. 1998;91(8):2679–88. PubMed PMID: 9531576.
128. Jagannath SA, Chanan-Khan A, Heffner LT, Avigan D, Lutz RJ, Uherek C, Osterroth F, Ruehle M, Haeder, Niemann G, Wartenberg-Demand A, Munshi NC, Anderson KC, editors. BT062, An antibody-drug conjugate directed against CD138, shows clinical activity in a phase I study in patients with relapsed or relapsed/refractory multiple myeloma. ASH annual meeting; 2010; Orlando: Blood. 2010;116:3060.
129. Chanan-Khan A, Wolf JL, Garcia J, Gharibo M, Jagannath S, Manfredi D, Sher T, Martin C, Zildjian SH, O'Leary J, Vescio R, editors. Efficacy analysis from phase I study of lorvotuzumab mertansine (IMGN901), used as monotherapy, in patients with heavily pre-treated CD56-positive multiple myeloma – a preliminary efficacy analysis. ASH annual meeting; 2010; Orlando: Blood. 2010;116:1962.
130. Berdeja JG, Hernandez-Ilizaliturri F, Chanan-Khan A, Patel M, Kelly KR, Running KL, Murphy M, Guild R, Carrigan C, Ladd S, Wolf BB, O'Leary JJ, Ailawadhi S, editors. Phase I study of Lorvotuzumab Mertansine (LM, IMGN901) in combination with lenalidomide (Len) and dexamethasone (Dex) in patients with CD56-positive relapsed or relapsed/refractory multiple myeloma (MM). ASH annual meeting; 2012; Atlanta: Blood. 2012;120;728.
131. Qiang YW, Kopantzev E, Rudikoff S. Insulinlike growth factor-I signaling in multiple myeloma: downstream elements, functional correlates, and pathway cross-talk. Blood. 2002;99(11):4138–46. PubMed PMID: 12010818.
132. Mitsiades CS, Mitsiades NS, McMullan CJ, Poulaki V, Shringarpure R, Akiyama M, et al. Inhibition of the insulin-like growth factor receptor-1 tyrosine kinase activity as a therapeutic strategy for multiple myeloma, other hematologic malignancies, and solid tumors. Cancer Cell. 2004;5(3):221–30. PubMed PMID: 15050914.
133. Bataille R, Robillard N, Avet-Loiseau H, Harousseau JL, Moreau P. CD221 (IGF-1R) is aberrantly expressed in multiple myeloma, in relation to disease severity. Haematologica. 2005;90(5):706–7. PubMed PMID: 15921396.
134. Moreau P, Cavallo F, Leleu X, Hulin C, Amiot M, Descamps G, et al. Phase I study of the anti insulin-like growth factor 1 receptor (IGF-1R) monoclonal antibody, AVE1642, as single agent and in combination with bortezomib in patients with relapsed multiple myeloma. Leukemia. 2011;25(5):872–4. PubMed PMID: 21321571.

135. Lacy MQ, Alsina M, Fonseca R, Paccagnella ML, Melvin CL, Yin D, et al. Phase I, pharmacokinetic and pharmacodynamic study of the anti-insulinlike growth factor type 1 receptor monoclonal antibody CP-751,871 in patients with multiple myeloma. J Clin Oncol. 2008;26(19):3196–203. PubMed PMID: 18474873.
136. Somlo G, Lashkari A, Bellamy W, Zimmerman TM, Tuscano JM, O'Donnell MR, et al. Phase II randomized trial of bevacizumab versus bevacizumab and thalidomide for relapsed/refractory multiple myeloma: a California Cancer Consortium trial. Br J Haematol. 2011;154(4):533–5. PubMed PMID: 21517811, Pubmed Central PMCID: 3272081.
137. White D, Kassim A, Bhaskar B, Yi J, Wamstad K, Paton VE. Results from AMBER, a randomized phase 2 study of bevacizumab and bortezomib versus bortezomib in relapsed or refractory multiple myeloma. Cancer. 2013;119(2):339–47. PubMed PMID: 22811009.
138. Humbert AT, RC, McFarland T, Go R, Markovina S, Miyamoto S, Williams EC, Asimakopoulos F, Kolesar J, Thompson MA, Kim K, Callander N, editors. Angiogenic blockade with bevacizumab does not increase response to lenalidomide and low dose dexamethasone in relapsed or refractory myeloma. ASH annual meeting; 2012; Atlanta: Blood. 2012;120:5041.
139. Moreaux J, Cremer FW, Reme T, Raab M, Mahtouk K, Kaukel P, et al. The level of TACI gene expression in myeloma cells is associated with a signature of microenvironment dependence versus a plasmablastic signature. Blood. 2005;106(3):1021–30. PubMed PMID: 15827134, Pubmed Central PMCID: 2408610.
140. Moreaux J, Legouffe E, Jourdan E, Quittet P, Reme T, Lugagne C, et al. BAFF and APRIL protect myeloma cells from apoptosis induced by interleukin 6 deprivation and dexamethasone. Blood. 2004;103(8):3148–57. PubMed PMID: 15070697, Pubmed Central PMCID: 2387243.
141. Tai YT, Li XF, Breitkreutz I, Song W, Neri P, Catley L, et al. Role of B-cell-activating factor in adhesion and growth of human multiple myeloma cells in the bone marrow microenvironment. Cancer Res. 2006;66(13):6675–82. PubMed PMID: 16818641.
142. Leng L, Metz CN, Fang Y, Xu J, Donnelly S, Baugh J, et al. MIF signal transduction initiated by binding to CD74. J Exp Med. 2003;197(11):1467–76. PubMed PMID: 12782713, Pubmed Central PMCID: 2193907.
143. Lantner F, Starlets D, Gore Y, Flaishon L, Yamit-Hezi A, Dikstein R, et al. CD74 induces TAp63 expression leading to B-cell survival. Blood. 2007;110(13):4303–11. PubMed PMID: 17846227.
144. Kaufman JL, RN, Stadtmauer EA, Chanan-Khan A, Siegel D, Horne H, Teoh N, Wegener WA, Goldenberg DM, editors. Dose-escalation trial of milatuzumab (humanized anti-CD74 monoclonal antibody) in multiple myeloma. ASCO annual meeting; 2009; Orlando: Journal of Clinical Oncology. 2009;27:8593.
145. Tai YT, Podar K, Gupta D, Lin B, Young G, Akiyama M, et al. CD40 activation induces p53-dependent vascular endothelial growth factor secretion in human multiple myeloma cells. Blood. 2002;99(4):1419–27. PubMed PMID: 11830495.
146. Tai YT, Podar K, Mitsiades N, Lin B, Mitsiades C, Gupta D, et al. CD40 induces human multiple myeloma cell migration via phosphatidylinositol 3-kinase/AKT/NF-kappa B signaling. Blood. 2003;101(7):2762–9. PubMed PMID: 12433678.
147. Urashima M, Chauhan D, Uchiyama H, Freeman GJ, Anderson KC. CD40 ligand triggered interleukin-6 secretion in multiple myeloma. Blood. 1995;85(7):1903–12. PubMed PMID: 7535594.
148. Gupta D, Treon SP, Shima Y, Hideshima T, Podar K, Tai YT, et al. Adherence of multiple myeloma cells to bone marrow stromal cells upregulates vascular endothelial growth factor secretion: therapeutic applications. Leukemia. 2001;15(12):1950–61. PubMed PMID: 11753617.
149. Hussein M, Berenson JR, Niesvizky R, Munshi N, Matous J, Sobecks R, et al. A phase I multidose study of dacetuzumab (SGN-40; humanized anti-CD40 monoclonal antibody) in patients with multiple myeloma. Haematologica. 2010;95(5):845–8. PubMed PMID: 20133895, Pubmed Central PMCID: 2864394.
150. Agura E, Niesvizky R, Matous J, Munshi N, Hussein M, Parameswaran RV, Tarantolo S, Whiting NC, Drachman JG, Zonder JA, editors. Dacetuzumab (SGN-40), lenalidomide, and weekly dexamethasone in relapsed or refractory multiple myeloma: multiple responses observed in a phase 1b study. ASH annual meeting; 2009; New Orleans: Blood. 2009;114:2870.
151. Bensinger W, Maziarz RT, Jagannath S, Spencer A, Durrant S, Becker PS, et al. A phase 1 study of lucatumumab, a fully human anti-CD40 antagonist monoclonal antibody administered intravenously to patients with relapsed or refractory multiple myeloma. Br J Haematol. 2012;159(1):58–66. PubMed PMID: 22861192.
152. Dimberg LY, Anderson CK, Camidge R, Behbakht K, Thorburn A, Ford HL. On the TRAIL to successful cancer therapy? Predicting and counteracting resistance against TRAIL-based therapeutics. Oncogene. 2013;32(11):1341–50. PubMed PMID: 22580613.
153. Belch A, Sharma A, Spencer A, Tarantolo A, Bahlis NJ, Doval D, Gallant G, Kumm E, Klein J, Chanan-Khan AA, editors. A multicenter randomized phase II trial of Mapatumumab, a TRAIL-R1 agonist monoclonal antibody, in combination with bortezomib in patients with relapsed/refractory multiple myeloma (MM). ASH annual meeting; 2010; Orlando: Blood. 2010;116:5031.
154. Campbell KS, Purdy AK. Structure/function of human killer cell immunoglobulin-like receptors:

lessons from polymorphisms, evolution, crystal structures and mutations. Immunology. 2011;132(3):315–25. PubMed PMID: 21214544, Pubmed Central PMCID: 3044898.

155. Benson DM Jr., Cohen AD, Munshi NC, Jagannath S, Spitzer G, Hofmeister CC, Zerbib R, Andre P, Efebera YA, Oxier S, Caligiuri MA, editors. A phase I trial of the anti-inhibitory KIR antibody, IPH2101, and lenalidomide in multiple myeloma: interim results. ASH annual meeting; 2012; Atlanta: Blood. 2012;120:4058.
156. Moreaux J, Hose D, Reme T, Jourdan E, Hundemer M, Legouffe E, et al. CD200 is a new prognostic factor in multiple myeloma. Blood. 2006;108(13):4194–7. PubMed PMID: 16946299.
157. Mahadevan D, Lanasa MC, Whelden M, Faas SJ, Ulery TL, Kukreja A, Li L, MS, Bedrosian CL, Heffner LT, editor. First-in-human phase I dose escalation study of a humanized anti-CD200 antibody (Samalizumab) in patients with advanced stage B cell chronic lymphocytic leukemia (B-CLL) or multiple myeloma (MM). ASH annual meeting; 2010; Orlando: Blood. 2010;116:2465.
158. Hofbauer LC, Neubauer A, Heufelder AE. Receptor activator of nuclear factor-kappaB ligand and osteoprotegerin: potential implications for the pathogenesis and treatment of malignant bone diseases. Cancer. 2001;92(3):460–70. PubMed PMID: 11505389.
159. Vij R, Horvath N, Spencer A, Taylor K, Vadhan-Raj S, Vescio R, et al. An open-label, phase 2 trial of denosumab in the treatment of relapsed or plateau-phase multiple myeloma. Am J Hematol. 2009;84(10):650–6. PubMed PMID: 19714603.
160. Henry DH, Costa L, Goldwasser F, Hirsh V, Hungria V, Prausova J, et al. Randomized, double-blind study of denosumab versus zoledronic acid in the treatment of bone metastases in patients with advanced cancer (excluding breast and prostate cancer) or multiple myeloma. J Clin Oncol. 2011;29(9):1125–32. PubMed PMID: 21343556.
161. Qiang YW, Barlogie B, Rudikoff S, Shaughnessy Jr JD. Dkk1-induced inhibition of Wnt signaling in osteoblast differentiation is an underlying mechanism of bone loss in multiple myeloma. Bone. 2008;42(4):669–80. PubMed PMID: 18294945.
162. Tian E, Zhan F, Walker R, Rasmussen E, Ma Y, Barlogie B, et al. The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. N Engl J Med. 2003;349(26):2483–94. PubMed PMID: 14695408.
163. Padmanabhan S, Beck JT, Kelly KR, Munshi NC, Dzik-Jurasz A, Gangolli E, Ettenberg S, Miner K, Bilic S, Whyte W, Mehdi F, Lu-May C, Rae PL, Spencer A, Shah J, Anderson KC, Giles FJ, Stewart AK, editors. A phase I/II study of BHQ880, a novel osteoblast activating, anti-DKK1 human monoclonal antibody, in relapsed and refractory multiple myeloma (MM) patients treated with zoledronic acid (Zol) and anti-myeloma therapy (MM Tx). ASH annual meeting; 2009; New Orleans: Blood. 2009;114:750.
164. Munshi NC, Abonour R, Beck JT MD, Bensinger W, Facon T, Stockerl-Goldstein K, Baz R, Siegel DS, Neben K, Lonial S, Suvannasankha A, Bilic S, Chica S, Mukhopadhyay S, Isaacs R, Jagannath S, editors. Early evidence of anabolic bone activity of BHQ880, a fully human anti-DKK1 neutralizing antibody: results of a phase 2 study in previously untreated patients with smoldering multiple myeloma at risk for progression. ASH annual meeting; 2012; Atlanta: Blood. 2012;120:331.

Immunopathology and Immunotherapy of Myeloid Leukemia

5

Sylvia Snauwaert, Bart Vandekerckhove, and Tessa Kerre

Contents

S. Snauwaert, MD, PhD
B. Vandekerckhove, MD, PhD
Department of Clinical Chemistry, Microbiology and Immunology, Ghent University, Ghent University Hospital, De Pintelaan 185, Ghent 9000, Belgium
e-mail: sylvia.snauwaert@ugent.be; bart.vandekerckhove@ugent.be

T. Kerre, MD, PhD (✉)
Department of Clinical Chemistry, Microbiology and Immunology, Ghent University, Ghent University Hospital, De Pintelaan 185, Ghent 9000, Belgium

Department of Hematology – Immunology, Ghent University Hospital, 9K12IE – Dienst Hematologie, Gent 9000, Belgium
e-mail: tessa.kerre@ugent.be

5.1 Introduction

Acute myeloid leukemia (AML) is the most common myeloid leukemia, with a median prevalence of 3–8 cases per 100,000. The median age at presentation is about 70 years, and men are more affected than women (ratio 3:2). Risk factors for acquiring AML include exposure to ionizing radiation, benzene, and cytotoxic chemotherapy [1]. AML is a heterogeneous clonal disorder of hematopoietic progenitor cells ("malignant blasts"), characterized by maturation arrest, uncontrolled proliferation, and resistance to apoptosis. Untreated, the disease is fatal within weeks to months, because of fatal infection, bleeding, or organ infiltration [1].

5.2 Immunopathology of Acute Myeloid Leukemia

It is now generally accepted that AML originates from genetic alterations in normal hematopoietic stem cells (HSC) or common myeloid progenitor cells (CMP), giving rise to the leukemic stem cell (LSC), from which the bulk of leukemic blasts arise, ultimately leading to the clinical presentation of AML.

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_5,

5.2.1 Causes of Genetic Alterations

5.2.1.1 Primary AML

Depending on the absence or presence of a pre-existing condition or therapy, we can discriminate between primary and secondary AML, respectively. According to the most recent, revised World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia, published in 2008 [2], primary acute myeloid leukemia (AML) can be classified, depending on the presence (acute myeloid leukemia with recurrent genetic abnormalities) or absence of known genetic alterations (acute myeloid leukemia, not otherwise specified), showing the importance of genetic abnormalities in AML: these can be of prognostic importance and can have therapeutic implications but, above all, are a major pathogenetic mechanism for AML.

In primary AML, the causes for the alterations are largely unknown, and the result of errors that appear during mitosis is considered as "bad luck." However, rare cases of familial hematological malignancies have been described [3, 4]. In this case, a probable genetic predisposition exists for multiple primary cancers. Mutations in genes as RUNX1 and CEBPA have been identified in these families [5]. This mechanism is also illustrated by the higher incidence of AML and other cancers in patients with specific genetic disorders such as Down syndrome [6] and Fanconi anemia [7].

5.2.1.2 Secondary AML

AML is considered secondary, if it evolves from a pre-existing myelodysplasia (MDS) or if the patient received chemotherapy and/or radiotherapy for an unrelated disease.

Acute Myeloid Leukemia with Myelodysplasia-Related Changes

In the case of MDS-AML, MDS is seen as a pre-malignant condition. It was shown by exome and whole genome sequencing that AML and MDS overall share only few common mutated genes, but still this number is higher than expected to occur by chance, suggesting that a fraction of recurrent mutations are involved in both AML and MDS [8].

Therapy-Related Myeloid Neoplasms (AML)

Risk factors for therapy-related AML are the type of chemotherapy (esp anthracyclines and epipodophyllotoxins), as well as host factors, such as specific polymorphisms of detoxification enzymes and of DNA repair genes (reviewed in [9]). Metabolomic analysis of samples before auto-HSCT for another disease showed that development of MDS/AML after the auto-HSCT was associated with dysfunctions in cellular metabolic pathways [10].

5.2.2 Genes Affected in AML

With regard to which genes are inducing AML, numerous studies have been done in mice with forced expression of oncogenes in normal bone marrow, resulting in the development of AML [11], but studies in human are more scarce [12]. Two recent reviews have nicely summarized the current knowledge of the translocations and/or mutations involved in AML and their interrelationship [13], the importance of next generation sequencing in the future management of AML [14], and the heterogeneous nature and complexity of the disease.

5.2.3 Models for Leukemogenesis Through Gene Alterations

AML can be the result of normal HSC acquiring a sequence of mutations, as evidenced by the shared $CD34^{+}CD38^{-}$ phenotype of both HSC and LSC [15–18], cytogenetic abnormalities in a proportion of the $CD34^{+}CD38^{-}$ cells of AML patients [19, 20], and the heterogeneity in LSC self-renewal potential and longevity [18]. Alternatively, more committed progenitor cells can undergo transforming events, partially reprogramming these cells, resulting in the reacquisition of stem cell characteristics such as self-renewal [17], supported by the heterogeneity within the LSC phenotype [21] and the fact that transduction of strictly defined populations of long-term repopulating HSC, short-term repopulating HSC, and lineage-committed CMP with a construct encoding

the leukemia-associated mixed lineage leukemia (MLL) protein resulted in a malignant phenotype from each of these subpopulations [22].

Based on the studies mentioned under Sect. 5.2.2, the classical two-hit leukemogenesis model was proposed (Fig. 5.1). In this model, it was suggested that AML blasts develop from normal blasts (affected by two types of genetic damage). The first (class 1) hit results in constitutive activation of cell-surface receptors or receptor tyrosine kinases, which leads to survival or proliferative advantage through various downstream pathways. Typical class 1 mutations affect RAS, FLT3, and KIT. However, in mouse models, it was shown that abnormalities in these genes are not sufficient to produce a typical AML, but rather result in a myeloproliferative disorder [23, 24]. The second hit (class 2) blocks myeloid differentiation, as is exemplified by the overexpression of homeobox (HOX) genes or by formation of fusion genes resulting from the translocation t(8;21) or inversion inv(16). Both the AML1-ETO and CBFβ-MYH11 fusion genes created by t(8;21) and inv(16), respectively, result in alterations of the AML1-CBFβ (core-binding factor β), a transcription factor which regulates a number of hematopoiesis-specific genes and is essential for normal development of the hematopoietic system [25–27]. Similarly to class 1 hits, these abnormalities alone do not cause leukemia in mouse models [28, 29]. Beside the knowledge from murine AML models, the observation in human AML that class 1 and class 2 lesions occur together more often than do two class 1 or two class 2 hits [23, 24, 28, 29] is in further support for this model. However, this "minimal two-hit"

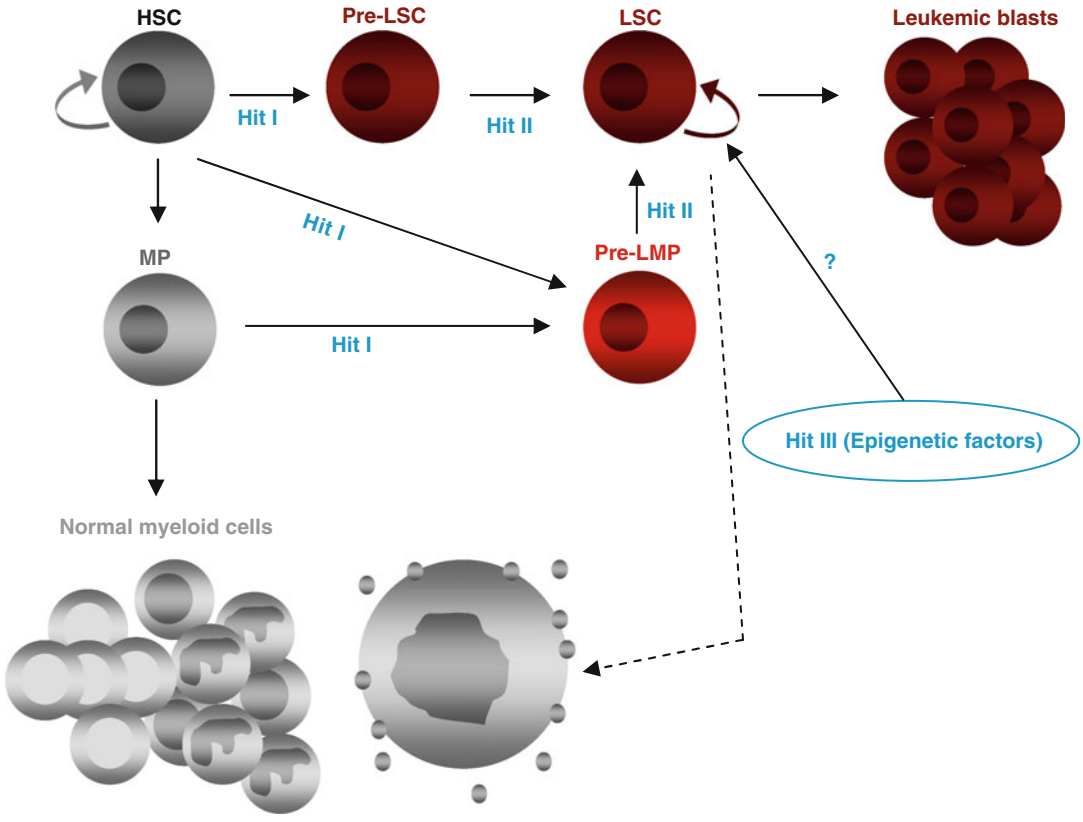


Fig. 5.1 Models of pathogenesis of AML. Three possible scenarios for the development of the LSC are shown. Hematopoietic stem cells (HSC) or myeloid progenitors (MP) or both populations are potential targets for primary (Hit I) and secondary (Hit II) hits. Usually, one single mutation leads to a preleukemic stem cell (pre-LSC) of myeloid progenitor (Pre-LMP), and a second mutation (Hit II) results in the formation of a leukemic stem cell (LSC) that finally gives rise to the bulk of the leukemic blasts, although the LSC can also – albeit to a lower extent – lead to normal differentiated cells. The role of epigenetic changes (Hit III) has recently become clear, but the exact mechanism has not been fully unraveled

model should be modified to take into account the recent booming knowledge about new mutations and especially the role of epigenetic factors. Epigenetic regulation of gene expression is mediated in part by DNA methylation and posttranslational modifications such as histone modifications to regulate chromatin structure. Beside gene mutations, tumor development also involves epigenetic changes such as hypomethylation of DNA and hypoacetylation of chromatin, as well as gene-specific hypo- or hypermethylation [30, 31], and these are nowadays considered as class 3 hits. In the past 5 years, the first genome-wide epigenetic studies focusing on DNA methylation in AML have been published [32, 33]. To understand the entire picture of molecular pathogenesis in AML, gene rearrangement, gene copy number, DNA methylation, and expression profiles are needed to be analyzed together with gene mutations [34].

The role of miRNAs in the development of AML, as in other cancers, has been shown by several studies comparing miRNA signature between AML and ALL, between AML and normal $CD34^+$ cells, and different AML samples (reviewed in [35]).

5.2.4 The Leukemic Stem Cell

5.2.4.1 Phenotype of the LSC

Most AML cells are unable to proliferate extensively, and only a subset of these cells preserves clonogenic properties, suggesting that, similar to normal hematopoiesis, leukemia may be maintained by a small population of stem cells [15–17, 36, 37]. In 1994, Dick and colleagues identified that AML-initiating cells or LSCs are $CD34^+CD38^-$ (a phenotype that is similar to normal HSC), based on transplantation experiments in SCID and later NOD/SCID mice [15, 16]. In addition, serial transplantation experiments performed by Shultz et al. in xenotransplant-permissive NOD/SCID/IL2R$\gamma^{-/-}$ (NSG) mice demonstrated that long-term engraftment and the self-renewal capacity of human AML cells resided exclusively in the $CD34^+CD38^-$ population [38]. LSCs were shown to be mainly in the G_0 phase of the cell cycle, confirming their quiescent nature [38, 39].

Despite these studies, controversy about the immunophenotype of the LSC arose (Table 5.1) [40]: it is now clear that although the LSC is contained within the $CD34^+CD38^-$ population in most patients, some exceptions exist where LSC can (also) be found in the $CD34^+CD38^+$ population [15, 16, 21] or the CD34low/$^-$ fraction (K, I) [21]. Moreover, identifying a more refined immunophenotype discriminating LSC from normal HSC would enable clinicians to better evaluate MRD after therapy and design LSC-targeted therapies. Some surface markers associated with LSC are C-type lectin-like 1(CLL-1/MICL/CLEC12A), CD123, CD44, CD47, CD96, and CD25 [41–47], but still a unique phenotype has not been established thus far.

Table 5.1 Adapted by permission from Nature Reviews Cancer (Copyright 2004. Bleakley and Riddell [62] and Immunotherapy. Copyright 2013 [108])

Antigen	Examples	Advantages	Disadvantage(s)
LSA	> mutations: Flt3, NPM1	Specificity	Expression restricted to defined AML subgroups → use limited to small patient populations
	> translocations: AML1-ETO, DEK-CAN, PML-RARα	Oncogenicity	
		LSC expression	
LAA	WT1, AurAkinase, Bcl2, Muc1, SSX21P	LSC expression	Low avidity of T cells for AG
		Broad applicability (AML and other types of cancer)	Potential toxicity to normal tissues
MIHA	HA-1, HA-2 (hematopoietic specific)	High-avidity T cells available	Use limited to allo-HSCT
		AG recognized by both $CD4^+$ and $CD8^+$ T cells	Limited number of AG defined
		Potential multivalent response	Potential cause of GVHD

Advantages and disadvantages of immunotherapy for acute myeloid leukemia, depending on the antigen targeted: *LSA* leukemia-specific antigen, *LAA* leukemia-associated antigen, *MIHA* minor histocompatibility antigen [57, 58]

5.2.4.2 Clinical Relevance of the LSC

If LSC, as defined in mouse models, were also relevant for AML patients, they may constitute the main targets for consolidation therapy against MRD [48]. In 2005, Van Rhenen et al. demonstrated that a high frequency of $CD34^+CD38^-$ LSCs at AML diagnosis predicts high frequencies of MRD after chemotherapy and poor overall, disease-free and relapse-free survival, both in an *in vivo* model and in correlation studies in patients [49]. Another study reported that the relative ability of AML cells to successfully engraft in immunodeficient mice (a property associated with LSCs) correlates with adverse clinical features [50]. Recently, two groups have independently demonstrated that HSC- and LSC-enriched populations share very similar transcriptional "stem cell-like" or "self-renewal" gene expression signatures that reflect stem cell function *in vivo* [51] and that are predictive of adverse clinical outcome in individuals with AML [51, 52]. The predictive value of this LSC score appeared to be independent of other risk factors in multivariate Cox regression analysis, which further supports the clinical relevance of LSC [51, 52].

5.2.5 How Do Gene Alterations in the LSC Lead to the Clinical Presentation of AML?

Genetic alterations lead to differentiation block and hyperproliferation, which further enhance the risk of genetic damage. This leads to a number of effects that are additive and ultimately lead to the clinical effects of this life-threatening disease:

- Clonal outgrowth and uncontrolled, limitless expansion, which is achieved by mutations that lead to constitutive activation of pathways involved in cell cycle, e.g., by activating mutations, overexpression of proto-oncogenes, and abrogation on restriction points [53].
- Inefficient maturation from the malignant blasts and also mature cells from the residual normal stem cells, caused by a maturation arrest (due to mutation), by cytokines that are produced by the malignant blasts and inhibit the normal differentiation, and – to a lesser extent – by the crowding effect
- Constitutive release of chemokines by the malignant blasts (that also express several chemokine receptors), which interact with other cytokines (esp hematopoietic growth factors and angioregulatory factors), and matrix metalloproteinases (MMP) system, also released by the AML cells [54]
- Expression of P-glycoprotein (Pgp, MDR1, ABCB1), plasma membrane transporters able to efflux a variety of substrates from the cytoplasm, including chemotherapeutic agents, leading to the development of resistance to chemotherapy [55].
- Resistance to apoptosis and defective or proficient DNA damage response [56]

5.3 Immunotherapy for AML

Despite the progress that has been made in the past decades, AML still remains a therapeutic challenge. A significant percentage of patients, especially the elderly, have primary induction failure, and even if chemotherapy is successful at inducing remission of AML, the probability of relapse is high [56]. Immunotherapy for AML was first put forward almost 40 years ago: the hypothesis that AML blasts were distinct from normal blasts led to preliminary attempts to improve immune responsiveness to AML by the administration of inactivated autologous AML blasts with BCG [57]. The most important insights in the role of the immune system in controlling AML, however, came from allogeneic HSCT and the observed graft-versus-leukemia (GVL) effect. Increasing evidence exists that the success of allo-HSCT in curing AML can be largely attributed to this GVL effect, especially in the context of non-myeloablative HSCT [58–62]. Both donor NK cells and donor T cells contribute to the suppression and elimination of leukemic cells [62]. Although allo-HSCT remains the most successful post-remission therapy in AML, it has its price of important morbidity and mortality, caused by infections, toxicity of the conditioning,

and acute and chronic graft-versus-host disease (GVHD) [58, 59]. These important complications limit its applicability in patients of older age and with comorbidities. Therefore, research groups investigated more targeted forms of immunotherapy that are more specific, do not require conditioning, and have less side effects. The biggest challenge here lies in identifying the ideal tumor antigen, present on LSCs, but low to absent on normal hematopoietic cells and other vital tissues.

5.3.1 Antigens to Target in AML

5.3.1.1 Antigens Presented by MHC After Internal Processing

Major histocompatibility complex (MHC)-presented antigens are targets for T cell-mediated immunotherapy, such as vaccination and adoptive T cell therapy. Three types of MHC-presented antigens can be described in AML: leukemia-specific antigens (LSA), leukemia-associated antigens (LAA), and minor histocompatibility antigens (MIHA). LSA arise from mutations or translocations, which lead to the formation of new antigens specific for the AML cells. LAA are expressed both on normal and leukemic cells, but they can be good targets if they are overexpressed on leukemic cells (including LSC) and/or their physiological expression is restricted to certain developmental stages (embryologic) [62–65]. Hematopoietic-specific MIHA differing between donor and recipient are interesting targets for AML in the context of allo-HSCT [61, 62, 66–70]. A ranking of the most promising cancer antigens is reviewed by Cheever et al. [67]. In addition, the authors and other groups have published an overview of AML antigens more recently [40, 71, 72]. Some examples, the advantages and disadvantages of these antigen types are summarized in Table 5.1.

5.3.1.2 Surface Antigens

Surface antigens are targets for monoclonal antibodies (mAbs) and chimeric antigen receptor-modified T cells (CAR T cells). Surface antigens on AML that are being targeted by mAbs are CD33, Flt3L, and those overexpressed on LSCs (CLL1, CD44, CD47, IL-3R (CD123)) (reviewed in [73]). These antigens have the disadvantage of being also expressed on normal tissues, resulting in important side effects, as seen with the anti-CD33 antibodies [73, 74] (See Sect. 5.3.2.2.1).

5.3.2 Current Immunotherapeutic Strategies for AML

Active immunotherapy (e.g., modified leukemic cells, peptide, DNA, or dendritic cell-based vaccinations) requires a patient with an intact immune system and can only exploit the available T cell receptor (TCR) of the patient. However, high-affinity TCR-bearing T cells specific to self-antigens (TAA) are expected to be deleted after negative selection in the thymus. In addition, the question is whether active immunotherapy will be able to combat the abundant negative influences of the host immune system and tumor microenvironment. Passive immunotherapeutic strategies (e.g., adoptive transfer of AML-specific T cells or NK cells) are expected to be more potent therapies to target LAA and MIHA. Also mAbs are considered passive immunotherapy and have proven efficacy in AML.

5.3.2.1 Active Immunotherapeutic Strategies

Peptide Vaccination

Known immunodominant and HLA-A2 (being highly prevalent among Caucasians)-binding nonamer peptide epitopes of WT1 and proteinase 3 (PR1) are most widely researched and developed as peptide vaccines for AML in clinical trials. Receptor for hyaluronan-mediated motility (RHAMM) has also been targeted in vaccine trials [75–77]. Vaccines have been combined with adjuvants such as montanide or keyhole limpet hemocyanin (KLH), with or without concurrently administered granulocyte-macrophage colony-stimulating factor (GM-CSF). As these studies comprise small and diverse groups of patients treated with different vaccines and schedules, it is difficult to draw meaningful conclusions about the true efficacy of peptide

vaccination in AML. Yet, immune responses were significantly associated with clinical response. Clinical responses ranged from reduction in marrow blasts to complete remissions in a low percentage of patients The potency of peptide vaccines may potentially be increased by genetically modifying peptides to enhance TCR affinity or by the use of synthetic long peptides (SLP) (instead of exact MHC-binding nonamer peptides), which deliver antigens in a more efficient and stable way to the patient's antigen presenting cells (APCs) [78, 79]. In addition, Toll-like receptor (TLR) ligand-peptide conjugates constitute an attractive vaccination modality, sharing the peptide antigen and a defined adjuvant in one single molecule [79, 80].

Dendritic Cell Vaccination

In order to circumvent the limitations inherent to peptide vaccines [81], researchers have intensely studied the role of antigen-loaded dendritic cells (DCs) as professional APCs which are able to prime naïve T cells [82]. Furthermore, the synthetic peptide approach which may miss immunodominant epitopes is replaced by the addition of whole protein or mRNA transfection [81]. Most of the strategies use DCs which are derived from monocytes, and only those strategies used in clinical trials are mentioned here:

- DCs pulsed with leukemic cell lysates [83], apoptotic leukemic cells [84], or modified WT1 peptides [84] have been successfully explored in small clinical trials.
- Even more promising are mRNA-electroporated DCs. In 2010, a phase I/II clinical trial (clinicaltrials.gov ID: NCT00834002) investigated the effect of vaccination with full-length WT1 mRNA-electroporated autologous dendritic cells in ten patients with AML, and in five of them, a molecular remission was reached, although not always persisting [85].
- In an attempt to generate WT1-presenting DCs with a longer *in vivo* persistence, Stripecke and colleagues recently developed a tricistronic lentiviral vector co-expressing a truncated form of WT1, granulocyte-macrophage colony-stimulating factor (GM-CSF), and interleukin-4 (IL-4), which was used for the transduction of human monocytes, leading to very rapid self-differentiation of these cells into "SmartDC/tWT1" that showed very promising potential for the use as immunotherapy against WT1-expressing tumors [86].

5.3.2.2 Passive Immunotherapeutic Strategies

Monoclonal Antibodies

Monoclonal antibodies given their antigen specificity and minimal toxicity may be an excellent AML- and even LSC-targeting therapy. This immunotherapeutic strategy functions through several mechanisms: Antibody-dependent cell-mediated cytotoxicity (ADCC), complement activation, a direct proapoptotic effect, and upon the inhibition of signal transduction cascades that are essential for homeostasis, proliferation, or interaction with the microenvironment [87]. In case mAbs are conjugated to radioisotopes or toxins, they can directly kill the recognized target. Anti-CD33 (present on 90 % of AML cells) mAbs are the most widely studied and have proven both clinical efficacy and important toxicity [72, 74, 88]. Current promising trials combine anti-CD33 mAbs in more fractionated (less toxic) administration with chemotherapy [89, 90]. Also radioisotope-coupled anti-CD45 antibodies have been used as part of the conditioning before allo-HSCT [91]. Alternative mAb tools include antibodies that block the immune-regulatory effect of molecules, such as cytotoxic T lymphocyte antigen-4 (CTL4) or programmed cell death-1 (PD-1), and thereby unleash cytotoxic T lymphocyte function [92–97].

Adoptive T Cell Transfer

The clinical results obtained with unmanipulated DLIs, a variety of T cell types/sources and *ex vivo* manipulations of T cells, point to a strong AML-directed therapeutic effect as well as a GVHD potential. Various research has been done to direct T cells more specifically toward the AML cells. In AML patients, autologous- or donor-derived antigen-specific T cells can be isolated from peripheral blood by pMHC-multimer staining, CD137- or CD154-based assays, cytokine (IFNγ)-secretion

techniques, or repeated *ex vivo* stimulation with antigen and subsequent expansion, but all of these techniques still require the availability of pre-existing high-affinity antigen-specific T cells in the patient or the donor (in the context of allo-HSCT) [98–100]. For LAA, these are usually absent due to negative selection in the thymus. In order to confine LAA-specificity to T cells, peripheral blood mononuclear cells (PBMC) can be transduced with a high-affinity TCR recognizing LAAs present on AML blasts, including LSCs, thereby circumventing the issue of tolerance. Such a high-affinity TCR can be isolated from LAA-specific T cells generated *in vivo* or *in vitro* in an autologous setting (e.g., from TILs of a patient with complete clinical responses after ACT) or, even more ideally, in an allogeneic MHC-mismatched setting [101–103]. Moreover, the availability of TCR genes specific for MIHA, such as HA-2, would increase the applicability of MIHA-directed immunotherapy, illustrated by the fact that 95 % of the population expresses the antigenic HA-2^v allele, and therefore, naturally mismatched recipient-donor pairs are infrequent [104].

A new promising immunotherapeutic strategy, using PBMC transduced with a CAR, a construct that encodes the VH and VL domain of a tumor antigen-specific antibody coupled to the CD3ζ chain (alone or combined with the signaling motifs of CD28 or CD137 to enhance the signal) of a TCR [105], has not yet been evaluated in clinical trials of AML.

Adoptive NK Cell Transfer

NK cells have an important antileukemic effect, and their role in other immunotherapeutic strategies has been established, e.g., in WT1-DC vaccination [85, 87] and KIR-mismatched haploidentical HSCT [106, 107]. Especially in the context of haploidentical HSCT, NK cell adoptive therapy is currently being explored in clinical trials (NCT 00799799, NTR 2818).

5.4 Concluding Remarks

This chapter reviewed both the immunopathology of AML and currently explored immunotherapeutic strategies targeting AML. The genetic alterations leading to the differentiation block and hyperproliferation which result in AML were discussed. The potential clinical relevance of the LSC concept in the pathogenesis of AML was emphasized. LSC might be one of the reasons why AML still remains a tremendous therapeutic challenge nowadays. New therapeutic strategies, including immunotherapy, are awaited. Various immunotherapeutic strategies and the possible target antigens were listed.

References

1. Estey E, Dohner H. Acute myeloid leukaemia. Lancet. 2006;368(9550):1894–907.
2. Swerdllow S, Campo E, Harris NL. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press; 2008.
3. Purtilo D, Paquin L, Gindhart T. Genetics of neoplasia–impact of ecogenetics on oncogenesis. A Rev Am J Pathol. 1978;91(3):609.
4. Kralovics R. Genetic complexity of myeloproliferative neoplasms. Leukemia. 2008;22(10):1841–8.
5. El Abed RBV, Huiart L, et al. Molecular study of CEBPA in familial hematological malignancies. Fam Cancer. 2009;8(4):581–4.
6. Hasle H. Pattern of malignant disorders in individuals with Down's syndrome. Lancet Oncol. 2001;2(7):429–36.
7. Mathew C. Fanconi anaemia genes and susceptibility to cancer. Oncogene. 2006;25(43):5875–84.
8. Graubert TA, Shen D, Ding L, Okeyo-Owuor T, Lunn CL, Shao J, et al. Recurrent mutations in the U2AF1 splicing factor in myelodysplastic syndromes. Nat Genet. 2012;44(1):53–7.
9. Hijiya N, Ness KK, Ribeiro RC, Hudson MM. Acute leukemia as a secondary malignancy in children and adolescents: current findings and issues. Cancer. 2009;115(1):23–35.
10. Cano KE, Li L, Bhatia S, Bhatia R, Forman SJ, Chen Y. NMR-based metabolomic analysis of the molecular pathogenesis of therapy-related myelodysplasia/acute myeloid leukemia. J Prot Res. 2011;10(6):2873–81.
11. McCormack E, Bruserud O, Gjertsen B. Review: genetic models of acute myeloid leukaemia. Oncogene. 2008;27(27):3765–79.
12. Abdul-Nabi AM, Yassin ER, Varghese N, Deshmukh H, Yaseen NR. In vitro transformation of primary human CD34+ cells by AML fusion oncogenes: early gene expression profiling reveals possible drug target in AML. PLoS One. 2010;5(8):e12464.
13. Sanders MA, Valk PJ. The evolving molecular genetic landscape in acute myeloid leukaemia. Curr Opin Hem. 2013;20(2):79–85.

14. Riva L, Luzi L, Pelicci PG. Genomics of acute myeloid leukemia: the next generation. Front Oncol. 2012;2:40.
15. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature. 1994;367(6464):645–8.
16. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med. 1997;3(7):730–7.
17. Passegue E, Jamieson CH, Ailles LE, Weissman IL. Normal and leukemic hematopoiesis: are leukemias a stem cell disorder or a reacquisition of stem cell characteristics? Proc Natl Acad Sci U S A. 2003;100 Suppl 1:11842–9.
18. Hope KJ, Jin L, Dick JE. Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity. Nat Immunol. 2004;5(7):738–43.
19. Haase D, Feuring-Buske M, Konemann S, Fonatsch C, Troff C, Verbeek W, et al. Evidence for malignant transformation in acute myeloid leukemia at the level of early hematopoietic stem cells by cytogenetic analysis of CD34+ subpopulations. Blood. 1995;86(8):2906–12.
20. Mehrotra B, George TI, Kavanau K, Avet-Loiseau H, Moore 2nd D, Willman CL, et al. Cytogenetically aberrant cells in the stem cell compartment (CD34+lin-) in acute myeloid leukemia. Blood. 1995;86(3):1139–47.
21. Taussig DC, Miraki-Moud F, Anjos-Afonso F, Pearce DJ, Allen K, Ridler C, et al. Anti-CD38 antibody-mediated clearance of human repopulating cells masks the heterogeneity of leukemia-initiating cells. Blood. 2008;112(3):568–75.
22. Cozzio A, Passegue E, Ayton PM, Karsunky H, Cleary ML, Weissman IL. Similar MLL-associated leukemias arising from self-renewing stem cells and short-lived myeloid progenitors. Gen Dev. 2003;17(24):3029–35.
23. Kelly LM, Gilliland DG. Genetics of myeloid leukemias. Annu Rev Genomics Hum Genet. 2002;3:179–98.
24. Frohling S, Scholl C, Gilliland DG, Levine RL. Genetics of myeloid malignancies: pathogenetic and clinical implications. J Clin Oncol. 2005;23(26):6285–95.
25. Okuda T, van Deursen J, Hiebert SW, Grosveld G, Downing JR. AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. Cell. 1996;84(2):321–30.
26. Erickson P, Gao J, Chang KS, Look T, Whisenant E, Raimondi S, et al. Identification of breakpoints in t(8;21) acute myelogenous leukemia and isolation of a fusion transcript, AML1/ETO, with similarity to Drosophila segmentation gene, runt. Blood. 1992;80(7):1825–31.
27. Liu P, Tarle SA, Hajra A, Claxton DF, Marlton P, Freedman M, et al. Fusion between transcription factor CBF beta/PEBP2 beta and a myosin heavy chain in acute myeloid leukemia. Science. 1993;261(5124):1041–4.
28. Downing JR. The core-binding factor leukemias: lessons learned from murine models. Curr Opin Genet Dev. 2003;13(1):48–54.
29. Wang YY, Zhao LJ, Wu CF, Liu P, Shi L, Liang Y, et al. C-KIT mutation cooperates with full-length AML1-ETO to induce acute myeloid leukemia in mice. Proc Natl Acad Sci U S A. 2011;108(6):2450–5.
30. Feinberg AP, Tycko B. The history of cancer epigenetics. Nat Rev Cancer. 2004;4(2):143–53.
31. Dawson MA, Kouzarides T, Huntly BJ. Targeting epigenetic readers in cancer. N Engl J Med. 2012;367(7):647–57.
32. Akalin A, Garrett-Bakelman FE, Kormaksson M, Busuttil J, Zhang L, Khrebtukova I, et al. Base-pair resolution DNA methylation sequencing reveals profoundly divergent epigenetic landscapes in acute myeloid leukemia. PLoS Genet. 2012;8(6):e1002781.
33. Figueroa ME, Lugthart S, Li Y, Erpelinck-Verschueren C, Deng X, Christos PJ, et al. DNA methylation signatures identify biologically distinct subtypes in acute myeloid leukemia. Cancer Cell. 2010;17(1):13–27.
34. Naoe T, Kiyoi H. Gene mutations of acute myeloid leukemia in the genome era. Int J Hematol. 2013;97(2):165–74.
35. Yuan Y, Kasar S, Underbayev C, Prakash S, Raveche E. MicroRNAs in acute myeloid leukemia and other blood disorders. Leuk Res Treatment. 2012;2012.
36. Ailles LE, Gerhard B, Kawagoe H, Hogge DE. Growth characteristics of acute myelogenous leukemia progenitors that initiate malignant hematopoiesis in nonobese diabetic/severe combined immunodeficient mice. Blood. 1999;94(5):1761–72.
37. Sabbath KD, Ball ED, Larcom P, Davis RB, Griffin JD. Heterogeneity of clonogenic cells in acute myeloblastic leukemia. J Clin Invest. 1985;75(2):746–53.
38. Ishikawa F, Yoshida S, Saito Y, Hijikata A, Kitamura H, Tanaka S, et al. Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. Nat Biotechnol. 2007;25(11):1315–21.
39. Guan Y, Gerhard B, Hogge DE. Detection, isolation, and stimulation of quiescent primitive leukemic progenitor cells from patients with acute myeloid leukemia (AML). Blood. 2003;101(8):3142–9.
40. Snauwaert S, Vandekerckhove B, Kerre T. Can immunotherapy specifically target acute myeloid leukemic stem cells? Oncoimmunology. 2013;2(2):e22943.
41. Jin L, Lee EM, Ramshaw HS, Busfield SJ, Peoppl AG, Wilkinson L, et al. Monoclonal antibody-mediated targeting of CD123, IL-3 receptor alpha chain, eliminates human acute myeloid leukemic stem cells. Cell Stem Cell. 2009;5(1):31–42.
42. Jin L, Hope KJ, Zhai Q, Smadja-Joffe F, Dick JE. Targeting of CD44 eradicates human acute myeloid leukemic stem cells. Nat Med. 2006;12(10):1167–74.
43. van Rhenen A, Moshaver B, Kelder A, Feller N, Nieuwint AW, Zweegman S, et al. Aberrant marker expression patterns on the CD34+CD38- stem cell compartment in acute myeloid leukemia allows to distinguish the malignant from the normal stem cell

compartment both at diagnosis and in remission. Leukemia. 2007;21(8):1700–7.
44. Saito Y, Kitamura H, Hijikata A, Tomizawa-Murasawa M, Tanaka S, Takagi S, et al. Identification of therapeutic targets for quiescent, chemotherapy-resistant human leukemia stem cells. Sci Transl Med. 2010;2(17):17ra9.
45. van Rhenen A, van Dongen GA, Kelder A, Rombouts EJ, Feller N, Moshaver B, et al. The novel AML stem cell associated antigen CLL-1 aids in discrimination between normal and leukemic stem cells. Blood. 2007;110(7):2659–66.
46. Majeti R, Chao MP, Alizadeh AA, Pang WW, Jaiswal S, Gibbs Jr KD, et al. CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. Cell. 2009;138(2):286–99.
47. Hosen N, Park CY, Tatsumi N, Oji Y, Sugiyama H, Gramatzki M, et al. CD96 is a leukemic stem cell-specific marker in human acute myeloid leukemia. Proc Natl Acad Sci U S A. 2007;104(26):11008–13.
48. Greaves M. Cancer stem cells renew their impact. Nat Med. 2011;17(9):1046–8.
49. van Rhenen A, Feller N, Kelder A, Westra AH, Rombouts E, Zweegman S, et al. High stem cell frequency in acute myeloid leukemia at diagnosis predicts high minimal residual disease and poor survival. Clin Cancer Res. 2005;11(18):6520–7.
50. Pearce DJ, Taussig D, Zibara K, Smith LL, Ridler CM, Preudhomme C, et al. AML engraftment in the NOD/SCID assay reflects the outcome of AML: implications for our understanding of the heterogeneity of AML. Blood. 2006;107(3):1166–73.
51. Eppert K, Takenaka K, Lechman ER, Waldron L, Nilsson B, van Galen P, et al. Stem cell gene expression programs influence clinical outcome in human leukemia. Nat Med. 2011;17(9):1086–93.
52. Gentles AJ, Plevritis SK, Majeti R, Alizadeh AA. Association of a leukemic stem cell gene expression signature with clinical outcomes in acute myeloid leukemia. JAMA. 2010;304(24):2706–15.
53. Schnerch D, Yalcintepe J, Schmidts A, Becker H, Follo M, Engelhardt M, et al. Cell cycle control in acute myeloid leukemia. Am J Cancer Res. 2012;2(5):508.
54. Hatfield KJ, Reikvam H, Bruserud O. The crosstalk between the matrix metalloprotease system and the chemokine network in acute myeloid leukemia. Curr Med Chem. 2010;17(36):4448–61.
55. Shaffer BC, Gillet J-P, Patel C, Baer MR, Bates SE, Gottesman MM. Drug resistance: still a daunting challenge to the successful treatment of AML. Drug Resist Updat. 2012;15(1):62–9.
56. Gale R, Foon K. Therapy of acute myelogenous leukemia. Semin Hematol. 1987;24:40–54.
57. Powles RL, Russell J, Lister TA, Oliver T, Whitehouse JM, Malpas J, et al. Immunotherapy for acute myelogenous leukaemia: a controlled clinical study 2 1/2 years after entry of the last patient. Br J Cancer. 1977;35(3):265–72.
58. Weiden PL, Flournoy N, Thomas ED, Prentice R, Fefer A, Buckner CD, et al. Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. N Engl J Med. 1979;300(19):1068–73.
59. Weiden PL, Sullivan KM, Flournoy N, Storb R, Thomas ED. Antileukemic effect of chronic graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. N Engl J Med. 1981;304(25):1529–33.
60. Kolb HJ, Schattenberg A, Goldman JM, Hertenstein B, Jacobsen N, Arcese W, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. Blood. 1995;86(5):2041–50.
61. Baron F, Storb R. Allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning as treatment for hematologic malignancies and inherited blood disorders. Mol Ther. 2006;13(1):26–41.
62. Bleakley M, Riddell SR. Molecules and mechanisms of the graft-versus-leukaemia effect. Nat Rev Cancer. 2004;4(5):371–80.
63. Kapp M, Stevanović S, Fick K, Tan S, Loeffler J, Opitz A, et al. CD8+ T-cell responses to tumor-associated antigens correlate with superior relapse-free survival after allo-SCT. Bone Marrow Transplant. 2009;43(5):399–410.
64. Zhong R-K, Lane TA, Ball ED. Generation of T-cell lines to autologous acute myeloid leukemia cells by competitive limiting dilution culture of acute myeloid leukemia mononuclear cells. Exp Hematol. 2008;36(4):486–94.
65. Ramírez F, Ghani Y, Gao L, Stauss H. Dendritic cell immunization induces nonprotective WT1-specific CTL responses in mouse. J Immunother. 2007;30(2):140–9.
66. Horowitz MM, Gale RP, Sondel PM, Goldman J, Kersey J, Kolb H, et al. Graft-versus-leukemia reactions after bone marrow transplantation. Blood. 1990;75(3):555–62.
67. Falkenburg J, van de Corput L, Marijt EW, Willemze R. Minor histocompatibility antigens in human stem cell transplantation. Exp Hematol. 2003;31(9):743–51.
68. Marijt WE, Heemskerk MH, Kloosterboer FM, Goulmy E, Kester MG, van der Hoorn MA, et al. Hematopoiesis-restricted minor histocompatibility antigens HA-1-or HA-2-specific T cells can induce complete remissions of relapsed leukemia. Proc Natl Acad Sci. 2003;100(5):2742–7.
69. Kloosterboer F, van Luxemburg-Heijs S, Van Soest R, Barbui A, Van Egmond H, Strijbosch M, et al. Direct cloning of leukemia-reactive T cells from patients treated with donor lymphocyte infusion shows a relative dominance of hematopoiesis-restricted minor histocompatibility antigen HA-1 and HA-2 specific T cells. Leukemia. 2004;18(4):798–808.
70. Bonnet D, Warren EH, Greenberg PD, Dick JE, Riddell SR. CD8+ minor histocompatibility antigen-specific cytotoxic T lymphocyte clones eliminate human acute myeloid leukemia stem cells. Proc Natl Acad Sci. 1999;96(15):8639–44.
71. Cheever MA, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT, et al. The prioritization of cancer anti-

gens: a national cancer institute pilot project for the acceleration of translational research. Clin Cancer Res. 2009;15(17):5323–37.
72. Anguille S, Van Tendeloo V, Berneman Z. Leukemia-associated antigens and their relevance to the immunotherapy of acute myeloid leukemia. Leukemia. 2012;26(10):2186–96.
73. Majeti R. Monoclonal antibody therapy directed against human acute myeloid leukemia stem cells. Oncogene. 2010;30(9):1009–19.
74. Petersdorf S, Kopecky K, Stuart RK, Larson RA, Nevill TJ, Stenke L et al., editors. Preliminary results of Southwest Oncology Group Study S0106: an International Intergroup phase 3 randomized trial comparing the addition of Gemtuzumab Ozogamicin to standard induction therapy versus standard induction therapy followed by a second randomization to post-consolidation Gemtuzumab Ozogamicin versus no additional therapy for previously untreated acute myeloid leukemia. ASH annual meeting abstracts. Blood. 2009:114, Abstract 790.
75. Keilholz U, Letsch A, Busse A, Asemissen AM, Bauer S, Blau IW, et al. A clinical and immunologic phase 2 trial of Wilms tumor gene product 1 (WT1) peptide vaccination in patients with AML and MDS. Blood. 2009;113(26):6541–8.
76. Rezvani K, Yong AS, Mielke S, Savani BN, Musse L, Superata J, et al. Leukemia-associated antigen-specific T-cell responses following combined PR1 and WT1 peptide vaccination in patients with myeloid malignancies. Blood. 2008;111(1):236–42.
77. Greiner J, Schmitt A, Giannopoulos K, Rojewski MT, Gotz M, Funk I, et al. High-dose RHAMM-R3 peptide vaccination for patients with acute myeloid leukemia, myelodysplastic syndrome and multiple myeloma. Haematologica. 2010;95(7):1191–7.
78. Heslop HE, Stevenson FK, Molldrem JJ. Immunotherapy of hematologic malignancy. Hematology. 2003:331–49
79. Melief CJ, van der Burg SH. Immunotherapy of established (pre)malignant disease by synthetic long peptide vaccines. Nat Rev Cancer. 2008;8(5):351–60.
80. Khan S, Bijker MS, Weterings JJ, Tanke HJ, Adema GJ, van Hall T, et al. Distinct uptake mechanisms but similar intracellular processing of two different toll-like receptor ligand-peptide conjugates in dendritic cells. J Biol Chem. 2007;282(29):21145–59.
81. Van Driessche A, Gao L, Stauss HJ, Ponsaerts P, Van Bockstaele DR, Berneman ZN, et al. Antigen-specific cellular immunotherapy of leukemia. Leukemia. 2005;19(11):1863–71.
82. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature. 1998;392(6673):245–52.
83. Lee JJ, Kook H, Park MS, Nam JH, Choi BH, Song WH, et al. Immunotherapy using autologous monocyte-derived dendritic cells pulsed with leukemic cell lysates for acute myeloid leukemia relapse after autologous peripheral blood stem cell transplantation. J Clin Apheresis. 2004;19(2):66–70.
84. Kitawaki T, Kadowaki N, Fukunaga K, Kasai Y, Maekawa T, Ohmori K, et al. Cross-priming of $CD8^+$ T cells in vivo by dendritic cells pulsed with autologous apoptotic leukemic cells in immunotherapy for elderly patients with acute myeloid leukemia. Exp Hematol. 2011;39(4):424–33.e2.
85. Van Tendeloo VF, Van de Velde A, Van Driessche A, Cools N, Anguille S, Ladell K, et al. Induction of complete and molecular remissions in acute myeloid leukemia by Wilms' tumor 1 antigen-targeted dendritic cell vaccination. Proc Natl Acad Sci U S A. 2010;107(31):13824–9.
86. Sundarasetty BS, Singh VK, Salguero G, Geffers R, Rickmann M, Macke L, et al. Lentivirus-induced dendritic cells for immunization against high-risk WT1(+) acute myeloid leukemia. Hum Gene Ther. 2013;24(2):220–37.
87. Cheson BD, Leonard JP. Monoclonal antibody therapy for B-cell non-Hodgkin's lymphoma. N Engl J Med. 2008;359(6):613–26.
88. Taksin A, Legrand O, Raffoux E, De Revel T, Thomas X, Contentin N, et al. High efficacy and safety profile of fractionated doses of Mylotarg as induction therapy in patients with relapsed acute myeloblastic leukemia: a prospective study of the alfa group. Leukemia. 2006;21(1):66–71.
89. Taksin A, Legrand O, Raffoux E, De Revel T, Thomas X, Contentin N, et al. High efficacy and safety profile of fractionated doses of Mylotarg as induction therapy in patients with relapsed acute myeloblastic leukemia: a prospective study of the alfa group. Leukemia. 2007;21(1):66–71.
90. Farhat H, Reman O, Raffoux E, Berthon C, Pautas C, Kammoun L, et al. Fractionated doses of gemtuzumab ozogamicin with escalated doses of daunorubicin and cytarabine as first acute myeloid leukemia salvage in patients aged 50–70-year old: a phase 1/2 study of the acute leukemia French association. Am J Hematol. 2012;87(1):62–5.
91. Chen Y, Kornblit B, Hamlin DK, Sale GE, Santos EB, Wilbur DS, et al. Durable donor engraftment after radioimmunotherapy using α-emitter astatine-211–labeled anti-CD45 antibody for conditioning in allogeneic hematopoietic cell transplantation. Blood. 2012;119(5):1130–8.
92. Hodi FS, Mihm MC, Soiffer RJ, Haluska FG, Butler M, Seiden MV, et al. Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. Proc Natl Acad Sci U S A. 2003;100(8):4712–7.
93. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature. 2006;439(7077):682–7.
94. Brahmer JR. PD-1-targeted immunotherapy: recent clinical findings. Clin Adv Hematol Oncol. 2012;10(10):674–5.
95. Lipson EJ, Sharfman WH, Drake CG, Wollner I, Taube JM, Anders RA, et al. Durable cancer regression off-treatment and effective reinduction therapy with an anti-PD-1 antibody. Clin Cancer Res. 2013;19(2):462–8.

96. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366(26):2443–54.
97. Sutmuller RP, van Duivenvoorde LM, van Elsas A, Schumacher TN, Wildenberg ME, Allison JP, et al. Synergism of cytotoxic T lymphocyte-associated antigen 4 blockade and depletion of CD25(+) regulatory T cells in antitumor therapy reveals alternative pathways for suppression of autoreactive cytotoxic T lymphocyte responses. J Exp Med. 2001;194(6):823–32.
98. Bornhauser M, Thiede C, Platzbecker U, Kiani A, Oelschlaegel U, Babatz J, et al. Prophylactic transfer of BCR-ABL-, PR1-, and WT1-reactive donor T cells after T cell-depleted allogeneic hematopoietic cell transplantation in patients with chronic myeloid leukemia. Blood. 2011;117(26):7174–84.
99. Yee C, Thompson JA, Byrd D, Riddell SR, Roche P, Celis E, et al. Adoptive T cell therapy using antigen-specific CD8+ T cell clones for the treatment of patients with metastatic melanoma: in vivo persistence, migration, and antitumor effect of transferred T cells. Proc Natl Acad Sci U S A. 2002;99(25):16168–73.
100. Schmitt TM, Ragnarsson GB, Greenberg PD. T cell receptor gene therapy for cancer. Hum Gene Ther. 2009;20(11):1240–8.
101. Griffioen M, van Egmond HE, Barnby-Porritt H, van der Hoorn MA, Hagedoorn RS, Kester MG, et al. Genetic engineering of virus-specific T cells with T-cell receptors recognizing minor histocompatibility antigens for clinical application. Haematologica. 2008;93(10):1535–43.
102. Sadovnikova E, Stauss HJ. Peptide-specific cytotoxic T lymphocytes restricted by nonself major histocompatibility complex class I molecules: reagents for tumor immunotherapy. Proc Natl Acad Sci U S A. 1996;93(23):13114–8.
103. Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. Science. 2006;314(5796):126–9.
104. Heemskerk MH, Hoogeboom M, Hagedoorn R, Kester MG, Willemze R, Falkenburg JF. Reprogramming of virus-specific T cells into leukemia-reactive T cells using T cell receptor gene transfer. J Exp Med. 2004;199(7):885–94.
105. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor–modified T cells in chronic lymphoid leukemia. N Engl J Med. 2011;365(8):725–33.
106. Lanier LL. NK cell recognition. Annu Rev Immunol. 2005;23:225–74.
107. Venstrom JM, Pittari G, Gooley TA, et al. HLAC-dependent prevention of leukemia relapse by donor activating KIR2DS1. N Engl J Med. 2012;367:805–16.
108. Lichtenegger FS, Schnorfeil FM, Hiddemann W, Subklewe M. Current strategies in immunotherapy for acute myeloid leukemia. Immunotherapy. 2013;5(1):63–78.

Immunopathology and Immunotherapy of Lymphoblastic Leukaemia

6

Thomas Stübig and Nicolaus Kröger

Contents

T. Stübig, MD • N. Kröger, MD (✉)
Department of Stem Cell Transplantation, University Medical Center Hamburg-Eppendorf (UKE), Martinistraße 52, Hamburg 20246, Germany
e-mail: tstuebig@uke.uni-hamburg.de; n.kroeger@uke.de

6.1 Introduction

Acute lymphatic leukaemia (ALL) is a disorder occurring from a lymphoid progenitor cell. Mostly ALL is known as leukaemia occurring in children, but there are a number of adult patients suffering from ALL. While both age groups can be affected by the "same" disease, the outcome is often different. There are plenty of molecular changes that can be found in ALL – however their prognostic impact may vary between both patient groups.

Mostly ALL is classified as leukaemia of the B-cell lineage, which is the case in 85 %; we therefore focused on the B-cell ALL and their biological background and immune therapeutical options.

This chapter will discuss the different pathobiological changes that occur in the development of ALL as well as their implication on the prognosis of the diseases. The second part will focus on the progress that has been made on different immune therapeutical approaches to treat and cure ALL. The therapies range from tyrosine kinase inhibitors, antibodies against different lymphatic antigens to cellular approaches like haematopoietic stem cell transplantation and chimeric antigen receptors (CARs)-transduced T cells. By incorporating the different therapeutic options, the treatment and opportunities have dramatically changed.

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_6,

6.2 Immunopathology of Lymphoblastic Leukaemia

6.2.1 General Considerations

The incidence of acute lymphoblastic leukaemia (ALL) is about 1–4/100.000 persons per year. Most of the cases occur in children below 6 years or in adults aged 80 years and more. Approximately 85 % of all ALL cases are of a B-cell phenotype.

6.2.2 Lymphocyte Development as Biological Basis of Disease

Acute lymphatic leukaemia rises from lymphoid progenitors. In humans $LIN^-/CD34^+/CD38^-$ cells are recognised as a stem and progenitor population in which three different sub-compartments can be found: $CD90^+/CD45RA^-$, $CD90^-/CD45RA^-$ and $CD90^-/CD45RA^+$. The $LIN^-/CD34^+/CD38^-/CD90^+/CD45RA^-$ fraction is highly enriched for haematopoietic stem cells HST [1]. The common lymphoid progenitor (CLP) can be defined as $LIN^-/CD10^+/CD34^+$ [2]. Originating from this CLP, B cells continue to differentiate into pre-pro-B cells, which then turned into pro-B cells, large pre-B cells and small pre-B cells and finally differentiate into immature B cells. This differentiation is highly regulated by various transcription factors which are specifically expressed over a time period to ensure the correct development of B cells (reviewed in [3]).

B-ALLs are heterogenic diseases, with an accumulation of abnormal cells. Traditionally B-ALL cells have been compared to their normal counter-partners in B-cell development. This was mostly done because of similarities in morphology and immune phenotype. However, this head-to-head comparison misses some ALL features, for example, up to 30 % of ALL cases express myeloid markers [4]. Research on chromosome changes in ALL has shown that some of the initiating changes occur very early (e.g. being of parental origin), while others occur at a later stage of development [3].

Genetical changes leading to the development of B-ALL are discussed below. The increasing role of the signalling of the pre-B-cell receptor and signal transduction by this receptor should be mentioned. During B-cell development, the pre-B-cell receptor has a dual function. It promotes survival and proliferation, and subsequently it induces differentiation in the B-cell compartment [5]. Two downstream targets which are mainly important for the tumor suppressive function of the pre-B-cell receptor are IKAROS and AIOLOS [6]. Interestingly, in 80 % of the $BCR\text{-}ABL^+$ ALL, the gene responsible for IKAROS (IKZF1) has been deleted [7], underlining the importance of those events in the signal transduction for the development of ALL.

Similar to AML, there is a two-hit model for ALL. The first "hit" is posed to be a chromosomal abnormality (the major are listed below); nonetheless, this first "hit" is not sufficient for the induction of an ALL. Therefore, a second hit such as the deletion of tumor suppressor genes is needed to fully generate an ALL.

6.2.3 Genetics in Acute Lymphatic Leukaemia

6.2.3.1 Numerical Chromosome Changes

Hyperdiploid

Hyperploidy (>50 chromosomes) can be found in up to 30 % of all cases in children [8]. In contrast, the number of hyperploid cases in adults is significantly lower (about 10 %). Hyperploidy is associated with good prognosis in children. This might be explained by the higher sensitivity to chemotherapy [9]. The impact of hyperploidy in adults is less clear; while some reports found a benefit, others deny this finding [10, 11].

Hypodiploid

Hypoploidy is defined as the presence of less than 46 chromosomes in a cell. Approximately 10 % of children and nearly 10 % of adult cases show a hypoploid chromosome content [12]. Patients with hypoploidy have a worse prognosis compared to those with a normal or hyperploid leukaemia [13].

This is even more of importance since the event-free survival depends on the number of chromosomes and patients having less than 44 chromosomes showed 8-year EFS of 30 % [14].

6.2.3.2 Structural Changes

MLL Rearrangements

Several rearrangements involving the MLL gene at chromosome 11q23 are present in ALL cells. The most common are t(4;11)(q21;q23), t(11;19)(q23;p13.3) and t(9;11)(p22;q23) which lead to the fusion of the 5′portion of the MLL with the 3′portion of AFF1, MLLT1 and MLLT3 [15, 16]. Beside these frequent translocation patterns, over 50 other translocations are known which fuse to MLL. Interestingly, there are two major breaking clustering regions in the MLL gene between exon 5 and exon 11 [17]. Regardless of the other fusion partner (e.g. AFF1), fusion proteins will keep the transcription repressing domain of MLL and gain the 3′portion of the partner, which is mostly a transcription factor. MLL rearrangement is usually associated with poor outcome [18–20].

BCR-ABL

The translocation of 9q to chromosome 22q leads to the formation of the Philadelphia (Ph) chromosome. This fusion protein is the hallmark of the chronic myeloid leukaemia (CML) but is also found in ALL. Around 5 % of the children and up to nearly 30 % of adults will show the t(9;22)(q34;q11.2) translocation which can be detected by conventional cytogenetics, FISH or PCR [12, 21, 22]. The latest is often used for quantification which makes this method extremely interesting for detection of minimal residual disease (MRD) [23].

Genetically the Ph chromosome is a fusion of the 5′portion of the BCR gene to the 3′portion of the Abelson leukaemia virus proto-oncogene (ABL1). The breaking points of BCR cluster occur in two regions. The major clustering region (M-bcr) is mostly found in CML, and the minor cluster (m-bcr) is predominantly seen in ALL. Therefore, two different translocation products can result depending on the involved breaking point, the p210 kDa and the smaller p180-190 kDa. Patients will show only one of the two possible fusion samples [24, 23]. Detection of the t(9;22) in patients with ALL leads to an adverse disease prognosis [25, 26].

Tyrosine kinase inhibitors are active in Ph^+ ALL; however, the majority of patients will relapse after initial response to treatment and even during treatment [27, 28].

ETV6-RUNX1

Translocation t(12;21)(p13:q22) leads to the production of the fusion protein ETV6-RUNX1. This is the most frequent structural chromosome change in paediatric ALL, which occurs in nearly 30 % of the cases [29, 30]. While being quite frequent in childhood lymphatic leukaemia, ETV6-RUNX1 transcripts are rare in adults with a frequency below 5 % [31, 32]. RUNX1 is a transcription factor that regulates various genes important for human haematopoiesis [33].

Occurrence of the ETV6-RUNX1 translocation is associated with a good prognosis in childhood ALL. This is especially seen in younger children (1–9 years of age) rather than in those older than 10 [14, 32, 34, 35]. A hypothesised mechanism is that the occurrence of the translocation sensitises malignant cells to classical chemotherapeutic drugs used in ALL protocols [36, 37].

6.3 Immune Phenotype and Targets in Lymphatic Leukaemia

6.3.1 Cell Surface Marker

ALL cells express a variety of antigens that are linked to normal B-cell development. In a more simplified way, three major subgroups can be defined which can be classified by their immune phenotype. The early precursor or pro-ALL is characterised by the expression of CD19, cytoplasmic CD79a, cytoplasmic CD22 and nuclear TdT. The intermediate stage or common ALL is recognised by CD10, and the late precursor or pre-B-ALL stage is marked by the cytoplasmic expression of the μ chain. Typical phenotypes are listed in Table 6.1.

Table 6.1 Phenotype of B-ALL and potential antibody-based targets

Stage	Immune phenotype	Target
Early precursor	HLA-DR, TdT, cCD22, CD79a, CD19	CD19
		Blinatumomab
Intermediate precursor (common)	HLA-DR, TdT, cCD22, CD79a, CD19, CD10, CD20 (variable)	CD19
		Blinatumomab
		CD20
		Rituximab, ofatumumab
Late precursor	HLA-DR, TdT (variable), cCD22, CD79a, CD19, CD10, CD20, cytoplasmic μ	CD19
		Blinatumomab
		CD20
		Rituximab, ofatumumab

Lymphoblasts are positive for CD10, surface CD22, CD24, Pax5 and TdT in most cases. The expression of CD34 and CD20 varies. CD45 may be absent. Myeloid markers such as CD13, CD15, CD33 and CD68 can also be expressed on lymphoblasts [38].

6.3.2 Tumor Antigens

6.3.2.1 WT1

Wilms tumor gene 1 (WT1) is a zinc finger transcription factor that was originally found as a mutated tumor suppressor in Wilms tumor. The expression of the transcription factor was also found in haematopoiesis, and the expression and relevance of its expression has been extensively studied in AML and to a lesser extent in ALL. Reports showed that a great number of ALL have an WT1 expression, but in contrast to childhood ALL where WT1 was inversely correlated to an inferior prognosis [39], a study on nearly 300 adult ALL cases missed to show any impact as an individual prognostic marker [40]. The expression varies in different ALL subtypes, and matured B-ALL were negative or showed low WT1 expression, while aberrant expression of myeloid marker led to the highest WT1 levels. However, in adult T-ALL an inferior outcome for patients harbouring a WT1 mutation in exon 7 was reported [41]. While data for WT1-specific T-cell therapy in ALL are missing, a report in CML patients used WT1-specific T cells to prevent relapse of leukaemia [42].

6.3.2.2 BCR-ABL

Most work with BCR-ABL as a tumor antigen was done in CML. Numbers of reports have shown that T cells specific for BCR-ABL contribute to the immune *vs.* CML effect [42–44]. However, in BCR-ABL-positive ALL, the potential use of BCR-ABL as an immune target is less promising. Data of allografted BCR-ABL-positive ALL patients showed a better overall survival (OS) and less relapse compared to patients treated with conventional chemotherapy [45, 46], suggesting that a graft-versus-leukaemia (GvL) effect also exists for BCR-ABL ALL; however the relapse rate is still 30 % [47], and ALL has shown to be less sensitive for donor lymphocyte infusion [48]. BCR-ABL-positive ALL has also been included into trail with bi-specific antibodies (discussed below), resulting in a considerable rate of relapse which however was short lasting [49]. These results suggest that either BCR-ABL itself is less immunogenic or that priming and expansion of BCR-ABL-specific T cells take too long in acute leukaemia to be effective as therapeutical approach.

6.3.3 Cancer/Testis Antigens

Cancer/testis antigens (CTAs) are a group of tumor antigens being limitedly expressed in somatic tissues and represent an attractive target for immunotherapy in cancer since the gonads are immune privileged organs and anti-CTA immune response can be tumor specific (reviewed in [50]). While CTA represents an attractive target in AML [51], the expression and practicability as an immunological target in ALL is less clear. In a small study, the expression of CTA in ALL patients could be detected [52].

6.4 Immunotherapy for Lymphatic Leukaemia

6.4.1 Cellular Approaches

6.4.1.1 T Cells and Modified T Cells

T cells as part of the adoptive immune system have the ability to recognise and kill tumor cells. This quality is part of the concept of donor lym-

phocyte infusions (DLI, discussed below) as cellular therapy against various types of haematological cancers [53–55]. However, the response rates of ALL to DLI are inferior compared to other haematological cancer types and mostly lower than 15 % [56, 57]. Possible explanations may be that ALL is an aggressive disease where time for priming the naïve T cells is lacking and ALL cells are missing co-stimulatory molecules [58]. Even *ex vivo* pre-stimulation of T cells with CD3/CD28 antibodies did not enhance the benefit of DLI for ALL patients [59]. Another approach to enhance the T-cell toxicity towards ALL is an *ex vivo* priming against known tumor antigens. WT1 and BCR-ABL are the best studied antigens so far, and first reports are promising that the priming may lead to a better control of tumor cells [60].

An alternative to conventional T cells for adoptive immunotherapy is the application of genetically modified T cells. Here the α and β subunits of the T-cell receptor (TCR) of a tumor-specific T-cell clone are used. First results in solid tumors such as melanoma were promising [61]; however, its use in haematological malignancies was limited due to the antigen restriction of the T-cell clone [62, 63]. In addition, many tumor cells downregulate HLA molecules and thereby lower the ability of recognition by T cells [64].

A possibility to avoid the limitations of TCR gene transfer may be the use of chimeric antigen receptors (CARs). CARs are composed of a single-chain variable-fragment (scFv) antibody specific to tumor antigen, fused to a transmembrane domain and a T-cell signalling moiety, most commonly either the CD3-ζ or Fc receptor γ cytoplasmic signalling domains [65]. The resulting receptor, when expressed on the surface of the T cell, mediates binding to the target tumor antigen through the scFv domain, which subsequently mediates an activating signal to the T cell inducing target cell lysis. Major advantages are the ability to produce large amounts of modified T cells in the lab and the ability that those cells kill HLA-independent T cells and that CAR-modified T cells can be further manipulated by co-expressing cytokines or co-stimulatory molecules [66–68].

By choosing the CD19 as an immunological target, some preclinical work reported beneficial effects of viral-transduced CD19-targeted CAR T cells [69, 70]. Further modifications of CAR T cells with co-stimulatory receptors have enhanced their potential in mice models [71–73]. Ongoing phase I trials are investigating the benefit of CAR-modified T cells in the context of ALL (NCT01044069 and NCT01029366), and it will be very interesting to see which impact this modified T cells will have on the management and cure of ALL. In a first proof-of-principle report, Porter et al. treated a patient with refractory CLL with modified autologous T cells. T cells were transduced with CD19, CD137 and CD3-ζ and infused at a dosage of 1.5×10^5/kg BW. A remarkable remission for 10 months was noted [74]. Interestingly in patients treated with CAR-modified T cells, a portion of memory CAR T cells could be found after 6 months [75].

In a more recent study, Grupp and co-workers used CD19 CAR-modified T cells with dosages of 1.4×10^6 to 1.2×10^7 T cells per kg/BW to treat two children with relapsed and refractory pre-B-ALL. Both children reached complete remission (CR) after treatment, and one remained in CR for 11 months, while the other child relapsed with a clone of non-CD19-expressing blasts [76]. Therefore alternative targets are investigated, and first reports show that CD22 can also be used as an immunological target for CAR in ALL [77]. However, this point remains critical as the chosen antigen determines the success of the cellular therapy.

6.4.1.2 NK Cell Approaches

NK cells are part of the innate immune system. In contrast to B or T cells, NK cells do not have receptors rearranged during their maturation, making them less specific for antigens. Indeed, receptors expressed on the NK cell surface have more function of carefully controlling NK cell activation. One of those receptors is the killer-cell immunoglobulin-like recepor (KIR, CD158) family, which consists of different members that have activating as well as inhibitory functions on NK cells. NK cell cytotoxicity is triggered by tumor cells, which lack the of expression of

Table 6.2 Function of antibody-based immunotherapy

CD name	Other name	Function	Antibody
CD19		Forms complex with CD21 and CD81, co-receptor for B cells, binds cytoplasmic tyrosine kinases and PI3K	Blinatumomab
CD20		Oligomer of CD20 is involved in Ca 2+ transport and B-cell activation	Rituximab, ofatumumab
CD22	BL-CAM	Binds sialoconjugates	Inotuzumab
CD52	CAMPATH-1, HE5	Unknown	Alemtuzumab
CD33		Binds sialoconjugates	Gemtuzumab

some self-MHC class I molecules referred to as "missing self" hypothesis [78]. Inhibitory KIRs recognise groups of HLA-A, HLA-B and HLA-C alleles. If KIR inhibitory NK cells target cells lacking the corresponding HLA-class I ligand, the target cell will be lysed (KIR-ligand model) [79].

Up to now, NK cell alloreactivity does not seem to be beneficial in the treatment of ALL [80], but some reports with genetic modified NK cells provide some encouraging data. Retroviral or electroporation of NK cells to induce a CD19 targeting CAR led to increased NK cell-mediated killing of ALL cell lines, as well as primary ALL blasts [81–83].

6.4.2 Antibodies (See Table 6.2)

6.4.2.1 CD20 Antibodies

The CD20 molecule is an integral membrane protein that is specific for B cells and seems to be important for calcium transport across the cell membrane [84]. The expression of CD20 is linked to poor prognosis [85]. CD20 is expressed on leukemic blast cells of about 50 % of the patients with B-lineage ALL.

Rituximab is a chimeric mouse/humane antibody that has dramatically changed the therapy of NHL. Since CD20 is also expressed in B-ALL cells, the antibodies have also been used in the ALL setting. Reports have shown that the addition of CD20 antibodies to conventional chemotherapy leads to a higher rate of complete response as well as a better overall survival. Of note the advantage seems only to be true in younger ALL patients [86, 87].

6.4.2.2 CD22 Antibody

CD22 molecule expression is found in more than 90 % of B-lineage ALL. Functionally, CD22 leads to downregulation of CD19 after its phosphorylation. CD22 is rapidly internalised after activation and therefore is highly attractive for toxin-linked antibodies [88]. Inotuzumab ozogamicin is an anti-CD22 antibody linked to calicheamicin. Calicheamicin is a toxic antibiotic which causes double-strand breaks in the DNA. In a first phase II trial of nearly 60 patients, 57 % responded to the immunotoxin and showed an OS of 5.1 months [89].

Epratuzumab is an unconjugated CD22 antibody. In a very small study with 15 paediatric patients, nine achieved CR with only moderate toxicity [90]. Furthermore, the addition of epratuzumab to standard chemotherapy improved the CR rate in a Children's Oncology Group (COG) study [91]. Moxetumomab pasudotox is a new-generation toxin-linked anti-CD22 antibody that is currently being investigated in a phase I trial [92].

6.4.2.3 CD52 Antibody

CD52 is a glycoprotein on the surface of lymphoid cells. CD52 can be found on T and B cells, making the antigen interesting for application in T- and B-ALL. Campath-1H is a humanised IgG1k antibody that showed major efficiency in NHL and CLL.

A small series of six patients with advanced ALL who had been treated with alemtuzumab (three times 30 mg IV) was reported, and nearly all patients showed infectious complications [93]. CALGB presented data from a phase 1 study including 24 patients in CR1 with alemtuzumab as treatment. The OS was 55 months, and

DFS was 53 months. Interestingly minimal residual disease (MRD) levels were 1 log lower in alemtuzumab-treated patients [94]. Again infection complications were a major side effect in the treatment.

6.4.2.4 CD19 Antibody

CD19 is expressed in nearly all B-cell malignancies due to the early expression of the CD19 molecule in B-cell development. Blinatumomab is a structured monoclonal antibody combining two single-chain antibodies to CD19 B cells and to CD3 T cells [95]. This antibody increases the contact of cytotoxic CD3 to malignant B cells which thereby gets lysed. The GMALL showed data on 21 patients who achieved MRD negativity after blinatumomab therapy. The response rate was 80 % with a probability of relapse-free survival of 78 % and only mild side effects [96].

6.5 Stem Cell Transplantation

6.5.1 Allogeneic Stem Cell Transplantation (Allo SCT)

Allogeneic stem cell transplantation is still the most effective immunotherapy for ALL. Donor T cells contribute to controlling malignant cells. This effect of GvL was first described in acute leukaemias including ALL [97]. However, there are also significant limiting factors in the treatment of ALL by allo SCT; even with the improved supportive care therapy, there is up to 30 % treatment-related mortality (TRM) [98], and GVHD accounts for up to 70 % of the cases [99].

The majority of ALL patients will achieve CR after the induction therapy [99], but if patients profit more from allo SCT or conventional chemotherapy as consolidation is still not fully answered. There are some patient subgroups defined by genetical features, delayed response (> day 28) and high leukocyte count at diagnosis that have been summarised by the term high-risk patients. In these patients allo SCT seems to be favourable as consolidation therapy [100–102]. Nevertheless, it has to be underlined that these data are mainly based on myeloablative treatment protocols and related donor transplantation. Of note some studies failed to support the beneficial use of allo SCT in high-risk ALL patients [103, 104].

Besides the group of high-risk ALL patients, debate exists on whether standard-risk ALL patients should be transplanted. The British MRC analysed 1,646 patients who were negative for the t(9;22). If a CR was achieved and the patient was eligible for an allo SCT, patients were biologically randomised on the donor/no donor base. Interestingly in this cohort of standard-risk ALL patients, the 5-year OS was significantly better in the transplant group compared to non-transplanted patients (62 % *vs.* 52 %, $p = 0.02$) [103].

High-resolution typing of the HLA locus resulted in improved survival after matched unrelated donor transplantation, and results become similar to those of HLA-identical sibling transplantation [105–107].

As an alternative stem cell source, umbilical cord blood (UCB) or haploidentical donors can be used for allogeneic stem cell transplantation. Retrospective studies resulted in similar outcome after UCB and matched related or unrelated stem cell transplantation [108, 109], and haploidentical donor transplantation has become a reasonable transplant option for those patients lacking a suitable HLA-matched donor [110].

One way to lower TRM is to Reduced-intensity conditioning (RIC). Patients in advanced stage of ALL, with older age or heavily pretreated can be transplanted after RIC [111, 112]. Registry data from the EBMT showed that using RIC protocols reduced TRM in the context of ALL, but also in this data set, there was an increase in relapse rate (RIC, 47 % *vs.* MAC 31 %, $p < 0.001$) [113].

6.6 Concluding Remarks

ALL are acute leukaemias arising from a common lymphatic progenitor. In a number of cases, chromosomal changes occur; some of them like BCR-ABL have direct impact on the biology of the disease. As the neoplastic cells are closely

related to normal lymphoid development, ALL express a vast of antigens, which are a target of antibody-based therapies. Incorporation, especially of the chimeric blinatumomab, has improved therapeutical outcome in ALL patients. Stem cell transplantation is mostly the therapy of choice in the case of an ALL relapse after conventional therapy or in case of high-risk features of the disease in upfront treatment. By replacing the haematopoietic system, the donor immune system is thought to control leukaemia growth by the GvL effect. This is mostly mediated by T cells as one of the effector arms of the adoptive immune system. Therefore the development of "more specialised" T cells by genetical modification of the T-cell receptor (CAR-Ts) is a logical progress. CAR-modified T cells have already shown high efficiency in the use against lymphatic leukaemias. In summary immune therapeutic approaches held great promise to optimise ALL therapy and lead to a longer and better survival of ALL patients.

References

1. Majeti R, Park CY, Weissman IL. Identification of a hierarchy of multipotent hematopoietic progenitors in human cord blood. Cell Stem Cell. 2007;1(6):635–45.
2. Haddad R, Guardiola P, Izac B, Thibault C, Radich J, Delezoide AL, et al. Molecular characterization of early human T/NK and B-lymphoid progenitor cells in umbilical cord blood. Blood. 2004;104(13):3918–26.
3. Campos-Sanchez E, Toboso-Navasa A, Romero-Camarero I, Barajas-Diego M, Sanchez-Garcia I, Cobaleda C. Acute lymphoblastic leukemia and developmental biology: a crucial interrelationship. Cell Cycle. 2011;10(20):3473–86.
4. Suggs JL, Cruse JM, Lewis RE. Aberrant myeloid marker expression in precursor B-cell and T-cell leukemias. Exp Mol Pathol. 2007;83(3):471–3.
5. Nahar R, Muschen M. Pre-B cell receptor signaling in acute lymphoblastic leukemia. Cell Cycle. 2009;8(23):3874–7.
6. Thompson EC, Cobb BS, Sabbattini P, Meixlsperger S, Parelho V, Liberg D, et al. Ikaros DNA-binding proteins as integral components of B cell developmental-stage-specific regulatory circuits. Immunity. 2007;26(3):335–44.
7. Mullighan CG, Miller CB, Radtke I, Phillips LA, Dalton J, Ma J, et al. BCR-ABL1 lymphoblastic leukaemia is characterized by the deletion of Ikaros. Nature. 2008;453(7191):110–4.
8. Moorman AV, Harrison CJ, Buck GA, Richards SM, Secker-Walker LM, Martineau M, et al. Karyotype is an independent prognostic factor in adult acute lymphoblastic leukemia (ALL): analysis of cytogenetic data from patients treated on the Medical Research Council (MRC) UKALLXII/Eastern Cooperative Oncology Group (ECOG) 2993 trial. Blood. 2007;109(8):3189–97.
9. Whitehead VM, Vuchich MJ, Lauer SJ, Mahoney D, Carroll AJ, Shuster JJ, et al. Accumulation of high levels of methotrexate polyglutamates in lymphoblasts from children with hyperdiploid (greater than 50 chromosomes) B-lineage acute lymphoblastic leukemia: a Pediatric Oncology Group study. Blood. 1992;80(5):1316–23.
10. Cytogenetic abnormalities in adult acute lymphoblastic leukemia: correlations with hematologic findings outcome. A Collaborative Study of the Group Francais de Cytogenetique Hematologique. Blood. 1996;87(8):3135–42.
11. Wetzler M, Dodge RK, Mrozek K, Carroll AJ, Tantravahi R, Block AW, et al. Prospective karyotype analysis in adult acute lymphoblastic leukemia: the cancer and leukemia Group B experience. Blood. 1999;93(11):3983–93.
12. Chessells JM, Hall E, Prentice HG, Durrant J, Bailey CC, Richards SM. The impact of age on outcome in lymphoblastic leukaemia; MRC UKALL X and XA compared: a report from the MRC Paediatric and Adult Working Parties. Leukemia. 1998;12(4):463–73.
13. Heerema NA, Nachman JB, Sather HN, Sensel MG, Lee MK, Hutchinson R, et al. Hypodiploidy with less than 45 chromosomes confers adverse risk in childhood acute lymphoblastic leukemia: a report from the children's cancer group. Blood. 1999;94(12):4036–45.
14. Schultz KR, Pullen DJ, Sather HN, Shuster JJ, Devidas M, Borowitz MJ, et al. Risk- and response-based classification of childhood B-precursor acute lymphoblastic leukemia: a combined analysis of prognostic markers from the Pediatric Oncology Group (POG) and Children's Cancer Group (CCG). Blood. 2007;109(3):926–35.
15. Domer PH, Fakharzadeh SS, Chen CS, Jockel J, Johansen L, Silverman GA, et al. Acute mixed-lineage leukemia t(4;11)(q21;q23) generates an MLL-AF4 fusion product. Proc Natl Acad Sci U S A. 1993;90(16):7884–8.
16. Gu Y, Nakamura T, Alder H, Prasad R, Canaani O, Cimino G, et al. The t(4;11) chromosome translocation of human acute leukemias fuses the ALL-1 gene, related to Drosophila trithorax, to the AF-4 gene. Cell. 1992;71(4):701–8.
17. Felix CA, Hosler MR, Slater DJ, Parker RI, Masterson M, Whitlock JA, et al. MLL genomic breakpoint distribution within the breakpoint cluster region in de novo leukemia in children. J Pediatr Hematol Oncol. 1998;20(4):299–308.

18. Pui CH, Gaynon PS, Boyett JM, Chessells JM, Baruchel A, Kamps W, et al. Outcome of treatment in childhood acute lymphoblastic leukaemia with rearrangements of the 11q23 chromosomal region. Lancet. 2002;359(9321):1909–15.
19. Pui CH, Chessells JM, Camitta B, Baruchel A, Biondi A, Boyett JM, et al. Clinical heterogeneity in childhood acute lymphoblastic leukemia with 11q23 rearrangements. Leukemia. 2003;17(4):700–6.
20. Marchesi F, Girardi K, Avvisati G. Pathogenetic, clinical, and prognostic features of adult t(4;11)(q21;q23)/MLL-AF4 positive B-cell acute lymphoblastic leukemia. Adv Hematol. 2011;2011:621627.
21. Bloomfield CD, Goldman AI, Alimena G, Berger R, Borgstrom GH, Brandt L, et al. Chromosomal abnormalities identify high-risk and low-risk patients with acute lymphoblastic leukemia. Blood. 1986;67(2):415–20.
22. Mullighan CG. The molecular genetic makeup of acute lymphoblastic leukemia. Hematol Am Soc Hematol Educ Program. 2012;2012:389–96.
23. Bruggemann M, Schrauder A, Raff T, Pfeifer H, Dworzak M, Ottmann OG, et al. Standardized MRD quantification in European ALL trials: proceedings of the second international symposium on MRD assessment in Kiel, Germany, 18–20 September 2008. Leukemia. 2010;24(3):521–35.
24. De Klein A, Hagemeijer A, Bartram CR, Houwen R, Hoefsloot L, Carbonell F, et al. bcr rearrangement and translocation of the c-abl oncogene in Philadelphia positive acute lymphoblastic leukemia. Blood. 1986;68(6):1369–75.
25. Fielding AK. How i, treat Philadelphia chromosome-positive acute lymphoblastic leukemia. Blood. 2010;116(18):3409–17.
26. Stock W. Current treatment options for adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia. Leuk Lymphoma. 2010;51(2):188–98.
27. Ottmann OG, Pfeifer H. Management of Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL). Hematology Am Soc Hematol Educ Program. 2009;371–81.
28. Hunger SP. Tyrosine kinase inhibitor use in pediatric Philadelphia chromosome-positive acute lymphoblastic anemia. Hematol Am Soc Hematol Educ Program. 2011;2011:361–5.
29. Pui CH, Evans WE. Acute lymphoblastic leukemia. N Engl J Med. 1998;339(9):605–15.
30. Barry E, DeAngelo DJ, Neuberg D, Stevenson K, Loh ML, Asselin BL, et al. Favorable outcome for adolescents with acute lymphoblastic leukemia treated on Dana-Farber Cancer Institute Acute Lymphoblastic Leukemia Consortium Protocols. J Clin Oncol. 2007;25(7):813–9.
31. Jabber Al-Obaidi MS, Martineau M, Bennett CF, Franklin IM, Goldstone AH, Harewood L, et al. ETV6/AML1 fusion by FISH in adult acute lymphoblastic leukemia. Leukemia. 2002;16(4):669–74.
32. Lee DS, Kim YR, Cho HK, Lee CK, Lee JH, Cho HI. The presence of TEL/AML1 rearrangement and cryptic deletion of the TEL gene in adult acute lymphoblastic leukemia (ALL). Cancer Genet Cytogenet. 2005;162(2):176–8.
33. Hart SM, Foroni L. Core binding factor genes and human leukemia. Haematologica. 2002;87(12):1307–23.
34. Loh ML, Goldwasser MA, Silverman LB, Poon WM, Vattikuti S, Cardoso A, et al. Prospective analysis of TEL/AML1-positive patients treated on Dana-Farber Cancer Institute Consortium Protocol 95-01. Blood. 2006;107(11):4508–13.
35. Borkhardt A, Cazzaniga G, Viehmann S, Valsecchi MG, Ludwig WD, Burci L, et al. Incidence and clinical relevance of TEL/AML1 fusion genes in children with acute lymphoblastic leukemia enrolled in the German and Italian multicenter therapy trials. Associazione Italiana Ematologia Oncologia Pediatrica and the Berlin-Frankfurt-Munster Study Group. Blood. 1997;90(2):571–7.
36. Krishna Narla R, Navara C, Sarquis M, Uckun FM. Chemosensitivity of TEL-AML1 fusion transcript positive acute lymphoblastic leukemia cells. Leuk Lymphoma. 2001;41(5–6):615–23.
37. Ramakers-van Woerden NL, Pieters R, Loonen AH, Hubeek I, van Drunen E, Beverloo HB, et al. TEL/AML1 gene fusion is related to in vitro drug sensitivity for L-asparaginase in childhood acute lymphoblastic leukemia. Blood. 2000;96(3):1094–9.
38. Sabattini E, Bacci F, Saqramoso C, Pileri SA. WHO classification of tumors of haematopoietic and lymphoid tissues in 2008: an overview. Pathologica. 2010;102(3):83–7.
39. Boublikova L, Kalinova M, Ryan J, Quinn F, O'Marcaigh A, Smith O, et al. Wilms' tumor gene 1 (WT1) expression in childhood acute lymphoblastic leukemia: a wide range of WT1 expression levels, its impact on prognosis and minimal residual disease monitoring. Leukemia. 2006;20(2):254–63.
40. Busse A, Gokbuget N, Siehl JM, Hoelzer D, Schwartz S, Rietz A, et al. Wilms' tumor gene 1 (WT1) expression in subtypes of acute lymphoblastic leukemia (ALL) of adults and impact on clinical outcome. Ann Hematol. 2009;88(12):1199–205.
41. Heesch S, Goekbuget N, Stroux A, Tanchez JO, Schlee C, Burmeister T, et al. Prognostic implications of mutations and expression of the Wilms tumor 1 (WT1) gene in adult acute T-lymphoblastic leukemia. Haematologica. 2010;95(6):942–9.
42. Bornhauser M, Thiede C, Platzbecker U, Kiani A, Oelschlaegel U, Babatz J, et al. Prophylactic transfer of BCR-ABL-, PR1-, and WT1-reactive donor T cells after T cell-depleted allogeneic hematopoietic cell transplantation in patients with chronic myeloid leukemia. Blood. 2011;117(26):7174–84.
43. Cai A, Keskin DB, DeLuca DS, Alonso A, Zhang W, Zhang GL, et al. Mutated BCR-ABL generates immunogenic T-cell epitopes in CML patients. Clin Cancer Res. 2012;18(20):5761–72.
44. Gannage M, Abel M, Michallet AS, Delluc S, Lambert M, Giraudier S, et al. Ex vivo characterization of multiepitopic tumor-specific CD8 T cells in patients with

chronic myeloid leukemia: implications for vaccine development and adoptive cellular immunotherapy. J Immunol. 2005;174(12):8210–8.
45. Fielding AK, Richards SM, Chopra R, Lazarus HM, Litzow MR, Buck G, et al. Outcome of 609 adults after relapse of acute lymphoblastic leukemia (ALL); an MRC UKALL12/ECOG 2993 study. Blood. 2007;109(3):944–50.
46. Laport GG, Alvarnas JC, Palmer JM, Snyder DS, Slovak ML, Cherry AM, et al. Long-term remission of Philadelphia chromosome-positive acute lymphoblastic leukemia after allogeneic hematopoietic cell transplantation from matched sibling donors: a 20-year experience with the fractionated total body irradiation-etoposide regimen. Blood. 2008;112(3):903–9.
47. Lee HJ, Thompson JE, Wang ES, Wetzler M. Philadelphia chromosome-positive acute lymphoblastic leukemia: current treatment and future perspectives. Cancer. 2011;117(8):1583–94.
48. Kolb HJ, Schattenberg A, Goldman JM, Hertenstein B, Jacobsen N, Arcese W, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. Blood. 1995;86(5):2041–50.
49. Hoelzer D. Novel antibody-based therapies for acute lymphoblastic leukemia. Hematol Am Soc Hematol. 2011;2011:243–9.
50. Lim SH, Zhang Y, Zhang J. Cancer-testis antigens: the current status on antigen regulation and potential clinical use. Am J Blood Res. 2012;2(1):29–35.
51. Atanackovic D, Luetkens T, Kloth B, Fuchs G, Cao Y, Hildebrandt Y, et al. Cancer-testis antigen expression and its epigenetic modulation in acute myeloid leukemia. Am J Hematol. 2011;86(11):918–22.
52. Niemeyer P, Tureci O, Eberle T, Graf N, Pfreundschuh M, Sahin U. Expression of serologically identified tumor antigens in acute leukemias. Leuk Res. 2003;27(7):655–60.
53. Sprangers B, Van Wijmeersch B, Fevery S, Waer M, Billiau AD. Experimental and clinical approaches for optimization of the graft-versus-leukemia effect. Nat Clin Pract Oncol. 2007;4(7):404–14.
54. Chang YJ, Huang XJ. Donor lymphocyte infusions for relapse after allogeneic transplantation: when, if and for whom? Blood Rev. 2013;27(1):55–62.
55. Roddie C, Peggs KS. Donor lymphocyte infusion following allogeneic hematopoietic stem cell transplantation. Expert Opin Biol Ther. 2011;11(4):473–87.
56. Choi SJ, Lee JH, Lee JH, Kim S, Lee YS, Seol M, et al. Treatment of relapsed acute lymphoblastic leukemia after allogeneic bone marrow transplantation with chemotherapy followed by G-CSF-primed donor leukocyte infusion: a prospective study. Bone Marrow Transplant. 2005;36(2):163–9.
57. Collins Jr RH, Goldstein S, Giralt S, Levine J, Porter D, Drobyski W, et al. Donor leukocyte infusions in acute lymphocytic leukemia. Bone Marrow Transplant. 2000;26(5):511–6.
58. Loren AW, Porter DL. Donor leukocyte infusions for the treatment of relapsed acute leukemia after allogeneic stem cell transplantation. Bone Marrow Transplant. 2008;41(5):483–93.
59. Porter DL, Levine BL, Bunin N, Stadtmauer EA, Luger SM, Goldstein S, et al. A phase 1 trial of donor lymphocyte infusions expanded and activated ex vivo via CD3/CD28 costimulation. Blood. 2006;107(4):1325–31.
60. Rezvani K, Yong AS, Savani BN, Mielke S, Keyvanfar K, Gostick E, et al. Graft-versus-leukemia effects associated with detectable Wilms tumor-1 specific T lymphocytes after allogeneic stem-cell transplantation for acute lymphoblastic leukemia. Blood. 2007;110(6):1924–32.
61. Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. Science. 2006;314(5796):126–9.
62. Dossett ML, Teague RM, Schmitt TM, Tan X, Cooper LJ, Pinzon C, et al. Adoptive immunotherapy of disseminated leukemia with TCR-transduced, CD8+ T cells expressing a known endogenous TCR. Mol Ther. 2009;17(4):742–9.
63. Xue SA, Gao L, Hart D, Gillmore R, Qasim W, Thrasher A, et al. Elimination of human leukemia cells in NOD/SCID mice by WT1-TCR gene-transduced human T cells. Blood. 2005;106(9):3062–7.
64. Khong HT, Restifo NP. Natural selection of tumor variants in the generation of "tumor escape" phenotypes. Nat Immunol. 2002;3(11):999–1005.
65. Park JH, Brentjens RJ. Adoptive immunotherapy for B-cell malignancies with autologous chimeric antigen receptor modified tumor targeted T cells. Discov Med. 2010;9(47):277–88.
66. Singh H, Figliola MJ, Dawson MJ, Huls H, Olivares S, Switzer K, et al. Reprogramming CD19-specific T cells with IL-21 signaling can improve adoptive immunotherapy of B-lineage malignancies. Cancer Res. 2011;71(10):3516–27.
67. Tammana S, Huang X, Wong M, Milone MC, Ma L, Levine BL, et al. 4-1BB and CD28 signaling plays a synergistic role in redirecting umbilical cord blood T cells against B-cell malignancies. Hum Gene Ther. 2010;21(1):75–86.
68. Hoyos V, Savoldo B, Quintarelli C, Mahendravada A, Zhang M, Vera J, et al. Engineering CD19-specific T lymphocytes with interleukin-15 and a suicide gene to enhance their anti-lymphoma/leukemia effects and safety. Leukemia. 2010;24(6):1160–70.
69. Brentjens RJ, Latouche JB, Santos E, Marti F, Gong MC, Lyddane C, et al. Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15. Nat Med. 2003;9(3):279–86.
70. Brentjens RJ, Santos E, Nikhamin Y, Yeh R, Matsushita M, La Perle K, et al. Genetically targeted T cells eradicate systemic acute lymphoblastic leukemia xenografts. Clin Cancer Res. 2007;13(18 Pt 1):5426–35.
71. Imai C, Mihara K, Andreansky M, Nicholson IC, Pui CH, Geiger TL, et al. Chimeric receptors with 4-1BB signaling capacity provoke potent cytotoxic-

ity against acute lymphoblastic leukemia. Leukemia. 2004;18(4):676–84.
72. Loskog A, Giandomenico V, Rossig C, Pule M, Dotti G, Brenner MK. Addition of the CD28 signaling domain to chimeric T-cell receptors enhances chimeric T-cell resistance to T regulatory cells. Leukemia. 2006;20(10):1819–28.
73. Milone MC, Fish JD, Carpenito C, Carroll RG, Binder GK, Teachey D, et al. Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo. Mol Ther. 2009;17(8): 1453–64.
74. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med. 2011;365(8):725–33.
75. Kalos M, Levine BL, Porter DL, Katz S, Grupp SA, Bagg A, et al. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Sci Transl Med. 2011;3(95):95ra73.
76. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med. 2013;368(16):1509–18.
77. Haso W, Lee DW, Shah NN, Stetler-Stevenson M, Yuan CM, Pastan IH, et al. Anti-CD22-chimeric antigen receptors targeting B-cell precursor acute lymphoblastic leukemia. Blood. 2013;121(7):1165–74.
78. Karre K, Ljunggren HG, Piontek G, Kiessling R. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. Nature. 1986;319(6055):675–8.
79. Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science. 2002;295(5562):2097–100.
80. Park JH, Sauter C, Brentjens R. Cellular therapies in acute lymphoblastic leukemia. Hematol Oncol Clin North Am. 2011;25(6):1281–301.
81. Li L, Liu LN, Feller S, Allen C, Shivakumar R, Fratantoni J, et al. Expression of chimeric antigen receptors in natural killer cells with a regulatory-compliant non-viral method. Cancer Gene Ther. 2010;17(3):147–54.
82. Imai C, Iwamoto S, Campana D. Genetic modification of primary natural killer cells overcomes inhibitory signals and induces specific killing of leukemic cells. Blood. 2005;106(1):376–83.
83. Shimasaki N, Campana D. Natural killer cell reprogramming with chimeric immune receptors. Methods Mol Biol. 2013;969:203–20.
84. Walshe CA, Beers SA, French RR, Chan CH, Johnson PW, Packham GK, et al. Induction of cytosolic calcium flux by CD20 is dependent upon B Cell antigen receptor signaling. J Biol Chem. 2008;283(25):16971–84.
85. Borowitz MJ, Shuster J, Carroll AJ, Nash M, Look AT, Camitta B, et al. Prognostic significance of fluorescence intensity of surface marker expression in childhood B-precursor acute lymphoblastic leukemia. A Pediatric Oncology Group Study. Blood. 1997;89(11):3960–6.
86. Thomas DA, O'Brien S, Faderl S, Garcia-Manero G, Ferrajoli A, Wierda W, et al. Chemoimmunotherapy with a modified hyper-CVAD and rituximab regimen improves outcome in de novo Philadelphia chromosome-negative precursor B-lineage acute lymphoblastic leukemia. J Clin Oncol. 2010; 28(24):3880–9.
87. Thomas DA, Faderl S, O'Brien S, Bueso-Ramos C, Cortes J, Garcia-Manero G, et al. Chemoimmunotherapy with hyper-CVAD plus rituximab for the treatment of adult Burkitt and Burkitt-type lymphoma or acute lymphoblastic leukemia. Cancer. 2006;106(7):1569–80.
88. de Vries JF, Zwaan CM, De Bie M, Voerman JS, den Boer ML, van Dongen JJ, et al. The novel calicheamicin-conjugated CD22 antibody inotuzumab ozogamicin (CMC-544) effectively kills primary pediatric acute lymphoblastic leukemia cells. Leukemia. 2012;26(2):255–64.
89. Kantarjian H, Thomas D, Jorgensen J, Jabbour E, Kebriaei P, Rytting M, et al. Inotuzumab ozogamicin, an anti-CD22-calecheamicin conjugate, for refractory and relapsed acute lymphocytic leukaemia: a phase 2 study. Lancet Oncol. 2012;13(4):403–11.
90. Raetz EA, Cairo MS, Borowitz MJ, Blaney SM, Krailo MD, Leil TA, et al. Chemoimmunotherapy reinduction with epratuzumab in children with acute lymphoblastic leukemia in marrow relapse: a Children's Oncology Group Pilot Study. J Clin Oncol. 2008;26(22):3756–62.
91. Raetz EA, Cairo MS, Borowitz MJ. Reinduction chemoimmunotherapy with epratuzumab in relapsed acute lymphoblastic leukemia (ALL) in children, adolescents and young adults: results from Children's Oncology Group (COG) study ADVL04P2 [abstract] Blood (ASH Annual Meeting Abstracts) 2011;118(21):573.
92. Kreitman RJ, Pastan I. Antibody fusion proteins: anti-CD22 recombinant immunotoxin moxetumomab pasudotox. Clin Cancer Res. 2011;17(20): 6398–405.
93. Tibes R, Keating MJ, Ferrajoli A, Wierda W, Ravandi F, Garcia-Manero G, et al. Activity of alemtuzumab in patients with CD52-positive acute leukemia. Cancer. 2006;106(12):2645–51.
94. Stock W, Sanford B, Lozanski G, Vij R, Byrd JC, Powell BL et al., editors. Alemtuzumab can be incorporated into front-line therapy of adult Acute Lymphoblastic Leukemia (ALL): Final Phase I results of a Cancer and Leukemia Group B Study (CALGB 10102). ASH annual meeting abstracts. 2009;114:abstract 838.
95. Bargou R, Leo E, Zugmaier G, Klinger M, Goebeler M, Knop S, et al. Tumor regression in cancer patients by very low doses of a T cell-engaging antibody. Science. 2008;321(5891):974–7.

96. Topp MS, Kufer P, Gokbuget N, Goebeler M, Klinger M, Neumann S, et al. Targeted therapy with the T-cell-engaging antibody blinatumomab of chemotherapy-refractory minimal residual disease in B-lineage acute lymphoblastic leukemia patients results in high response rate and prolonged leukemia-free survival. J Clin Oncol. 2011;29(18):2493–8.
97. Weiden PL, Flournoy N, Thomas ED, Prentice R, Fefer A, Buckner CD, et al. Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. N Engl J Med. 1979;300(19):1068–73.
98. Gokbuget N, Hoelzer D. Treatment of adult acute lymphoblastic leukemia. Semin Hematol. 2009;46(1):64–75.
99. Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. N Engl J Med. 2006;354(2):166–78.
100. Hahn T, Wall D, Camitta B, Davies S, Dillon H, Gaynon P, et al. The role of cytotoxic therapy with hematopoietic stem cell transplantation in the therapy of acute lymphoblastic leukemia in adults: an evidence-based review. Biol Blood Marrow Transplant. 2006;12(1):1–30.
101. Yanada M, Matsuo K, Suzuki T, Naoe T. Allogeneic hematopoietic stem cell transplantation as part of postremission therapy improves survival for adult patients with high-risk acute lymphoblastic leukemia: a metaanalysis. Cancer. 2006;106(12):2657–63.
102. Thomas X, Boiron JM, Huguet F, Dombret H, Bradstock K, Vey N, et al. Outcome of treatment in adults with acute lymphoblastic leukemia: analysis of the LALA-94 trial. J Clin Oncol. 2004;22(20):4075–86.
103. Goldstone AH, Richards SM, Lazarus HM, Tallman MS, Buck G, Fielding AK, et al. In adults with standard-risk acute lymphoblastic leukemia, the greatest benefit is achieved from a matched sibling allogeneic transplantation in first complete remission, and an autologous transplantation is less effective than conventional consolidation/maintenance chemotherapy in all patients: final results of the International ALL Trial (MRC UKALL XII/ECOG E2993). Blood. 2008;111(4):1827–33.
104. Ribera JM, Oriol A, Bethencourt C, Parody R, Hernandez-Rivas JM, Moreno MJ, et al. Comparison of intensive chemotherapy, allogeneic or autologous stem cell transplantation as post-remission treatment for adult patients with high-risk acute lymphoblastic leukemia. Results of the PETHEMA ALL-93 trial. Haematologica. 2005;90(10):1346–56.
105. Chim CS, Lie AK, Liang R, Au WY, Kwong YL. Long-term results of allogeneic bone marrow transplantation for 108 adult patients with acute lymphoblastic leukemia: favorable outcome with BMT at first remission and HLA-matched unrelated donor. Bone Marrow Transplant. 2007;40(4):339–47.
106. Dahlke J, Kroger N, Zabelina T, Ayuk F, Fehse N, Wolschke C, et al. Comparable results in patients with acute lymphoblastic leukemia after related and unrelated stem cell transplantation. Bone Marrow Transplant. 2006;37(2):155–63.
107. Nishiwaki S, Terakura S, Yasuda T, Imahashi N, Sao H, Iida H, et al. Outcome of allogeneic bone marrow transplantation from unrelated donors for adult Philadelphia chromosome-negative acute lymphocytic leukemia in first complete-remission. Int J Hematol. 2010;91(3):419–25.
108. Atsuta Y, Suzuki R, Nagamura-Inoue T, Taniguchi S, Takahashi S, Kai S, et al. Disease-specific analyses of unrelated cord blood transplantation compared with unrelated bone marrow transplantation in adult patients with acute leukemia. Blood. 2009;113(8):1631–8.
109. Kumar P, Defor TE, Brunstein C, Barker JN, Wagner JE, Weisdorf DJ, et al. Allogeneic hematopoietic stem cell transplantation in adult acute lymphocytic leukemia: impact of donor source on survival. Biol Bone Marrow Transplant. 2008;14(12):1394–400.
110. Aversa F. Haploidentical haemotopoietic stem cell transplantation for acute leukaemia in adults: experience in Euvope and the united states. Bone Marrow Transplant. 2008;41(5):473–81.
111. Ram R, Storb R, Sandmaier BM, Maloney DG, Woolfrey A, Flowers ME, et al. Non-myeloablative conditioning with allogeneic hematopoietic cell transplantation for the treatment of high-risk acute lymphoblastic leukemia. Haematologica. 2011;96(8):1113–20.
112. Stein AS, Palmer JM, O'Donnell MR, Kogut NM, Spielberger RT, Slovak ML, et al. Reduced-intensity conditioning followed by peripheral blood stem cell transplantation for adult patients with high-risk acute lymphoblastic leukemia. Biol Blood Marrow Transplant. 2009;15(11):1407–14.
113. Mohty M, Labopin M, Volin L, Gratwohl A, Socie G, Esteve J, et al. Reduced-intensity versus conventional myeloablative conditioning allogeneic stem cell transplantation for patients with acute lymphoblastic leukemia: a retrospective study from the European Group for Blood and Marrow Transplantation. Blood. 2010;116(22):4439–43.

Immunopathology and Immunotherapy of Hodgkin Lymphoma

7

Maryam Ebadi, Yi Zeng, Maria Gkotzamanidou, and Nima Rezaei

Contents

7.1 Introduction

The majority of patients with early-stage Hodgkin lymphoma (HL) experience recovery by conventional chemo- and/or radiotherapy, yet considerable morbidity is posed by combined chemotherapy and radiotherapy [1]. On the other hand, refractory or relapsed patients comprise a considerable proportion, all of which could explain for the waning popularity of conventional

M. Ebadi, MD
Department of Immunology, Molecular Immunology Research Center, Children's Medical Center, Tehran University of Medical Sciences, Tehran 14194, Iran
e-mail: mary.ebadi@yahoo.com

Y. Zeng, MD, PhD
Department of Pediatrics, Steele Children's Research Center, University of Arizona,Tucson, AZ 85724, USA

M. Gkotzamanidou, MD, PhD
Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02215, USA
e-mail: maria_gkotzamanidou@dfci.harvard.edu

N. Rezaei, MD, PhD (✉)
Research Center for Immunodeficiencies, Children's Medical Center, Pediatrics Center of Excellence, Tehran University of Medical Sciences, Tehran 14194, Iran

Department of Immunology, Molecular Immunology Research Center, School of Medicine, Tehran University of Medical Sciences, Tehran 14194, Iran

Universal Scientific Education and Research Network (USERN), Tehran, Iran
e-mail: Rezaei_nima@tums.ac.ir

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_7,

therapies. Development of resistant cell clones is acknowledged as the main reason for tumor relapse; therefore, alternative techniques are mandated to circumvent these limitations. Monoclonal antibodies (mAbs) have emerged as promising anticancer therapies over the last several years [2, 3]. Various effector functions including ADCC, CDC, and direct apoptosis induction are mediated by mAbs [4]. Novel therapies are hinting toward immunotherapy-based treatments. Various antibodies have been targets of antibody-based immunotherapy, including CD20, CD30, CD40, IL13 receptor, RANK ligand, and DR4 [5]. The efficacy of antibody-based immunotherapy has been enhanced by a variety of approaches including radioimmunoconjugation and antibody-cytokine and antibody-toxin conjugation, in addition to biphasic Abs [6]. The treatment paradigm of classical HL (cHL) has considerably improved during the last two decades [7]. Among the four broad treatment modalities, immunotherapy is gaining superiority [6]. In this chapter, we aim to tackle immunotherapy and immunopathology of HL.

7.2 Immunopathology of Hodgkin Lymphoma

Proper insight into the immunopathology of HL seems mandatory for the development of efficient immunotherapeutic strategies; therefore, studies have been conducted in this regard.

HL is from B-cell origin, yet immunity impairment is a predominant feature, which is attributed to both T-cell and B-cell dysfunction. Immune deficiency is a characteristic feature of HL [8], leading to the patients' ineffective immune response toward Hodgkin and Reed-Sternberg (HRS) cells, which is recognized as a mechanism in the pathogenesis of HL. Infiltrating immune cells are present in an immune response toward HL; however, the activated immune system mostly resembles an acquired cellular immune deficiency [7]. A variety of immunopathologic features have made HL a suitable candidate for antibody-based therapeutic approaches: (a) the expression of various cell surface Ags including CD15, CD25, CD30, CD40, and CD80. The paucity of expression of these markers on normal human cells, preceded by low cross-reactivity, has made HL an ideal target for immunotherapy with minimal side effects. (b) Selective immunotherapy could be intensified by applying cocktails of mAbs against different Ags. As a result, the resistance of one malignant cell clone to a specific Ab leaves the opportunity for it to be targeted by other present Abs. (c) Malignant cells comprise the minority of the tumor mass, as the majority of cells are innocent bystander cells. (d) Rich vascularization is observed in HL, leading to feasible intravenous connection. (e) HL is acknowledged to possess a high response rate to standard therapeutic regimens, leaving a small proportion being targeted by immunotherapy (a high clinical remission rate is achieved after standard therapy). The immune environment surrounding HRS cells is depicted in Fig. 7.1. A distinguishing feature of HL is the paucity of malignant cells in lymph nodes surrounded by immune effector cells, including dense inflammatory infiltrates enriched in inhibitory T-regulatory cells which fail to recognize and invade malignant cells [9, 10]. The cytokines and chemokines produced by HL tumors which promote a Th2-type T-cell response are recognized as potential contributors to immune evasion [11]. Polarization toward T-helper-2 (Th2) immune response is activated by various cytokines including IL-10 and chemokines produced by HRS cells, leading to restrained cellular reactivity and poor prognosis [3, 6–10]. Multiple signaling pathways are deregulated in HRS cells. Both canonical and noncanonical NF-kB signaling pathways are frequently activated in HRS cells, playing an essential role in the survival of HRS cells. Signaling through CD40 and RANK receptors triggers the NF-kB signaling pathway [12], while in approximately 40 % of classical HL, the HRS cells are latently infected by EBV and, consequently, the EBV-encoded latent membrane protein 1 (LMP1) is expressed, which activates also NF-kB by mimicking an activated CD40 receptor [13]. Therefore, interference with this pathway by the targeting Ab is considered profitable [5]. Death of activated cytotoxic T lymphocyte and NK cells is induced by the upregulation of immunomodulatory surface

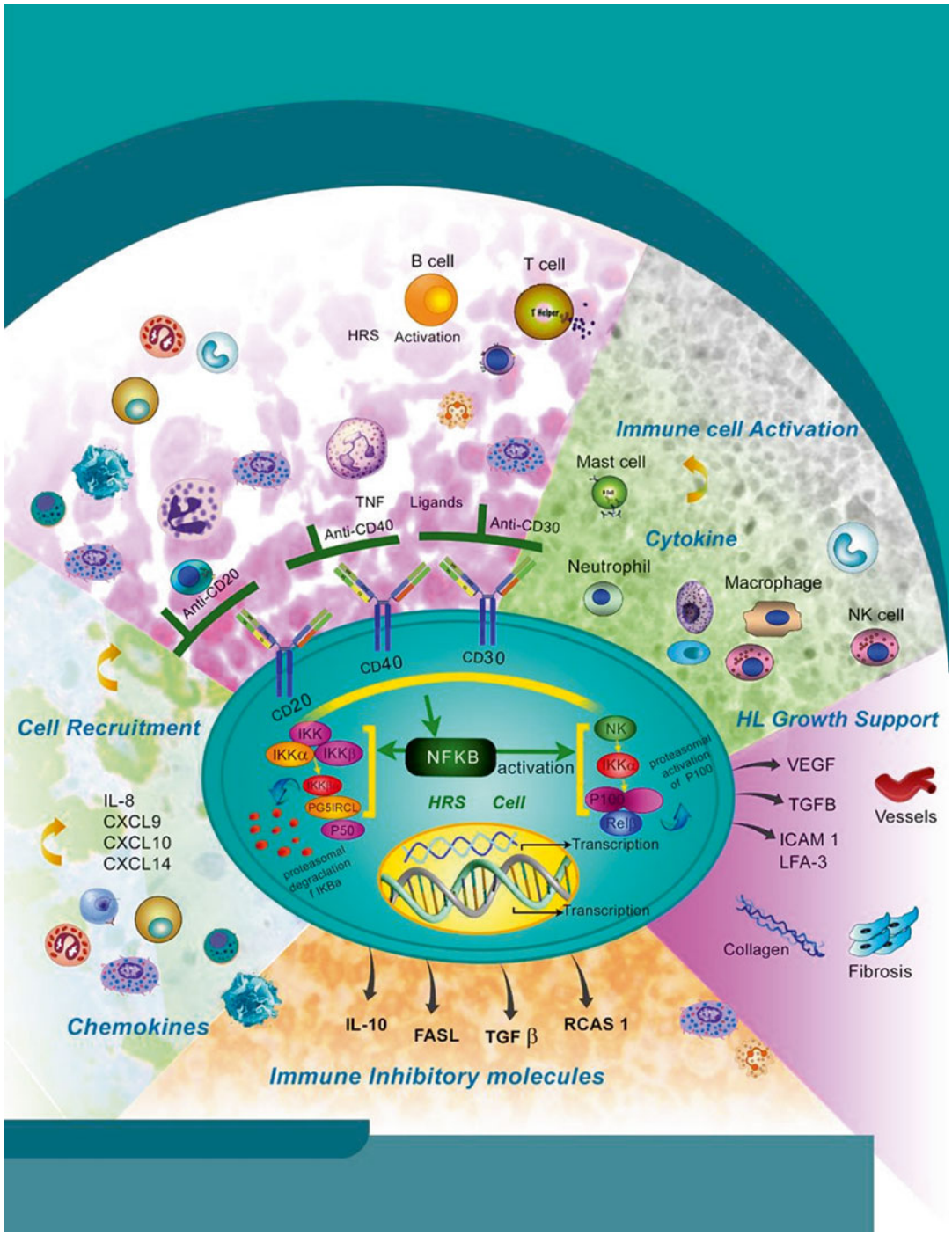


Fig. 7.1 The figure demonstrates the immune environment of HRS cells in HL, as well as the antibodies located on the cell surface which are considered potential targets for immunotherapy

receptors including RCAS1 or FAS ligand on HRS cells. Furthermore, resistance to CD95-mediated cell death triggered by inflammatory cells is manifested in HRS cells [14]. Reiners et al. [9] demonstrated impairment of the HL-derived target cell line L428 in peripheral

NK cells in HL. Furthermore, it was shown that healthy NK cells lyse L428 target cells mainly by NKG2D. Remarkably, a significant reduction in the NK surface expression of NKG2D is indicated in HL-derived p-NK cells [9]. The novel NKp30 ligand, BAG6, is found to suppress NK cell function in its soluble form. In a study conducted by Reiners et al., it was indicated that surface ligands for NKp30 are not expressed in HL cell line L428. Of note, trace expression of NKp30-ligand BAG6/BAT3 in L428 cell line was found [9]. In patients with HL, MICA, the ligand for NKG2D, is elevated. Moreover, serum levels of MIF and BAG6 are increased. It has been reported that MIF downregulates NKG2D and, hence, reduces NK cell function [15]. In addition, elevated serum BAG6 levels are proposed to reduce the function of NK cells [16]. The release of MICA and ULBP3, as well as TGF-β from bystander and HRS cells, leads to downregulation of NKG2D on effector lymphocytes and results in impaired NKG2D-dependent killing of malignant cells [17]. Mamessier et al. [16] described that despite the efficiency of IL-2 in restoring NK cell activity via RAS/MAPK, JAK/STAT, and PI3-kinase/Akt signaling pathways in normal cell environments, this pathway fails to activate NK cells in the serum of patients with HL, proposing the inhibitory role of serum factors in HL patients. In the same line, reversible suppression of peripheral NK cells has been manifested in other tumors including breast cancer [18, 19]. The JAK/STAT pathway is activated by many cytokines including IL-13 and IL-21, which ultimately leads to the translocation of STAT homo- or heterodimers into the nucleus; moreover, SOCS1, encoding the main inhibitor of JAK/STAT signaling, is inactivated by mutations in 40 % of classical HLs. Activated STAT3, STAT5, and STAT6 factors are accumulated in HRS cells [12]. MEK/ERK and PI3K/AKT pathways are also activated in HRS cells. In addition, other signaling pathways including multiple receptor tyrosine kinases, the T-cell transcription factors Notch-1 and GATA-3, the NK cell factor ID2, and the myeloid receptor CSF1R are triggered, all together contributing to the mixed phenotype of HRS cells [12, 20, 21].

Naked Abs are dependent on immune effector cell activation for implying their therapeutic effects. In addition, effector immune cells are targeted by T-regulatory cells. Thus, an immunosuppressive environment is induced, explaining the low therapeutic efficacy of naked mAbs in clearance of HRS cells [22]. All the above-mentioned mechanisms provide explanations to the low efficacy of several therapeutic Abs employed in HL involving effector cell-dependent antitumor activities [5]. The low mitotic index (0.5 %), due to mitotic defects and high degrees of apoptosis [23], contributes to the low efficacy of Abs directed toward tumor Ags, targeting as low as 0.5–5 % of the tumor mass cell population [24].

HL tumors, classified as a liquid tumor, possess a solid tumorlike appearance and composition. Antibodies and antibody-drug conjugates are poorly effective on solid tumors, and only 0.001–0.01 % of the injected antibody permeates the tumor [25]. Therefore, the efficacy of immunotherapy in HL is mitigated due to the solid tumorlike composition, as well as the low frequency of HRS cells [5].

Tumor necrosis factor receptor (TNFR) superfamily includes CD30, CD40, Fas (CD95), and OX40 (CD134), just a few to point to [26]. CD30-ligand binding or cross-linking by immobilized Abs, ultimately provokes biological signals including cell proliferation and apoptosis [5]. CD30-CD30 ligand interactions on the surface of HRS cells are recognized in the pathogenesis of HL [27]. Due to the considerable difference between the ability of anti-CD30 Abs in the interference with NF-kB signaling pathway, their therapeutic efficacy is variable [5]. MAP kinases and NF-kB are the hallmark events regulated by CD30 signaling pathway [28, 29], leading to cell proliferation and survival, in addition to induction of antiproliferative responses and cell death [30]. Activation of NF-kB leads to the expression of antiapoptotic genes such as cFLIP [31], XIAP [32], and Bcl X_L [33], hence posing an additional level of complexity to the treatment of HL [34]. Therefore, inhibition of the NF-kB signaling pathway by therapeutic Abs seems a beneficial interference [34].

The active, phosphorylated form of the mitogen-activated protein kinase kinase/extracellular signal-regulated kinase (MAPK, EPK; p44/42) is observed in cultured and primary HRS cells. Furthermore, inhibition of the upstream kinase EPK has been shown to be associated with decreased growth of HL cell lines [35]. Hetero- and homodimers of Jun, Fos, and other members of the activating transcription factor (ATF) family, c-Jun, and JunB are found to be expressed excessively in HL and ALCL, but not in other subtypes [36]. On the other hand, inhibitors of the phosphatidylinositol kinase/AKT pathway are recognized as potential inducers of apoptosis in HRS cells [37]. The certain morphological feature of HL tumor, along with alterations in major signaling pathways, justifies the lack of the efficacy of conventional antibody-based immunotherapeutic treatments and highlights the inevitable need to novel therapeutic strategies [5]. Moreover, the expression of delayed hypersensitivity is found to be impaired in these patients.

T cells play a critical role in the HL cell microenvironment, as they compromise the majority of infiltrating cells. HRS cells are directly surrounded by $CD4^+$ T cells, which do not have a Gemcitabine and carboplatin (GC) Th-cell phenotype, and comprise a mixed population of Th cells with Th2 phenotype and regulatory T cells (T reg) [38, 39]. MHC II, a crucial mediator of the interaction between T cells and B cells, is downregulated in 40 % of HRS cells [40]. HRS cells attract chemokine receptor CCR4-Th and Treg cells via the secretion of large amounts of the CCL17 chemokine [38]. Overall, studies have indicated that the HRS cell/T-cell interaction plays an important role in the pathogenesis of HRS cells. Various factors contribute to the interaction between $CD4^+$ T cells with HRS cells, including the production of IL-13, an HRS cell growth factor produced by T cells [41], and stimulation of HRS cells through interaction between CD28 (expressed on T-cell surface), CD80 and CD86 (expressed by HRS cells), and CD40 on HRS. Tumor-infiltrating Treg cells preserve HRS cells from the attack of cytotoxic T and NK cells, via the production of IL-10 and the expression of T lymphocyte-associated protein 4 (CTLA4) [10]. Therefore, Treg cells play a suppressive role in the HL microenvironment. HRS cells are recognized to draw Treg cells into the lymphoma microenvironment; in addition, they play a direct role in the differentiation of $CD4^+$ T cells to Treg cells [42]. HRS cells directly inhibit cytotoxic T lymphocyte and NK cells in cHL, through production of IL-10, TGF, gelatin-1, tissue inhibitor of metalloproteinase 1 (TIMP1), and prostaglandin E2 (PGE2) [43, 44]. On the other hand, ligands PD1 and 2, which inhibit PD1 T cells and express CD95 ligand, are also expressed on HRS cells, resulting in the apoptosis of CD95-activated CD8 and Th1 T cells [43, 45]. Figure 7.1 well depicts the microenvironment of an HRS cell.

As explained by the immunopathology, the main focus of immunotherapy in HL is directed toward activation of T-cell responsiveness, particularly around HRS cells. Reactivating an antitumor immune response by pinpointing cytokines capable of reversing the polarized immune response has been advocated in this regard; in addition, antibody-targeted cytokines which accumulate in the lymphoma lesion have been found to be more efficacious compared to unmodified cytokines. These findings shed light on the importance of the application of antibody-cytokine fusion proteins for targeting cytokines toward HRS tumor cells, combined with the advantages of local reactivation of immune responses [46]. HRS cells express multiple cell surface Abs, among which a variety are considered potent targets for immunotherapy [47].

CD1d and NK cells also play a pivotal role in the pathogenesis of HL. CD1d, normally expressed on hematopoietic cells of myelomonocytic and B-cell lineages, is a marker for malignancies originating from the corresponding tissues.

B-cell malignancies have also been found to display CD1d. Studies on murine models have demonstrated the expression of CD1d on many leukemia and lymphoma cell lines. Moreover, NKTs have exhibited a protective role in the A20 murine B-cell lymphoma model [48], which is correlated to the level of CD1d expression on lymphoma cells, and was lost in NKT-deficient mice. Studies on human lymphomas have

revealed that CD1d is expressed on the surface HRS cells in half of the cHL cases [49]. Notably, NKTs were present at high frequencies in primary cHL tumors and reactive lymph nodes irrespective of CD1d expression on tumor cells. However, the functional role of tumor-infiltrating NKTs in cHL biology and disease outcome is yet to be determined. It is postulated that NKTs may co-localize with CD1d-positive tumor-associated monocytes/macrophages (TAMs) in the microenvironment of CD1d-negative tumors [50]. In addition, the increased number of TAMs is significantly correlated to decreased survival rates in patients with cHL [51]. Targeting both HRS cells and TAMs for immunotherapy with NKTs and/or their ligands seems a promising approach [52]. The strongest known risk factor for the development of lymphoma is immunosuppression, predominantly NK cell dysfunction. NK cells are critical effectors in tumor immunology and were usually regarded as effector cells of innate immunity. However, more recently, it has been shown that they attribute to both innate and adaptive immunity, playing a regulatory role in shaping antigen-specific T- and B-cell responses [53]. NK cell activity is significantly impaired in HL compared to controls, irrespective of histological type and clinical stage. Notably, the most profound NK cell dysfunction, present and persistent in HL, is associated with increased LDH release activity from peripheral blood mononuclear cells. NK cell function is greatly impaired in HL; in addition, impaired NK cell activity is associated with increased spontaneous release activity of LDH from patients' PBL, which is indicative of cell membrane damage, followed by the release of cytotoxic proteins, and eventually impaired NK cell activity [4].

7.3 General Concepts of Monoclonal Antibodies

7.3.1 The Structure of Monoclonal Antibodies

Antibodies used in immunotherapy target the Ags specifically present on tumor cells. The mAb technology was developed by Kochler and Milstein [54]. Monoclonal antibodies consist of a Fab and an Fc region, resulting in a Y-shape structure. The Fab fragment contains the complementary-determining region (CDR), which defines the specificity of a mAb toward the Ags, whereas the Fc fragment, an isotype IgG, is responsible for the Ab's mechanism of action and interacts with cells expressing Fcg receptors (FcgR) on immune cells including natural killer (NK) cells, macrophages, and neutrophils [55]. FcgR stimulation leads to activation of the ADCC pathway and results in cytotoxic events. In addition, complements are fixated by Fc fragments, ensuing the activation of the Complement-dependent cytotoxicity (CDC). Direct intracellular signal, resulting in an antiproliferative effect and apoptosis, is proposed as an alternative mechanism of action, which is activated by direct binding of the mAb to its Ag [55].

7.3.2 Choosing the Optimal Antibody

The exquisite specificity of Abs renders them ideal targets for immunotherapy in malignancies including HL and NHL. Selective cancer immunotherapy was first proposed by Paul Ehrlich [56]. Successful immunotherapeutic approaches rely on appropriate target Ag selection. In selecting the appropriate antibody-based therapies, certain conditions should be taken into account, including (1) selectivity of the antigen expressed on the target cell and its sufficient expression; (2) maintenance of the antigen on the cell membrane; (3) no internalization after mAb binding, and (4) its ability in initiating a cytotoxic effect upon binding to its target.

IgG1 is the most widely used human therapeutic Ab isotype [57]. It has the appeal of prolonged half-life compared to other human IgGs [58]. In addition, it exhibits significantly greater specificity and affinity to activation and inhibition of FcgR. Finally, it results in greater ADCC induction [59].

Monoclonal antibodies are named based on their origin; their general nomenclature is as

follows: The addition of -omab, -amab, and -emab to the generic name of the Ab each implies murine, rat, and hamster origins, respectively, whereas adding -imab, -ximab, -zamab, and -umab each indicates primate, chimeric, humanized, and human origins, respectively [55].

Herein, Ags targeted in immunotherapy of HL, along with the advances made in immunotherapeutic approaches, have been discussed.

7.4 CD30

CD30, a member of TNF receptor superfamily, is expressed on active B and T lymphocytes and NK cells. In addition, it is considered a diagnostic immunomarker for classical HL (cHL), as it contributes to the proinflammatory tumor microenvironment [60]. Through interaction with the TNF receptor-associated factor 2 and 5 (TRAF 2 and 5), activation of NF-kB is pursued. Thereafter, apoptosis and proliferative potential of autoreactive T cells are regulated [55]. CD30 is expressed on the cell surface of HRS cells [61]. Due to the paucity of CD30 expression on nonimmune system cells, it is considered a potential target for immunotherapeutic approaches for HL [62]. CD30-CD30 ligand interaction on the surface of HRS cells is recognized in the pathobiology of HL (Fig. 7.1) [27]. Under normal conditions, CD30 is solely expressed on activated NK cells, monocytes, eosinophils, and a small proportion of large lymphoid cells in sections of lymph nodes, tonsil, thymus, and endometrial cells. Serum CD30 level has been found to correlate with the prognosis of HL [63]. Due to the paucity of expression in nonneoplastic cells outside the immune system, it is regarded as an exquisite candidate for mAb therapy [64]. Nonetheless, the paucity of malignant $CD30^+$ (HRS) cells poses an additional level of complexity to CD30-targeted therapy [65]. The tumor mass in HL is defined as $CD30^+$ malignant cells surrounded by massive infiltrations of immune effector cells, which apparently have failed to clear the cell mass in the involved lymph nodes [9]. Due to considerable differences between the ability of anti-CD30 Abs in interfering with NF-kB signaling pathway, their therapeutic efficacy is variable [5]. MAP kinases and NF-kB are the hallmark events regulated by CD30 signaling pathway [28, 29], leading to cell proliferation and survival, as well as induction of antiproliferative responses and cell death [30]. Activation of NF-kB leads to the expression of antiapoptotic genes such as cFLIP [31], XIAP [32], and Bcl X_L [33], hence posing an additional level of complexity to the treatment of HL [34].

Various mAbs targeting CD30 have been evaluated in preclinical tumor models (Table 7.1) [75–78]. CD30 monoclonal Abs may trigger cell death directly or indirectly through ADCC or

Table 7.1 CD30-directed immunotherapy in HL

Drug	Study type	*N*	CR + PR (%)	Reference
Murine anti-CD30-saporin conjugate (Ber-H2/SO6)	Pilot	4	75	[63]
Murine anti-CD16/CD30	Phase I/II	15	13	[66]
Murine anti-CD16/CD30 combined with IL-2, GM-CSF	Pilot	16	25	[67]
Anti-CD64/CD30	Phase I	10	40	[68]
Murine anti-CD30-ricin-A conjugate (Ki-4.dgA)	Phase I	17	7	[69]
Murine anti-CD30-131 iodine-conjugate	Phase I	22	27	[70]
Chimerized anti-CD30 mAb (cAC10, SGN-30)	Phase I	13	15	[5]
cAC10, SGN-30	Phase II	35	0	[71]
Humanized anti-CD30-mAb, (MDX-060)	Phase I/II	72	8	[72]
cAC10-auristatin conjugate (cAC10-vcMMAE, SGN-35)	Phase I	39	45	[73]
Humanized, effector cell-enhanced anti-CD30 mAb (parental MDC-060, MDX-1401)	Phase II	72	8	[74]

CR complete response, *PR* partial response

CDC or via Ag-dependent cellular phagocytosis (ADCP). Gerber et al. [5] described different physiobiochemical properties of various CD30 mAbs, leading to the induction of unique set of pharmacodynamic responses, discrepancy in epitope recognition, binding affinities, and effector cell activation characteristics, only a few outcomes to point to. Accordingly, various anti-CD30 Abs including AC10, Ki-1, 5F11, M67, and Ber-H2 demonstrated distinct domain recognition during cross-blocking competition studies [75, 79]. Moreover, only 5F11 and AC10 are found to interfere with human HL cell line growth in culture [80–82]. More precise descriptions on each anti-CD30 Ab are provided below.

7.4.1 CD30 Monoclonal Antibodies

7.4.1.1 MDX-060 (5F11)

5F11, a hybridoma-derived antihuman CD30 IgG1 Ab, is a well-established inhibitor of the growth of cells expressing CD30, *in vitro*, acting via the induction of growth inhibitory cell signaling and ADCC pathways and eventually leading to efficient cell apoptosis. It is generated in human transgenic mice, and its optimal anti-HL effect was established in disseminated and solid murine models with human HL [75]. Borchmann et al. demonstrated that treating mice implanted with solid or disseminated HD tumor cells (L540cy) leads to reduced tumor volume and increased survival [75]. Moreover, its additive effect was observed in combination with conventional cytotoxic drugs, particularly with gemcitabine and etoposide, *in vitro*, leading to increased sensitivity to chemotherapy [78]. During a phase I clinical study on patients with refractory HL, a dose up to 15 mg/kg was proved to be safe [72]. However, further studies regarding combination therapy with cytotoxic drugs seem mandatory.

7.4.1.2 MDX-1401

MDX-1401, the non-fucosylated version of MDX-060, is superior to MDX-060 due to increased ADCC activity. Hence, lower doses are required to achieve the same ADCC activity [74].

7.4.1.3 Chimeric-AC10

Chimeric-AC10, which is similar to human IgG1 subclass in structure, promotes growth arrest and DNA fragmentation of CD30 positive tumor cells, thereby inducing its antitumor effects [83]. *In vitro* experiments revealed that ADCP plays a pivotal role in the antitumor activity of chimeric-AC10 [22]. Moreover, chimeric-AC10 was found to boost the antitumor activity of bleomycin in HL xenografts [84]. The domain on CD30 recognized by AC10 differs from the domain bonded by Ki-11, 5F11, or Ber-H2 [75, 79].

7.4.1.4 SGN-30

SGN-30, a chimeric IgG1 mAb derived from the murine AC10 anti-CD30 mAb, has demonstrated an antiproliferative effect *in vitro* and a potent anti-HL effect in xenografts [83]. Macrophages play a critical role in the activity of SCN-30, proven by the abolished effect of SGN-30 in the absence of macrophages in experimental studies [83]. It has proved as a safe and well-tolerated mAb, yielding adequate response in patients with HL during phase I and phase II [85, 86]. Remarkably, HL cell lines treated with SGN-30 were sensitized to conventional cytotoxic drugs, including bleomycin and etoposide [84]. However, combination of SGN-30 with conventional chemotherapy in a phase III study led to development of pneumonitis in a considerable proportion of patients, posing a potential limitation to its administration combined with chemotherapy. Particularly, FcgRIIIa-158 V/F polymorphism was associated with an increased risk [87].

7.4.2 CD30 mAb-Drug Conjugates

The combination of chemotherapy with immunotherapy has revealed enhanced efficacy and has been clearly effective in extending survival [88]. Some limitations to effective immunotherapy are overcome by applying antibody-drug conjugates (ADCs). Since ADCs apply their therapeutic effect independent of inflammatory cells, immune evasion mechanisms are not involved. Hence, lower exposure levels are required compared to naked Abs. In addition, restricted access of

macromolecules to tumor cells has been overcome by combining ADCs to cytoreductive chemotherapeutic agents. Interestingly, immense increase in the amount of local active drug in malignant cells is observed by applying ADCs, which could compensate for the small fraction of malignant HRS cells in HL tumor mass [5]. ADCs have the appeal of being administered over a prolonged time with no treatment holidays, as their toxicity profile has been reduced compared to cytotoxic drugs [5]. In the following CD30 mAb-drug conjugates, studies in HL are discussed.

7.4.2.1 Brentuximab Vedotin

Brentuximab vedotin (SNG-35, ADCETRIS™, Seattle Genetics) was approved by the FDA for the treatment of cHL and ALCL in 2011. Brentuximab, a CD30 mAb, comprises the Ab section, whereas a microtubule-disrupting agent, monomethyl auristatin E (MMAE, three to five units), comprises the drug section. Once brentuximab vedotin binds to the CD30 receptor on the cell surface, CD30-drug complex is internalized, and cytotoxic components are released as a consequence of proteolytic cleavage in the lysosome. Remarkably, few brentuximab vedotin molecules are sufficient to achieve clinical efficacy, making it favorable for HLs with low CD30 expression [89]. Moreover, brentuximab vedotin leads to decreased levels of chemokines and cytokines (TARC), which resolve the inflammatory infiltrate and disrupt the microenvironment, and in turn, facilitate the antitumor immune response [90]. It has been suggested that combining brentuximab vedotin with chemotherapeutic regimens yields promising results.

7.5 CD20

CD20, a protein of 297 amino acids with four transmembrane regions, is exclusively expressed on the lymphocytic and histiocytic cells of nodular lymphocyte predominant HL (NLPHL). The expression of CD20 in this subtype is recognized as a diagnostic hallmark, distinguishing it from cHL [91, 92]. Some studies have investigated its efficacy in NLPHL, which would be briefly discussed in the following.

7.5.1 Rituximab

Wirth et al. have described beneficial outcome with the administration of rituximab in patients with relapsed stage IA NLPHL. The GHSG and Stanford trials conducted on relapsed patients observed a 93 and 100 % ORR, respectively. Four weekly administrations of rituximab (375 mg/m^2) as a frontline therapy in the GHSG study on 28 patients yielded an ORR of 100 % and an 86 % CR. As concluded in their study, rituximab-based combination treatment holds promising potential as the frontline treatment [93]. Nineteen newly diagnosed NLPHL patients recruited in the study by the Stanford group manifested a 100 % ORR and a 63 % CR [93]. The study was extended to 2 years with repeated four weekly infusions every 6 months which improved CR to 88 %. In the same line, limited rituximab therapy in NLPHL patients in the GHSG study resulted in 94 % ORR and 53 % CR [94]. Moreover, a phase one half trial has been conducted on relapsed NLPHL patients using tositumomab, a first-generation type II CD20 mAb, and ^{131}I-tositumomab. Patients received 450 mg single dose, leading to CR in all patients. Cytopenia was regarded as the most common adverse event [95].

Nonetheless, the application of CD20 mAbs is restricted in HL and is mainly specific to NHL. Accordingly, further description on the application of CD20 mAbs is provided in the next chapter.

7.6 CD40

CD40, a member of the tumor necrosis factor receptor family, is highly expressed on neoplastic B cells. Stimulation of CD40 leads to immunoglobulin isotype switching and activation of B cells, eventually resulting in enhanced proliferation and survival. Remarkably, it is recognized as an independent risk factor for some hematological malignancies [96]. Most studies in the literature are conducted on NHL patients, with only a handful of data available on its effect in HL.

7.6.1 Lucatumumab (HCD122)

Lucatumumab (HCD122), a CD40-targeted mAb, was studied in a phase IA/II study in order to determine its maximum tolerated dose (MTD) and activity. Escalating doses of lucatumumab administered intravenously once weekly for 4 weeks of an 8-week cycle were administered in 37 patients with relapsed HL. Finally a MTD of 4 mg/kg and modest activity were manifested, necessitating further investigations to establish its benefits in the clinical setting [97].

7.7 CD80

CD80 (B7-1) is a co-stimulatory molecule aberrantly expressed on HRS. Various anti-CD80 mAbs have been developed, most of which have been studied in NHL, whereas only a few studies have addressed their implication in HL.

7.7.1 Galiximab (IDEC-114)

Galiximab, a chimeric mAb against CD80, has manifested favorable toxicity profile in NHL, while its activity in HL is rarely studied. Just recently, the Cancer and Leukemia Group B (CALGB) 50602 (Alliance) investigated its efficacy in highly refractory HL patients who had previously received a median of three prior regimens, 83 % failing after prior stem cell transplant. Disappointingly, an ORR as low as 10.3 % and only 1.6 months PFS was achieved, indicating its limited activity in heavily pretreated HL patients. However, galiximab was well tolerated [98].

7.8 Therapeutic Efficacy of Cytokines

Targeting cytokines in immunotherapy for HL is restricted due to a variety of challenges. Since structural properties, binding avidity, and retention time in the tumor tissue as well as the pharmacodynamics and pharmacokinetics are all affected by the optimal design of each individual domain in the fusion protein, an optimized molecular design is mandatory for efficient treatment with antibody-targeted cytokines [7]. HL sheds the targeted cell surface antigen CD30 in substantial amounts which competes with the tumor cell-bound Ag in binding, leading to substantial rise in serum levels of soluble CD30 (sCD30). Therefore, greater affinity to the solid-phase-bound Ag in the presence of high amounts of soluble Ag is needed. In addition, high systemic toxicity is observed with the administration of some cytokines, hence limiting their application for specifically targeted tumor tissues. As a result, when they are delivered by a targeting antibody fused to the cytokine, healthy tissues are left free from toxic cytokine concentrations. On the other hand, fusion to other protein domains may decrease functional properties of cytokines. Hence, the potentiality of the Ab-targeted cytokine depends on the binding avidity and the immunomodulatory capacity of the fusion protein [7]. Various cytokines are applied in this regard which are discussed herein.

7.8.1 Interleukin-2 (IL-2)

The first clinical trial on the administration of recombinant IL-2 (rIL-2) was conducted in 1984. Thereafter, several clinical trials have been conducted to examine the efficacy of rIL-2 with or without LAK cells in patients with refractory HL. Even though previous clinical trials have approached patients with relapsed or refractory HL which had poor prognosis, the results seemed promising. More recent studies have approached those requiring maintenance regimen alone or combined with other cytokines after high-dose chemotherapy. Intensification of immune-mediated effector mechanism holds great potential in reducing relapse rates after peripheral blood stem cell transplantation. Side effects were those expected from IFN-α and IL-2 when given as single agents, including fever, chills, fatigue, flu-like symptoms, anorexia, nausea, vomiting, and diarrhea which were transient and reversible. Prospective randomized studies are required to confirm the promising results of

combined IFN-α/r-IL-2 maintenance therapy after autologous bone marrow transplantation. The application of low-dose IL-2 expands and activates NK cells in both animal models and cancer patients [99, 100].

A therapeutic whole-cell vaccine consisting of IL-2 adsorbed onto aluminum hydroxide as cytokine-depot formulation exhibited potent antitumor immunity, induced delayed tumor growth, controlled tumor dissemination, and led to longer survival in mice challenged with A20 lymphoma. It proved to overcome the adverse effects of intratumoral Treg cells. However, clinical studies are mandated [101].

7.8.2 An IL-2-IL-12 Fusion Protein Targeting Hodgkin Lymphoma

It is hypothesized that an IL-12 polymorphism concomitant with Th2 polarization leading to decreased IL-12 production is a determinant of increased susceptibility in young adult HL [102]. Low IL-12 levels lead to reduced cellular immune response during the disease [103]; therefore, targeting IL-12 may reverse the situation locally in lymphoma lesions. In addition to the crucial role of IL-12 in stimulating cytotoxic T-cell and NK cell activities, it plays a predominant role in Ag processing and presentation. As a result, immunotherapies targeting IL-12 are considered to induce a strong cell-based immune response with enhanced tumor cell killing [104, 105] and an efficient antitumor response [106, 107]. Since a major limitation of current immunotherapy regimens is unintended activation of effector cells beyond target sites, attempts have been made to overcome this inadequacy. T and NK cells express upregulated amount of IL-2 and IL-12 receptors and transient CD30. Therefore, binding of anti-CD30 fusion proteins and unintended off-target effector cell activation may occur. Remarkably, the Ab-binding domains of these proteins bind to tumor cells with higher avidity, and these specific bindings are more resistant to blocking by soluble target Ag. Lines of evidence suggest that by simultaneous targeting of cooperating cytokines, a broad immune response is activated, hence providing a valuable response in cancer immunotherapy [7]. An overlapping activity in mustering T and NK cells has been described between IL-2 and IL-12 [108]; IL-2 leads to potent proliferation induction, while IL-12 stimulates cytokine secretion including IFN-c. A synergic effect between these two cytokines with respect to these functions is manifested, resulting in more efficient lysis of target cells [108]. In order to mobilize both adoptive and innate immune cells for an antitumor attack, IL-2 and IL-12 have been fused to an anti-CD30 scFv Ab; hence both cytokines are accumulated on the malignant $CD30^+$ HRS cells in the lymphoma lesion [7]. This dual cytokine-antibody fusion protein has revealed significant activity and superior efficacy in activating resting T and NK cells compared to the corresponding fusion proteins containing either IL-2 or IL-12 in mouse model [108]. Cytolysis in resting NK cells and reactivated IL-2-deprived T cells was induced by this dual cytokine protein, benefiting immunotherapy of HL. Interestingly, simultaneous application of both single-cytokine proteins was less effective in delivering both cytokines to the same cell at the same time. Using this technique facilitated dimerization of the molecule via integration of various domains, thus leading to favorable binding to solid-phase Ag, even in the presence of the soluble antigen, as well as higher and specific retention in the targeted tissues and lower tissue penetration *in vivo* studies. Overall, dimerized fusion proteins appear to be more suitable for site-specific immunotherapy compared to the corresponding monomeric proteins [7]. Due to the predominant expression of CD30 on activated Th2 cells [109] which are present in high numbers in the tissue of HL [110], the tumor environment of HL may be modulated by shifting T cells to Th1 reactivity by the application of anti-CD30-IL-12-IL-2 dual cytokine fusion protein. In addition, both types of cytolytic effector cells, T and NK cells, may be reactivated by simultaneous synergistic action of IL-2 and IL-12. It is hypothesized that IL-2 and IL-12 lead to NK and T-cell activation followed by increased IFN-c secretion and shift the Th2 imbalance in the lymphoma lesion toward Th1

reactivity; therefore counteracting T-cell hyporesponsiveness in HL [111].

After injection of tumor-targeted IL-12-IL-2 to immune-competent mice with established antigen-positive tumors, it accumulated at the tumor site and induced a remarkable tumor regression in mice model [7].

Even though antibody-cytokine fusion proteins have demonstrated clinical efficacy in phase II studies for the therapy of solid tumors [112], clinical trials on hematologic diseases are lacking. Various cytokine fusion proteins have been studied in preclinical models for the immunotherapy of HL.

Improved antitumor activity was achieved by Abs targeting IL-2 in the lymphoma lesions after systemic application compared with unconjugated IL-2 [113]. In addition, the combination of rituximab with the antibody-targeted IL-2 improved infiltration and activation of immune effector cells in lymphoma lesions, leading to eradication of those lesions that were not cured with rituximab alone. This could be explained by the sustained anti-lymphoma activity of rituximab by targeting IL-2 which acts through promoting the recruitment of NK cells and macrophages into lymphoma [113].

7.9 Bispecific Monoclonal Antibodies

Bispecific antibody-mediated activation of NK cells along with T cells in the lymphoma lesion has exhibited therapeutic effects [46]. It is well established that peripheral NK cells are inactive in HL, resulting in elevated serum levels of ligands engaging NKG2D (MICA) and NKp30 (BAG6/BAT3). Hence, immunotherapeutic strategies targeting NK cells were proposed. NK cell activation by the bispecific construct has been exclusively observed in the $CD30^+$ target cells, which is recognized as an efficient inducer of CD69 expression on NK cells. CD69 triggers cytotoxic activity and costimulates cytokine production via phosphotyrosine kinases of the Src family. Therefore, cytokines are activated toward HRS tumor cells by the application of antibody-cytokine fusion proteins. Bispecific antibody-mediated activation of NK cells along with T cells in lymphoma lesions demonstrated relative therapeutic effect; hence, local reactivation of the immune response seems to be beneficial in the therapy of HL [66, 67]. A recombinant bispecific antibody targeting CD30 on HL cells and the Fc receptor (CD64) on monocytes which triggers CD64-mediated effector functions has been developed and proved promising results [114]. Reiners et al. [9] developed a tetravalent bispecific Ab which targets CD30 on HRS cells and the activating receptor CD16A on NK cells, leading to selective cross-linking between tumor and NK cells. CD30xCD16A is a NKG2D/NKp30-independent bispecific antibody construct, which artificially links the tumor receptor CD30 with NK cells' cytotoxicity receptor, CD16A. Furthermore, it triggered activation of NK cells and restoration of cytotoxicity against HL target cells [9]. Moreover, AFM 13, another bispecific Ab construct which simultaneously targets CD16A on effector cells and CD30 on malignant cells, is proven to maintain NK cell function [66]. The tetravalent binding construction, consisting of two polypeptides pairing head-to-tail with each other, leads to a high avidity parallel to that of IgG. Fc-mediated side effects observed with HRS-3/A9, the previous CD30-CD16 targeting Ab, is circumvented by its distinctive nature, as it is solely comprised of variable domains. A favorable outcome in terms of restoring the function of NK cells has been observed with the administration of a bivalent CD16-CD30 mAb against xenotransplanted solid human HL [60]. CD16 is a low-affinity IgG receptor on the surface of NK cells, which leads to ADCC through degranulation of NK cells and enhances target cell lysis [115] (Table 7.2).

7.10 Novel Immunotherapeutic Treatment Strategies in HL

It has been postulated that eradication of human cancers may be accomplished by combining cancer treatment modalities [117]. Lack of specificity is acknowledged as the major shortcoming of conventional cancer therapies [118]. Promising results have been yielded by combined immunotherapy and conventional treatment pro-

Table 7.2 Bispecific monoclonal antibodies in HL

Bispecific antibody	Antibody	Year	Reference	Notable features
HRS-3/A9	CD16-CD30	1997	[66]	Limited efficacy
AFM13	CD16-CD30	1997	[66]	Maintains NK cell function
Blinatumomab	CD19-CD3	2000	[116]	Efficient anti-lymphoma cytotoxicity at extremely low doses (10 pg/ml, 100,000-folds lower than rituximab
CD30xCD64	CD30-CD64	2009	[114]	Promising results
CD30xCD16A	CD16-CD30	2013	[9]	

cedures. Due to their different therapeutic mechanism, side effects differ, and toxicity is not elevated [119]. In addition, the combination is proven to yield a synergic effect [120]. Furthermore, Abs are considered ideal vehicles for drug and radionuclide delivery due to their high specificity [120]. Multiple clinical trials have been conducted in this regard, yet their clinical applications need to be established.

7.10.1 Immunotoxins

In order to overcome the low specificity of conventional therapies, immunotoxins were developed, consisting of two sections: a cell-binding part combined with a cell death-inducing agent [118]. In the attempt to develop less immunogenic compounds, the fourth generation of immunotoxins were developed by Mathew and Verma in 2009. In this new generation, both comprising moieties (the cell-binding ligand and proapoptotic enzyme) are humanized [121]. Granzymes, which are effective serine proteases, are considered exquisite candidates for antitumor immunotherapy. Granzyme B (GrB) is recognized as the most effective subtype [122]. It is found to effectively invade transformed tumor cells and virus-infected cells [123]; nonetheless, PI-9, co-expressed in a variety of cancer cells including cHL [124], is known to irreversibly inhibit its effect [125].

7.11 Concluding Remarks

Overall, the emergence of immunotherapy and its ongoing development has resulted in immense improvement in the outcome of patients with HL. It has the potential to replace all other conventional treatment modalities and has changed the insight to the prognosis of lymphomas. However, studies are ongoing and hold the hope to achieving optimal disease outcome in the near future.

References

1. Lunning MA, Vose JM. Management of indolent lymphoma: where are we now and where are we going. Blood Rev. 2012;26(6):279–88.
2. Czuczman MS, Weaver R, Alkuzweny B, Berlfein J, Grillo-López AJ. Prolonged clinical and molecular remission in patients with low-grade or follicular non-Hodgkin's lymphoma treated with rituximab plus CHOP chemotherapy: 9-year follow-up. J Clin Oncol. 2004;22(23):4711–6.
3. Robert N, Leyland-Jones B, Asmar L, Belt R, Ilegbodu D, Loesch D, et al. Randomized phase III study of trastuzumab, paclitaxel, and carboplatin compared with trastuzumab and paclitaxel in women with HER-2–overexpressing metastatic breast cancer. J Clin Oncol. 2006;24(18):2786–92.
4. Konjevic G, Jurisic V, Jovic V, Vuletic A, Martinovic KM, Radenkovic S, et al. Investigation of NK cell function and their modulation in different malignancies. Immunol Res. 2012;52(1–2):139–56.
5. Gerber H-P. Emerging immunotherapies targeting CD30 in Hodgkin's lymphoma. Biochem Pharmacol. 2010;79(11):1544–52.
6. Carter PJ. Potent antibody therapeutics by design. Nat Rev Immunol. 2006;6(5):343–57.
7. Jahn T, Zuther M, Friedrichs B, Heuser C, Guhlke S, Abken H, et al. An Il12-Il2-antibody fusion protein targeting Hodgkin's lymphoma cells potentiates activation of Nk and T cells for an anti-tumor attack. PLoS One. 2012;7(9):e44482.
8. O'Shea JJ, Holland SM, Staudt LM. JAKs and STATs in immunity, immunodeficiency, and cancer. N Engl J Med. 2013;368(2):161–70.
9. Reiners KS, Kessler J, Sauer M, Rothe A, Hansen HP, Reusch U, et al. Rescue of impaired NK cell activity in Hodgkin's lymphoma with bispecific antibodies in vitro and in patients. Mol Ther. 2013;21(4):895–903.

10. Marshall NA, Christie LE, Munro LR, Culligan DJ, Johnston PW, Barker RN, et al. Immunosuppressive regulatory T cells are abundant in the reactive lymphocytes of Hodgkin's lymphoma. Blood. 2004;103(5):1755–62.
11. Skinnider BF, Mak TW. The role of cytokines in classical Hodgkin's lymphoma. Blood. 2002;99(12):4283–97.
12. Schmitz R, Stanelle J, Hansmann M-L, Küppers R. Pathogenesis of classical and lymphocyte-predominant Hodgkin's lymphoma. Ann Rev Pathol Mech Dis. 2009;4:151–74.
13. Brune V, Tiacci E, Pfeil I, Döring C, Eckerle S, van Noesel CJ, et al. Origin and pathogenesis of nodular lymphocyte–predominant Hodgkin's lymphoma as revealed by global gene expression analysis. J Exp Med. 2008;205(10):2251–68.
14. Re D, Hofmann A, Wolf J, Diehl V, Staratschek-Jox A. Cultivated H-RS cells are resistant to CD95L-mediated apoptosis despite expression of wild-type CD95. Exp Hematol. 2000;28(1):31–5.
15. Krockenberger M, Dombrowski Y, Weidler C, Ossadnik M, Hönig A, Häusler S, et al. Macrophage migration inhibitory factor contributes to the immune escape of ovarian cancer by down-regulating NKG2D. J Immunol. 2008;180(11):7338–48.
16. Mamessier E, Sylvain A, Bertucci F, Castellano R, Finetti P, Houvenaeghel G, et al. Human breast tumor cells induce self-tolerance mechanisms to avoid NKG2D-mediated and DNAM-mediated NK cell recognition. Cancer Res. 2011;71(21):6621–32.
17. Zocchi MR, Catellani S, Canevali P, Tavella S, Garuti A, Villaggio B, et al. High ERp5/ADAM10 expression in lymph node microenvironment and impaired NKG2D ligands recognition in Hodgkin's lymphomas. Blood. 2012;119(6):1479–89.
18. Waldhauer I, Goehlsdorf D, Gieseke F, Weinschenk T, Wittenbrink M, Ludwig A, et al. Tumor-associated MICA is shed by ADAM proteases. Cancer Res. 2008;68(15):6368–76.
19. Jinushi M, Takehara T, Tatsumi T, Kanto T, Groh V, Spies T, et al. Expression and role of MICA and MICB in human hepatocellular carcinomas and their regulation by retinoic acid. Int J Cancer. 2003;104(3):354–61.
20. Lamprecht B, Walter K, Kreher S, Kumar R, Hummel M, Lenze D, et al. Derepression of an endogenous long terminal repeat activates the CSF1R proto-oncogene in human lymphoma. Nat Med. 2010;16(5):571–9.
21. Stanelle J, Döring C, Hansmann M-L, Küppers R. Mechanisms of aberrant GATA3 expression in classical Hodgkin's lymphoma and its consequences for the cytokine profile of Hodgkin's and Reed/Sternberg cells. Blood. 2010;116(20):4202–11.
22. Oflazoglu E, Stone IJ, Gordon KA, Grewal IS, van Rooijen N, Law C-L, et al. Macrophages contribute to the antitumor activity of the anti-CD30 antibody SGN-30. Blood. 2007;110(13):4370–2.
23. Spina D, Leoncini L, Close P, Megha T, Pacenti L, Tosi P, et al. Growth vs. DNA strand breaks in Hodgkin's disease: impaired proliferative ability of Hodgkin's and Reed-Sternberg cells. Int J Cancer. 1996;66(2):179–83.
24. Klimm B, Schnell R, Diehl V, Engert A. Current treatment and immunotherapy of Hodgkin's lymphoma. Haematologica. 2005;90(12):1680–92.
25. Epenetos AA, Snook D, Durbin H, Johnson PM, Taylor-Papadimitriou J. Limitations of radiolabeled monoclonal antibodies for localization of human neoplasms. Cancer Res. 1986;46(6):3183–91.
26. Smith CA, Gruss H-J, Davis T, Anderson D, Farrah T, Baker E, et al. CD30 antigen, a marker for Hodgkin's lymphoma, is a receptor whose ligand defines an emerging family of cytokines with homology to TNF. Cell. 1993;73(7):1349–60.
27. Al-Shamkhani A. The role of CD30 in the pathogenesis of haematopoietic malignancies. Curr Opin Pharmacol. 2004;4(4):355–9.
28. Horie R, Aizawa S, Nagai M, Ito K, Higashihara M, Ishida T, et al. A novel domain in the CD30 cytoplasmic tail mediates NFkappaB activation. Int Immunol. 1998;10(2):203–10.
29. Duckett CS, Gedrich RW, Gilfillan MC, Thompson CB. Induction of nuclear factor kappaB by the CD30 receptor is mediated by TRAF1 and TRAF2. Mol Cell Biol. 1997;17(3):1535–42.
30. Hsu P-L, Hsu S-M. Autocrine growth regulation of CD30 ligand in CD30-expressing Reed-Sternberg cells: distinction between Hodgkin's disease and anaplastic large cell lymphoma. Lab Invest. 2000;80(7):1111–9.
31. Mathas S, Lietz A, Anagnostopoulos I, Hummel F, Wiesner B, Janz M, et al. c-FLIP mediates resistance of Hodgkin/Reed-Sternberg cells to death receptor–induced apoptosis. J Exp Med. 2004;199(8):1041–52.
32. Kashkar H, Haefs C, Shin H, Hamilton-Dutoit SJ, Salvesen GS, Krönke M, et al. XIAP-mediated caspase inhibition in Hodgkin's lymphoma–derived B cells. J Exp Med. 2003;198(2):341–7.
33. García JF, Camacho FI, Morente M, Fraga M, Montalbán C, Álvaro T, et al. Hodgkin's and Reed-Sternberg cells harbor alterations in the major tumor suppressor pathways and cell-cycle checkpoints: analyses using tissue microarrays. Blood. 2003;101(2):681–9.
34. Horie R, Higashihara M, Watanabe T. Hodgkin's lymphoma and CD30 signal transduction. Int J Hematol. 2003;77(1):37–47.
35. Zheng B, Fiumara P, Li YV, Georgakis G, Snell V, Younes M, et al. MEK/ERK pathway is aberrantly active in Hodgkin's disease: a signaling pathway shared by CD30, CD40, and RANK that regulates cell proliferation and survival. Blood. 2003;102(3):1019–27.
36. Mathas S, Hinz M, Anagnostopoulos I, Krappmann D, Lietz A, Jundt F, et al. Aberrantly expressed c-Jun and JunB are a hallmark of Hodgkin lymphoma cells, stimulate proliferation and synergize with NF-κB. EMBO J. 2002;21(15):4104–13.
37. Georgakis GV, Li Y, Rassidakis GZ, Medeiros LJ, Mills GB, Younes A. Inhibition of the phosphatidylinositol-3 kinase/Akt promotes G1 cell cycle arrest

and apoptosis in Hodgkin lymphoma. Br J Haematol. 2006;132(4):503–11.
38. Ishida T, Ishii T, Inagaki A, Yano H, Komatsu H, Iida S, et al. Specific recruitment of CC chemokine receptor 4–positive regulatory T cells in Hodgkin lymphoma fosters immune privilege. Cancer Res. 2006;66(11):5716–22.
39. Ma Y, Visser L, Blokzijl T, Harms G, Gcaron Ç, dem Atayar SP, et al. The CD4+ CD26− T-cell population in classical Hodgkin's lymphoma displays a distinctive regulatory T-cell profile. Lab Invest. 2008;88(5):482–90.
40. Diepstra A, van Imhoff GW, Karim-Kos HE, van den Berg A, te Meerman GJ, Niens M, et al. HLA class II expression by Hodgkin's Reed-Sternberg cells is an independent prognostic factor in classical Hodgkin's lymphoma. J Clin Oncol. 2007;25(21):3101–8.
41. Atayar Ç, Poppema S, Visser L, van den Berg A. Cytokine gene expression profile distinguishes CD4+/CD57+ T cells of the nodular lymphocyte predominance type of Hodgkin's lymphoma from their tonsillar counterparts. J Pathol. 2006;208(3):423–30.
42. Tanijiri T, Shimizu T, Uehira K, Yokoi T, Amuro H, Sugimoto H, et al. Hodgkin's Reed-Sternberg cell line (KM-H2) promotes a bidirectional differentiation of CD4+ CD25+ Foxp3+ T cells and CD4+ cytotoxic T lymphocytes from CD4+ naive T cells. J Leukoc Biol. 2007;82(3):576–84.
43. Chemnitz JM, Eggle D, Driesen J, Classen S, Riley JL, Debey-Pascher S, et al. RNA fingerprints provide direct evidence for the inhibitory role of TGFβ and PD-1 on CD4+ T cells in Hodgkin lymphoma. Blood. 2007;110(9):3226–33.
44. Juszczynski P, Ouyang J, Monti S, Rodig SJ, Takeyama K, Abramson J, et al. The AP1-dependent secretion of galectin-1 by Reed–Sternberg cells fosters immune privilege in classical Hodgkin lymphoma. Proc Natl Acad Sci. 2007;104(32):13134–9.
45. Yamamoto R, Nishikori M, Kitawaki T, Sakai T, Hishizawa M, Tashima M, et al. PD-1–PD-1 ligand interaction contributes to immunosuppressive microenvironment of Hodgkin lymphoma. Blood. 2008;111(6):3220–4.
46. Driessens G, Kline J, Gajewski TF. Costimulatory and coinhibitory receptors in anti-tumor immunity. Immunol Rev. 2009;229(1):126–44.
47. Re D, Thomas RK, Behringer K, Diehl V. From Hodgkin disease to Hodgkin lymphoma: biologic insights and therapeutic potential. Blood. 2005;105(12):4553–60.
48. Renukaradhya GJ, Khan MA, Vieira M, Du W, Gervay-Hague J, Brutkiewicz RR. Type I NKT cells protect (and type II NKT cells suppress) the host's innate antitumor immune response to a B-cell lymphoma. Blood. 2008;111(12):5637–45.
49. Xu C, de Vries R, Visser L, Diepstra A, Gadola SD, Poppema S, et al. Expression of CD1d and presence of invariant NKT cells in classical Hodgkin lymphoma. Am J Hematol. 2010;85(7):539–41.
50. Song L, Asgharzadeh S, Salo J, Engell K, Wu HW, Sposto R, Ara T, Silverman AM, DeClerck YA, Seeger RC, Metelitsa LS. Valpha24-invariant NKT cells mediate anti-tumor activity via killing of tumor-associated macrophages. J Clin Invest. 2009;119:1524–36.
51. Steidl C, Lee T, Shah SP, Farinha P, Han G, Nayar T, et al. Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. N Engl J Med. 2010;362(10):875–85.
52. Metelitsa LS. Anti-tumor potential of type-I NKT cells against CD1d-positive and CD1d-negative tumors in humans. Clin Immunol. 2011;140(2):119–29.
53. Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, Lanier LL, et al. Innate or adaptive immunity? The example of natural killer cells. Science. 2011;331(6013):44–9.
54. Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature. 1975;256(5517):495–7.
55. van Meerten T, Hagenbeek A. Novel antibodies against follicular non-Hodgkin's lymphoma. Best Pract Res Clin Haematol. 2011;24(2):231–56.
56. Ehrlich P. The relationship existing between chemical constitution, distribution, and pharmacological action. The collected papers of Paul Ehrlich. 1956;1:596–618
57. Reichert JM, Valge-Archer VE. Development trends for monoclonal antibody cancer therapeutics. Nat Rev Drug Discov. 2007;6(5):349–56.
58. Waldmann TA, Strober W. Metabolism of immunoglobulins. Prog Allergy. 1968;13:1–110.
59. Peipp M, van de Winkel JG, Valerius T. Molecular engineering to improve antibodies' anti-lymphoma activity. Best Pract Res Clin Haematol. 2011;24(2):217–29.
60. Yurchenko M, Sidorenko S. Hodgkin's lymphoma: the role of cell surface receptors in regulation of tumor cell fate. Exp Oncol. 2010;32(4):214–23.
61. Kaudewitz P, Stein H, Burg G, Mason DY, Braun-Falco O. Atypical cells in lymphomatoid papulosis express the Hodgkin cell-associated antigen Ki-1. J Invest Dermatol. 1986;86(4):350–4.
62. Chiarle R, Podda A, Prolla G, Gong J, Thorbecke GJ, Inghirami G. CD30 in normal and neoplastic cells. Clin Immunol. 1999;90(2):157–64.
63. Falini B, Flenghi L, Aversa F, Barbabietola G, Martelli M, Comeli P, et al. Response of refractory Hodgkin's disease to monoclonal anti-CD30 immunotoxin. Lancet. 1992;339(8803):1195–6.
64. Deutsch YE, Tadmor T, Podack ER, Rosenblatt JD. CD30: an important new target in hematologic malignancies. Leuk Lymphoma. 2011;52(9):1641–54.
65. Kennedy-Nasser AA, Hanley P, Bollard CM. Hodgkin's disease and the role of the immune system. Pediatr Hematol Oncol. 2011;28(3):176–86.
66. Hartmann F, Renner C, Jung W, Deisting C, Juwana M, Eichentopf B, et al. Treatment of refractory Hodgkin's disease with an anti-CD16/CD30 bispecific antibody. Blood. 1997;89(6):2042–7.

67. Hartmann F, Renner C, Jung W, da Costa L, Tembrink S, Held G, et al. Anti-CD16/CD30 bispecific antibody treatment for Hodgkin's disease role of infusion schedule and costimulation with cytokines. Clin Cancer Res. 2001;7(7):1873–81.
68. Borchmann P, Schnell R, Fuss I, Manzke O, Davis T, Lewis LD, et al. Phase 1 trial of the novel bispecific molecule H22xKi-4 in patients with refractory Hodgkin lymphoma. Blood. 2002;100(9):3101–7.
69. Kaudewitz P, Burg G, Stein H, Klepzig K, Mason D, Braun-Falco O. Monoclonal antibody patterns in lymphomatoid papulosis. Dermatol Clin. 1985;3(4):749.
70. Schnell R, Dietlein M, Staak JO, Borchmann P, Schomaecker K, Fischer T, et al. Treatment of refractory Hodgkin's lymphoma patients with an iodine-131–labeled murine anti-CD30 monoclonal antibody. J Clin Oncol. 2005;23(21):4669–78.
71. Diefenbach CS, Leonard JP. Targeting CD30 in Hodgkin lymphoma: antibody-drug conjugates make a difference. Am Clin Oncol Educ Book. 2012;32:162–6.
72. Ansell SM, Horwitz SM, Engert A, Khan KD, Lin T, Strair R, et al. Phase I/II study of an anti-CD30 monoclonal antibody (MDX-060) in Hodgkin's lymphoma and anaplastic large-cell lymphoma. J Clin Oncol. 2007;25(19):2764–9.
73. Younes A, Bartlett NL, Leonard JP, Kennedy DA, Lynch CM, Sievers EL, et al. Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas. N Engl J Med. 2010;363(19):1812–21.
74. Cardarelli PM, Moldovan-Loomis M-C, Preston B, Black A, Passmore D, Chen T-H, et al. In vitro and in vivo characterization of MDX-1401 for therapy of malignant lymphoma. Clin Cancer Res. 2009;15(10):3376–83.
75. Borchmann P, Treml JF, Hansen H, Gottstein C, Schnell R, Staak O, et al. The human anti-CD30 antibody 5F11 shows in vitro and in vivo activity against malignant lymphoma. Blood. 2003;102(10):3737–42.
76. Gruss H, Boiani N, Williams D, Armitage R, Smith C, Goodwin R. Pleiotropic effects of the CD30 ligand on CD30-expressing cells and lymphoma cell lines. Blood. 1994;83(8):2045–56.
77. Gruss H-J, DaSilva N, HU Z-B, Uphoff C, Goodwin R, Drexler H. Expression and regulation of CD30 ligand and CD30 in human leukemia-lymphoma cell lines. Leukemia. 1994;8(12):2083–94.
78. Heuck F, Ellermann J, Borchmann P, Rothe A, Hansen H, Engert A, et al. Combination of the human anti-CD30 antibody 5F11 with cytostatic drugs enhances its antitumor activity against Hodgkin and anaplastic large cell lymphoma cell lines. J Immunother. 2004;27(5):347–53.
79. Horn-Lohrens O, Tiemann M, Lange H, Kobarg J, Hafner M, Hansen H, et al. Shedding of the soluble form of CD30 from the Hodgkin-analogous cell line L540 is strongly inhibited by a new CD30-specific antibody (Ki-4). Int J Cancer. 1995;60(4):539–44.
80. Zambello R, Trentin L, Facco M, Siviero M, Galvan S, Piazza F, et al. Analysis of TNF-receptor and ligand superfamily molecules in patients with lymphoproliferative disease of granular lymphocytes presented in part in abstract form at the 87th Meeting of the American Association for Cancer Research, San Diego, CA, April 1997 (abstract no. 3698). Blood. 2000;96(2):647–54.
81. Franke AC, Jung D, Ellis TM. Characterization of the CD30L binding domain on the human CD30 molecule using anti-CD30 antibodies. Hybridoma. 2000;19(1):43–8.
82. Mir SS, Richter BW, Duckett CS. Differential effects of CD30 activation in anaplastic large cell lymphoma and Hodgkin disease cells. Blood. 2000;96(13):4307–12.
83. Wahl AF, Klussman K, Thompson JD, Chen JH, Francisco LV, Risdon G, et al. The anti-CD30 monoclonal antibody SGN-30 promotes growth arrest and DNA fragmentation in vitro and affects antitumor activity in models of Hodgkin's disease. Cancer Res. 2002;62(13):3736–42.
84. Cerveny C, Law C, McCormick R, Lenox J, Hamblett K, Westendorf L, et al. Signaling via the anti-CD30 mAb SGN-30 sensitizes Hodgkin's disease cells to conventional chemotherapeutics. Leukemia. 2005;19(9):1648–55.
85. Bartlett NL, Younes A, Carabasi MH, Forero A, Rosenblatt JD, Leonard JP, et al. A phase 1 multidose study of SGN-30 immunotherapy in patients with refractory or recurrent CD30+ hematologic malignancies. Blood. 2008;111(4):1848–54.
86. Forero-Torres A, Leonard JP, Younes A, Rosenblatt JD, Brice P, Bartlett NL, et al. A Phase II study of SGN-30 (anti-CD30 mAb) in Hodgkin lymphoma or systemic anaplastic large cell lymphoma. Br J Haematol. 2009;146(2):171–9.
87. Blum KA, Jung S, Johnson JL, et al. Serious pulmonary toxicity in patients with Hodgkin's lymphoma with SGN-30, gemcitabine, vinorelbine, and liposomal doxorubicin is associated with an Fc{gamma} RIIIa-158 V/F polymorphism. Ann Oncol. 2010;21(11):2246–54.
88. Berinstein N, Grillo-Lopez A, White C, Bence-Bruckler I, Maloney D, Czuczman M, et al. Association of serum Rituximab (IDEC-C2B8) concentration and anti-tumor response in the treatment of recurrent low-grade or follicular non-Hodgkin's lymphoma. Ann Oncol. 1998;9(9):995–1001.
89. Fromm JR, McEarchern JA, Kennedy D, Thomas A, Shustov AR, Gopal AK. Clinical binding properties, internalization kinetics, and clinicopathologic activity of brentuximab vedotin: an antibody-drug conjugate for CD30-positive lymphoid neoplasms. Clin Lymphoma Myeloma Leuk. 2012;12(4):280.
90. Theurich S, Malcher J, Wennhold K, Shimabukuro-Vornhagen A, Chemnitz J, Holtick U, et al. Brentuximab vedotin combined with donor lymphocyte infusions for early relapse of Hodgkin lymphoma after allogeneic stem-cell transplantation induces tumor-specific immunity and sustained clinical remission. J Clin Oncol. 2013;31(5):e59–63.

91. Fanale M. Lymphocyte-predominant Hodgkin lymphoma: what is the optimal treatment? ASH Educ Program Book. 2013;2013(1):406–13.
92. Advani RH, Hoppe RT. How I treat nodular lymphocyte predominant Hodgkin lymphoma. Blood. 2013;122(26):4182–8.
93. Advani R, Horning S, Hoppe R, Daadi S, Allen J, Natkunam Y, editors. Frontline therapy of nodular lymphocyte predominant Hodgkin lymphoma with rituximab. The Stanford University Experience. ASH annual meeting abstracts. 2011;118(21):2686
94. Schulz H, Rehwald U, Morschhauser F, Elter T, Driessen C, Rüdiger T, et al. Rituximab in relapsed lymphocyte-predominant Hodgkin lymphoma: long-term results of a phase 2 trial by the German Hodgkin Lymphoma Study Group (GHSG). Blood. 2008;111(1):109–11.
95. Jacene HA, Kasamon YL, Ambinder RF, Kasecamp W, Serena D, Wahl RL, editors. Phase I/II dose-escalation study of tositumomab and iodine I 131 tositumomab for relapsed/refractory classical or lymphocyte-predominant Hodgkin's lymphoma: feasibility and initial safety. Blood. 2008;112(11):10512
96. Hock BD, McKenzie JL, Patton NW, Drayson M, Taylor K, Wakeman C, et al. Circulating levels and clinical significance of soluble CD40 in patients with hematologic malignancies. Cancer. 2006;106(10):2148–57.
97. Fanale M, Assouline S, Kuruvilla J, Solal-Céligny P, Heo DS, Verhoef G, et al. Phase IA/II, multicentre, open-label study of the CD40 antagonistic monoclonal antibody lucatumumab in adult patients with advanced non-Hodgkin or Hodgkin lymphoma. Br J Haematol. 2014;164(2):258–65.
98. Smith SM, Schöder H, Johnson JL, Jung S-H, Bartlett NL, Cheson BD. The anti-CD80 primatized monoclonal antibody, galiximab, is well-tolerated but has limited activity in relapsed Hodgkin lymphoma: Cancer and Leukemia Group B 50602 (Alliance). Leuk Lymph. 2013;54(7):1405–10.
99. Gluck WL, Hurst D, Yuen A, Levine AM, Dayton MA, Gockerman JP, et al. Phase I studies of interleukin (IL)-2 and rituximab in B-cell non-Hodgkin's lymphoma IL-2 mediated natural killer cell expansion correlations with clinical response. Clin Cancer Res. 2004;10(7):2253–64.
100. Eisenbeis CF, Grainger A, Fischer B, Baiocchi RA, Carrodeguas L, Roychowdhury S, et al. Combination immunotherapy of B-cell non-Hodgkin's lymphoma with rituximab and interleukin-2 a preclinical and phase I study. Clin Cancer Res. 2004;10(18):6101–10.
101. Grille S, Brugnini A, Nese M, Corley E, Falkenberg FW, Lens D, et al. A B-cell lymphoma vaccine using a depot formulation of interleukin-2 induces potent antitumor immunity despite increased numbers of intratumoral regulatory T cells. Cancer Immunol Immunother. 2010;59(4):519–27.
102. Cozen W, Gill PS, Salam MT, Nieters A, Masood R, Cockburn MG, et al. Interleukin-2, interleukin-12, and interferon-γ levels and risk of young adult Hodgkin lymphoma. Blood. 2008;111(7):3377–82.
103. Slivnick DJ, Ellis TM, Nawrocki JF, Fisher RI. The impact of Hodgkin's disease on the immune system. Semin Oncol. 1990;17(6):673–82.
104. Heuser C, Diehl V, Abken H, Hombach A. Anti-CD30-IL-12 antibodycytokine fusion protein that induces IFN-gamma secretion of T cells and NK cell-mediated lysis of Hodgkin's lymphoma-derived tumor cells. Int J Cancer. 2003;106:545–52.
105. Rooney C, Bollard C, Huls M, Gahn B, Gottschalk S, Wagner H, et al. Immunotherapy for Hodgkin's disease. Ann Hematol. 2002;81:S39.
106. Wang B, Xiong Q, Shi Q, Le X, Xie K. Genetic disruption of host interferon-gamma drastically enhances the metastasis of pancreatic adenocarcinoma through impaired expression of inducible nitric oxide synthase. Oncogene. 2001;20:6930–7.
107. Qin Z, Schwartzkopff J, Pradera F, Kammertoens T, Seliger B, et al. A critical requirement of interferon gamma-mediated angiostasis for tumor rejection by CD8+ T cells. Cancer Res. 2003;2003:4095–100.
108. Hombach A, Pohl C, Heuser C, Sircar R, Koch D, Diehl V, et al. Generation of the single chain antibody fragment conserves the idiotypic profile of the anti-CD30 monoclonal antibody HRS3. Scand J Immunol. 1998;48(5):497–501.
109. Toennies HM, Green JM, Arch RH. Expression of CD30 and Ox40 on T lymphocyte subsets is controlled by distinct regulatory mechanisms. J Leukoc Biol. 2004;75(2):350–7.
110. Schreck S, Friebel D, Buettner M, Distel L, Grabenbauer G, Young LS, et al. Prognostic impact of tumour-infiltrating Th2 and regulatory T cells in classical Hodgkin lymphoma. Hematol Oncol. 2009;27(1):31–9.
111. C-y W, Ferrante J, Gately MK, Magram J. Characterization of IL-12 receptor beta1 chain (IL-12Rbeta1)-deficient mice: IL-12Rbeta1 is an essential component of the functional mouse IL-12 receptor. J Immunol. 1997;159(4):1658–65.
112. Shusterman S, London WB, Gillies SD, Hank JA, Voss SD, Seeger RC, et al. Antitumor activity of hu14. 18-IL2 in patients with relapsed/refractory neuroblastoma: a Children's Oncology Group (COG) phase II study. J Clin Oncol. 2010;28(33):4969–75.
113. Schliemann C, Palumbo A, Zuberbühler K, Villa A, Kaspar M, Trachsel E, et al. Complete eradication of human B-cell lymphoma xenografts using rituximab in combination with the immunocytokine L19-IL2. Blood. 2009;113(10):2275–83.
114. Ranft K, Thepen T, Fischer R, Barth S, Stöcker M. Recombinant bispecific single chain antibody fragments induce Fc gamma-receptor-mediated elimination of CD30+ lymphoma cells. Cancer Lett. 2009;282:187–94.
115. Lanier LL. Up on the tightrope: natural killer cell activation and inhibition. Nat Immunol. 2008;9(5):495–502.

116. Löffler A, Kufer P, Lutterbüse R, Zettl F, Daniel PT, Schwenkenbecher JM, et al. A recombinant bispecific single-chain antibody, CD19× CD3, induces rapid and high lymphoma-directed cytotoxicity by unstimulated T lymphocytes. Blood. 2000;95(6):2098–103.
117. Lundqvist A, Berg M, Smith A, Childs RW. Bortezomib treatment to potentiate the anti-tumor immunity of ex-vivo expanded adoptively infused autologous natural killer cells. J Cancer. 2011;2:383.
118. Schiffer S, Hansen H, Hehmann-Titt G, Huhn M, Fischer R, Barth S, et al. Efficacy of an adapted granzyme B-based anti-CD30 cytolytic fusion protein against PI-9-positive classical Hodgkin lymphoma cells in a murine model. Blood Cancer J. 2013;3(3):e106.
119. Maunch PM, Armitage JO, Diehl V, Hoppe RT, Weiss LM, editors. Hodgkin's disease. New York: Lippincott Williams & Wilkins; 1999.
120. Chinn P, Braslawsky G, White C, Hanna N. Antibody therapy of non-Hodgkin's B-cell lymphoma. Cancer Immunol Immunother. 2003;52(5):257–80.
121. Mathew M, Verma RS. Humanized immunotoxins: a new generation of immunotoxins for targeted cancer therapy. Cancer Sci. 2009;100(8):1359–65.
122. Kanatani I, Lin X, Yuan X, Manorek G, Shang X, Cheung LH, et al. Targeting granzyme B to tumor cells using a yoked human chorionic gonadotropin. Cancer Chemother Pharmacol. 2011;68(4):979–90.
123. Veugelers K, Motyka B, Goping S, Shostak I, Sawchuk T, Bleackley RC. Granule-mediated killing by granzyme B and perforin requires a mannose 6-phosphate receptor and is augmented by cell surface heparan sulfate. Mol Biol Cell. 2006;17(2):623–33.
124. Bladergroen BA, Meijer CJ, Rosita L, Hack CE, Muris JJ, Dukers DF, et al. Expression of the granzyme B inhibitor, protease inhibitor 9, by tumor cells in patients with non-Hodgkin and Hodgkin lymphoma: a novel protective mechanism for tumor cells to circumvent the immune system? Blood. 2002;99(1):232–7.
125. Jiang X, Ellison S, Alarid E, Shapiro D. Interplay between the levels of estrogen and estrogen receptor controls the level of the granzyme inhibitor, proteinase inhibitor 9 and susceptibility to immune surveillance by natural killer cells. Oncogene. 2007;26(28):4106–14.

Immunopathology and Immunotherapy of Non-Hodgkin Lymphoma

8

Maryam Ebadi, Nishitha M. Reddy, and Nima Rezaei

Contents

M. Ebadi, MD
Department of Immunology,
Molecular Immunology Research Center,
Children's Medical Center, Tehran University
of Medical Sciences, Tehran 14194, Iran
e-mail: mary.ebadi@yahoo.com

N.M. Reddy, MBBS, MSCI
Hematology and Stem Cell Transplantation Section,
Division of Hematology/Oncology, Department
of Medicine, Vanderbilt-Ingram Cancer Center,
Vanderbilt University Medical Center,
Nashville, TN 37232, USA
e-mail: Nishitha.reddy@vanderbilt.edu

N. Rezaei, MD, PhD (✉)
Research Center for Immunodeficiencies,
Children's Medical Center, Pediatrics Center
of Excellence, Tehran University of Medical
Sciences, Tehran 14194, Iran

Department of Immunology, Molecular Immunology
Research Center, School of Medicine, Tehran
University of Medical Sciences, Tehran 14194, Iran

Universal Scientific Education and Research
Network (USERN), Tehran, Iran
e-mail: Rezaei_nima@tums.ac.ir

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_8, © Springer-Verlag Berlin Heidelberg 2015

8.1 Introduction

The majority of patients with early-stage non-Hodgkin lymphoma (NHL) experience recovery by conventional chemo- and/or radiotherapy, yet combined chemotherapy and radiotherapy pose a significant morbidity to NHL patients. Cytotoxic chemotherapy cocktails were the treatment of choice in patients with NHL [1]. Disease relapse resulting from the development of resistant cell clones comprises a considerable proportion. Therefore, alternative techniques are warranted to overcome these limitations. Monoclonal antibodies (mAbs) have emerged as promising anticancer therapies over the last several years [2, 3]. After the emergence of hybridoma technology by Köhler and Milstein [4], mAbs against B-cell-related antigens (Ags), including CD19 and CD20, were among the foremost mAbs developed [5]. Albeit their rapid move towards clinical studies, results were disappointing. In an attempt to overcome the encountered limitations, chimerized murine antibodies (Abs) were developed [4]. With respect to recruiting human effector cells for Antibody-dependent cell-mediated cytotoxicity (ADCC) and triggering complement-dependent cytotoxicity (CDC), functional studies revealed that human Fc portion was significantly more effective than most of their murine counterparts [6]. Their extended serum half-life compared to murine Abs could be explained by identification of FcR and its function in IgG homeostasis [6]. Various effector functions including ADCC, CDC, and direct apoptosis induction are mediated by mAbs [7]. Novel therapies are hinting towards immunotherapy-based treatments. Various antigens expressed by the tumor have been targets of antibody-based immunotherapy, including CD20, CD30, CD40, IL13 receptor, RANK ligand, and DR4 [8]. The efficacy of antibody-based immunotherapy has been enhanced by a variety of approaches including radioimmunoconjugation and antibody–cytokine and antibody–toxin conjugation, in addition to biphasic Abs [9].

The treatment paradigm of NHL has considerably improved during the last two decades [10]. Among the four broad treatment modalities, immunotherapy is gaining superiority [9], and active immunotherapy has yielded promising results [11], possibly explained by the exquisite specificity of Abs in NHL. In this chapter, we aim to tackle immunotherapy and immunopathology of the wide clinical continuum, that is, NHL. Since successful immunotherapy in NHL is hampered by the striking unresponsiveness of lymphoma-infiltrating immune cells, the authors seek to discuss novel immunotherapeutic approaches developed to overcome the limitations of previous therapies.

8.2 Immunopathology of NHL

The majority of NHLs originate from clonal B-cell expansions; thus, they are potential Ag presenters [12]. Follicular lymphoma (FL) cells express cell surface molecules that can be targeted by mAbs, including pan B-cell antigens: CD19, CD22, CD23, CD79a, and CD79b, as well as GC surface markers like CD10 and bcl-6 [13].

In the clinical setting, MHC-II expression on diffuse large B-cell lymphoma (DLBCL) cells is correlated with higher numbers of tumor-infiltrating lymphocytes and a significantly prolonged survival rate [14, 15]. These results

suggest that MHC-II loss in B-cell lymphomas may be a pathway to tumor immune evasion, resulting in compromised patient survival. These co-stimulatory signals are provided by the B7 family [B7-1 (CD80) and B7-2 (CD86)] expressed on antigen-presenting cells (APCs) [16]. In addition, CD40–CD40L interaction plays a critical role in the generation of effective T-cell-mediated immune response, as it upregulates CD80 and CD86 expression on B cells [17]. One report has demonstrated that CD40 expression on DLBCL tumors correlates with improved prognosis [18]. Another study identified a correlation between loss of CD86 expression and decreased tumor-infiltrating lymphocytes in aggressive human B-cell lymphomas; however, the relationship between co-stimulatory molecule expression and prognosis in patients with B-cell malignancies expressing MHC-II has not been extensively investigated [19]. Moreover, mAb-resistant tumor cells remain sensitive to ADCC. Hence, ADCC enhancement will improve the clinical efficacy of mAb-based anticancer therapies. Currently, nonfucosylated next-generation Ab ingredients that elicit high ADCC are being developed. However, effective antitumor immune response is absent as the expression of co-stimulatory molecules which are essential for T-cell activation besides T-cell receptor (TCR) engagement is lacking [16]. Tiemessen et al. indicated that Tcf12/2 mice normally have a very small thymus, as a result of the impairment in T-cell development. Inextricably, a considerable proportion are doomed to spontaneous development of thymic lymphomas. Approximately 50 % of the mice population developed a thymic lymphoma/leukemia by the age of 16 weeks, conferring to T-cell deregulation in the pathogenesis of lymphoma [20]. Anergy of TCR may result due to the absence of co-stimulatory signals [16].

More recent studies have unveiled other mechanisms involved in the immunopathogenesis of lymphoma: Vera-Recabarren et al. demonstrated that the presence of anti-Ro⁄SS-A Abs, prevalent Abs among many autoimmune diseases, is associated with 16.7- and 10.6-fold increased risk of T-cell lymphoma and NHL development, respectively [21]. On the other hand, serum Abs derived from patients with hematological malignancies including lymphomas have been demonstrated to bind to DNA and several autoimmune disease nuclear Ags including Ro ⁄SS-A, possibly indicating its role in the immunopathogenesis of these malignancies. However, the exact mechanism remains enigmatic [22].

Herein, Abs targeted in the immunotherapy of NHL, along with the advances made, are discussed.

8.3 CD20

CD20, a protein of 297 amino acids with four transmembrane regions, is the most prevalent monoclonal targeted Ag [36]. It is a unique molecule resistant to internalization. Human CD20 molecule is exclusively expressed on the B lymphocyte lineage, which can be detected during all stages of maturation, from the pre-B-cell stage to the memory B-cell stage. Nonetheless, it is not expressed on very early pro-B-cell stage and plasma cells [37]. It is minimally modulated or shed from the cell surface of more than 95 % of B-cell lymphomas at high copy numbers. CD20 is also minimally internalized on binding Abs [38]. These characteristics make CD20 an attractive target for immunotherapy. Due to its stability, it has emerged as an ideal target for mAb therapy [39]. The CD20 Ag comprises only two extracellular loops [40]. CD20 mAbs are classified into two groups based on their different binding characteristics, altered lipid raft associations, and different modes of action [41]. Both types are known to trigger ADCC; however, type I Abs (e.g., rituximab, ofatumumab) predominantly act through the CDC mechanism, whereas type II antibodies (e.g., tositumomab, obinutuzumab) preferentially exert their antitumor properties through apoptosis [42].

8.3.1 Effector Mechanisms of CD20 mAbs

CD20 mAbs act through Fab-mediated effector mechanisms including inhibition of proliferation and induction of apoptosis, as well as through Fc-mediated effector mechanisms such as CDC

and ADCC [41]. However, all potential effector mechanisms involved *in vivo* remain to be unveiled. As evident by tumor cell clearance associated by caspase activation in patients with B chronic lymphocytic leukemia, contribution of direct induction of apoptosis has been proposed as a possible mechanism [43]. As rituximab lost its therapeutic activity in C1q knockout mice *in vivo* studies, CDC is proposed as another important effector mechanism [44]; nonetheless, the contribution of CDC to the *in vivo* activity in patients still remains controversial. Importantly, the critical role of Fc–Fc receptor (FcR) interactions for the therapeutic activity of rituximab is well established [45]. ADCC is well described as an essential mechanism of action for CD20 Abs in the literature [46]. Experimental evidence pointed to the important role of phagocytic cells in the clearance of B cells after CD20 Ab therapy [47]; nevertheless, effector mechanisms vary in different compartments such as the blood, bone marrow, or lymph nodes [48].

8.3.2 Rituximab

The chimeric CD20 Ab C2B8 (rituximab, Rituxan, Genentech, San Francisco, Calif) was the first mAb to demonstrate consistent therapeutic activity in clinical trials. It received FDA approval for the treatment of patients with relapsed low-grade follicular lymphoma (FL) on November 26, 1997, and is known as the first FDA-approved mAb applied in cancer patients [49], which led to a new era in the treatment of NHL. Since its approval, it has fundamentally changed the treatment concepts of most B-cell lymphomas [48, 50, 51].

The most efficient chemotherapy regimens yield objective responses in about 50 % of the patients with FL. Interestingly, the addition of rituximab to chemotherapy improves the response rates to 80 % [52]. In addition, its efficacy as a single agent in maintenance and re-treatment strategies with a favorable toxicity profile has been well established [13].

To develop murine anti-CD20 mAb, mice were immunized with human lymphoblastoid $CD20^+$ B cells [53]. Human antibody responses to rituximab were minimal, both due to the immunocompromised nature of NHL patients and the significant efficacy of rituximab [54]. It is the established standard therapy for B-cell NHL alone or as part of combination therapies [55]. The success of this therapy is far-reaching. Not only it has improved survival rates in lymphoma patients [56] but has also introduced a new paradigm in the therapy of other diseases associated with lymphoid dysregulation such as autoimmune diseases and graft-versus-host disease (GVHD). Although rituximab is established to act through CDC, ADCC, induction of apoptosis, and antiproliferative effects *in vitro*, the relative value of these mechanisms in patients remains enigmatic. In addition, the ultimate mechanism of action depends on the localization of the tumor and its microenvironment [57].

8.3.2.1 Mechanisms of B-Cell Depletion by Rituximab

Rituximab-binding sites are located in close proximity to the cell surface, contributing to the efficiency of humoral and cellular effector cells in killing these cells [58]. Several mechanisms are involved in rituximab's mechanism of action; however, ADCC is regarded as the major mechanism of action. Other modes including CDC and programmed cell death are also recognized [41]. Rituximab triggers and modifies various intracellular signaling pathways in NHL B-cell lines, leading to apoptosis and chemosensitization. It downregulates tumor-derived IL-10 transcription, followed by downregulation of Bcl-2 gene expression. In addition, it leads to the inhibition of STAT3 activity and Bcl-2 expression, as well as sensitization to the apoptotic effects of various chemotherapeutic drugs, through the p38 MAPK pathway [40].

A plethora of different half-lives have been reported for rituximab. As measured by Maloney et al. via ELISA assay in a phase I study, the half-life of rituximab after first infusion was 33.2 h, which increased to 76.6 h after the fourth infusion [59], whereas 445 ± 361 h (range: 0.5–58.5 days) was yielded by Japanese investigators in two groups of patients who received 250 and 375 mg/m^2 [60, 61]. Technical and racial variations are recognized to contribute to this variation. It is

understood that the efficacy of B-cell depletion by rituximab is enhanced by persistence of the CD20–Ab complex on the cell surface and long half-life of rituximab. Notably, the location of the B cells was found to have a considerable influence on the kinetics of B-cell depletion in a mouse model. Moreover, its surrounding microenvironment was suggested to contribute to its resistance [62]. In mouse model, the most efficient B-cell depletion occurred in the circulating system where >90 % depletion reached within minutes, followed by slow rate of depletion in the lymph nodes and spleen (approximately 60–70 % depletion in 24 h) and the slowest depletion in the peritoneal cavity (significant depletion after a week) [62, 63]. With respect to the lymphoid organs, the position of B cells in the organ itself also determines the rate of depletion by rituximab, which could explain for the relative resistance of mantle zone B cells [64]. Importantly, no dose-limiting toxicity at a single dose of 500 mg/m^2 was observed in a phase I clinical study; moreover, weekly dose schedule of 375 mg/m^2 was well tolerated [59, 65]. Despite successes obtained with rituximab therapy, resistance still remains a challenging issue in FL, as disease relapse occurs in almost 60 % of patients during the first 5 years and finally occurs in all patients with FL in the long term. The precise mechanism of resistance yet remains to be unveiled; nonetheless, it is postulated that it may be lymphoma related or host related. Low expression of CD20 on the cell surface [74], high expression of complement regulatory proteins [75], expression of anti-apoptotic genes, and blockade of effector cells due to the deposition of C3 fragments of the complement system [76] are postulated mechanisms which lead to resistance of lymphoma cells [13]. Furthermore, loss of targeted CD20 epitope after rituximab infusion [77] or consumption of the extracellular part of CD20 mAb complexes by phagocytic cells is also involved [45]. Survival signals secreted by the microenvironment may impede death of lymphoma cells induced by mAb [78]. A considerable difference in B-cell depletion by rituximab in the peripheral blood, lymph nodes, and spleen has been elucidated; B cells in the lymph nodes were found more susceptible [79].

Considerable progress has been made in the treatment of B-NHL; in addition, the outcome has improved by the use of rituximab either as a single agent or in combination with chemotherapy. Nonetheless, relapse with loss of response to treatment is an inevitable fate in a considerable proportion of patients, evoking the need for new therapeutic modalities [80]. As revealed by genetic analysis of FcγR polymorphism in cancer patients treated with mAb-based rituximab and trastuzumab, ADCC is one of the critical factors responsible for the clinical efficacy of therapeutic Abs [81–83].

8.3.2.2 Rituximab in Diffuse Large B-Cell Lymphoma (DLBCL)

Initial clinical trials on relapsed or refractory DLBCL patients revealed that rituximab was well tolerated and resulted in complete or partial response (PR) in one-third of the patients [84].

The addition of rituximab (R) to CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) chemotherapy was regarded as a significant breakthrough in the treatment of DLBCL. R-CHOP regimen has become widely accepted in this regard. The final outcome is influenced by various pathways: the number of patients who reach high-dose-therapy ASCT is increased with prior R-CHOP salvage therapy; moreover, the outcome is improved when used as posttransplantation maintenance therapy. Overall, rituximab salvage therapy for DLBCL is effective and well tolerated [94]. Coiffier et al. [60] compared the efficacy of CHOP and R-CHOP in elderly DLBCL and concluded that the addition of 375 mg/m^2 rituximab on day 1 of each cycle significantly improves CR/Cru (73 % *vs.* 63 %, $P=0.005$), leading to a significantly greater EFS (47 % *vs.* 29 %, $P=0.00002$), PFS (54 % *vs.* 30 %, $P<0.0001$), disease-free survival (DFS) (66 % *vs.* 45 %, $P<0.00031$), and OS (58 % *vs.* 45 %; $P<0.0037$) after a medium of 5-year follow-up [95]. The addition of rituximab didn't significantly add to the toxicity of the regimen, and no long-term toxicity was observed [60, 95, 96]. The addition of rituximab to anthracycline-containing chemotherapy or CHOP is considered as the first-line therapy in DLBCL in various stages [94].

8.3.2.3 Rituximab in Mantle

Lower response rates to rituximab have been observed in mantle cell lymphoma (MCL) compared to other subtypes; only 27 % response rate with an event-free survival (EFS) of 6–12 in patients aged 41–83 years (median 65) was yielded in patients with newly diagnosed or relapsed/refractory MCL [85]. As a result, rituximab isn't routinely used as a single agent in MCL, whereas its benefit in combination with chemotherapy has been demonstrated [86–88]. In a clinical study, the role of rituximab and chemotherapy combination therapy as the first-line therapy was evaluated in MCL patients over the age of 65 (median age 74) within 6 months of diagnosis, among whom 85 % had a performance status of 0, 89 % had an NCI comorbidity index of 0–1, and 20 % were stage I or II. Median survival was 37 months, and the 2-year OS rate was 63 %, compared to 27 months and 52 % for chemotherapy alone. Nonetheless, no difference in the time to next treatment was observed [89]. Therefore, rituximab combined with chemotherapy is recommended as the first-line therapy in MCL, regardless of age [90].

8.3.2.4 Rituximab in Follicular Lymphoma

The introduction of rituximab was considered a breakthrough with significant efficacy and an excellent toxicity profile in FL [91]. CD20 is expressed in more than 90 % of FLs. Rituximab has displayed promising results when applied as a single agent [92] and in combination with different chemotherapy regimens (chemoimmunotherapy). Notably, the addition of rituximab to chemoimmunotherapy significantly improved the ORR, PFS, and OS [93]. Rituximab maintenance therapy following first-line induction with chemoimmunotherapy is currently being evaluated in international phase III randomized trials [91]. Significant single-agent efficacy with an overall response rate of 46 % was obtained during phase II clinical studies [50]. By applying the weekly dosing strategy, an overall response rate (ORR) of 48 and 47 % was achieved in confirmatory phase II studies by a multicenter international consortium and the German low-grade lymphoma study group (GLSG), respectively. Progression was observed after 13 and 7 months, respectively. Despite the desirable results, bone marrow involvement was found to contribute to poor response [49, 66, 67]. By the administration of the confirmed weekly dose for 4-week cycles in patients with low disease burden advance stage NHL, ORRs of 47 and 73 % were achieved after one cycle in two different studies [68, 69]. Furthermore, a randomized three-armed study by Ardeshna et al. in advanced low burden disease patients compared the outcome of (a) watchful waiting, (b) rituximab monotherapy (four weekly doses), and (c) one standard induction dose followed by maintenance therapy with rituximab given every 2 months. The primary endpoint, regarded as time to first therapy, radiation, or chemotherapy, was first met by arm a. After 7 months of randomization, rituximab single-agent plus maintenance therapy yielded an ORR of 85 %, with complete remission (CR) observed in 39 %, whereas 17 % had progressive disease in the watch and wait subgroup. Surprisingly, in neither arm with single-agent rituximab nor rituximab plus maintenance, the median time to first chemotherapy was met in 4 years, whereas chemotherapy was started for patients in the watch and wait arm after 33 months of randomization. Therefore, rituximab was advocated as a promising therapy for patients with newly diagnosed disease who do not meet GELF criteria [70]. Prolonged overall survival (OS) has been observed in patients with FL receiving rituximab combined with chemotherapy [52, 71]. As proven in the literature, progression-free survival (PFS) in patients with a good initial response to immunochemotherapy is significantly prolonged by rituximab maintenance therapy [72, 73].

8.3.2.5 Rituximab Incorporated with Carboplatin-/Cisplatin-Based Chemotherapy

Various studies have investigated the efficacy and safety profile of rituximab plus platinum-based chemotherapies in NHL. An ORR of 56–100 % and CR of 10–67 % have been achieved by adding rituximab to chemotherapies including

ifosfamide, carboplatin, and etoposide (ICE); cisplatin, high-dose Ara-C, and dexamethasone (DHAP); etoposide, doxorubicin, methylprednisolone, cytarabine, and cisplatin (ESHAP); and dexamethasone and oxaliplatin, which make progression to autologous stem cell transplantation (ASCT) more likely. Notably, the response rate relies on several factors including previous rituximab therapy, relapse or refraction, and the international prognostic index (IPI) score at relapse [94].

R-ICE has been evaluated in patients with relapsed or refractory DLBCL; after standard anthracycline-based therapies, an OR of 78 % and CR of 53 % have been yielded which were significantly greater compared to the controls receiving ICE alone ($P=0.006$) [97]. In addition, OR was remarkably higher in relapsed *vs*. refractory patients (96 % *vs*. 46 %) ($P<0.001$) [98]. The addition of rituximab to DHAP led to a 62 % OR in refractory and relapsed NHL patients, with a more remarkable OR (82 %) in patients treated at the first relapse. An overall OS of 8.2 months and 20.4 months was achieved in the overall patients and those proceeding to HDT-ASCT, respectively [99]. In the same line, a prospective randomized clinical trial on 225 patients with refractory or relapsed DLBCL exhibited a 75 % *vs*. 54 % PR/CR in the R-DHAP and DHAP arms, respectively, after two courses of chemotherapy. R-DHAP significantly improved failure-free survival (FFS), 50 % *vs*. 24 % ($P<0.001$) at 24 months follow-up, whereas OS did not improve significantly (52 % *vs*. 59 %, $P=0.15$). Finally, rituximab treatment was found to have a significant effect on FFS and OS when adjusted for age, performance status, time since treatment, and secondary age-adjusted IPI [100]. In addition, the efficacy of R-EPOCH therapy was explored in a phase II clinical trial in NHL. By adding the standard dose of 375 mg/m^2 rituximab to the first day of each cycle of the standard six cycles in patients with NHL (pretreated primary DLBCL, transformed DLBCL and MCL) an OR of 68 % and CR of 28 % were achieved, with 19/31 patients under the age of 60 proceeding to HDT-ASCT. In a study on patients with relapsed/refractory B-cell lymphoma, the efficacy and safety of the combination of gemcitabine plus oxaliplatin, with and without rituximab, were studied in which an ORR and CR of 57 and 30 % (95 % CI, 15–49) in the GEMOX and 78 and 50 % (95 % CI, 32–68) in the R-GEMOX arm were yielded. With respect to the safety profile, grade 3/4 neutropenia occurred in 57 and 47 % of cycles for GEMOX and R-GEMOX, respectively, while grade 3/4 thrombocytopenia was observed in 26 and 17 % of courses in the same groups. The FFS was 7 % (95 % CI, 0–16) for GEMOX and 28 % (95 % CI, 9–47) for R-GEMOX ($P=0.014$), with overall survivals of 7 (95 % CI, 0–16) and 37 % (95 % CI, 20–55), respectively ($P=0.016$). Even though tolerability and appealing response rate were achieved by both regimens, FFS was more prolonged in R-GEMOX. However, continued relapse without a clear plateau on survival curves was seen [101]. The efficacy and safety of the combination of dexamethasone, high-dose cytarabine, and oxaliplatin combined with rituximab as salvage therapy in 70 patients with relapsed or refractory aggressive NHL and HL were studied. Notably, high-grade non-hematologic toxicity and renal- or neurotoxicity weren't observed. Overall, this combined therapy proved as an effective and feasible outpatient regimen for salvage therapy in patients affected by relapsed or refractory lymphoma [102]. These studies highlight the improvement in OR and CR with the addition of rituximab to platinum-based salvage chemotherapy [94, 103]. However, the most optimal chemotherapy regimen for rituximab to bind to needs to be defined [94].

Since the presence of GC phenotype in DLBCL plays a prognostic role, it is used to divide chemotherapy-treated patients into low- and high-risk groups. A study on patients with DLBCL lymphoma treated with R-CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone), in which the presence of GC phenotype was evaluated immunohistochemically, demonstrated that patients with a GC phenotype display a significantly better outcome [104].

A summary of studies on the combination of rituximab and chemotherapy are summarized in Table 8.1.

Table 8.1 Rituximab in combination with chemotherapy

Regimen	Disease status	*n*	ORR	CR	Survival	Reference
R-GEM	High-grade B-NHL (elderly: 64–78 years)	7 (1 previously untreated)	71 %	29 %	PFS:10 m	[344]
					OS:11 m	
R-GEMOX	Aggressive NHL	46	83 % at the end of fourth cycle	50 %	OS: 66 %	[345]
			74 % at the end of treatment	72 % at the end of treatment	EFS:43 %	
					FU:28 m	
R-GIFOX	Aggressive NHL	13	77 %	54 %	EFS:80 %	[346]
					FU: 6 m	
GaRD	Aggressive NHL	19	79 %	42 %	NG	[103]
GaRD	Aggressive B-NHL	22	55 %	27 %	NG	[347]
R + E	DLBCL	6	67 %	50 %	NG	[348]
R + E	DLBCL	15	47 %	33 %	PFS:6 m	[139]
					FU:11 m	
R + CMD	DLBCL (elderly: 65–79 years)	30	74 %	57 %	OS:45 %	[349]
					PFS:37 %	
					FU:2 years	
R + TTP	Aggressive NHL	71	70 %	25 % in primary refractory	DR:21 m	[350]
				56% in relapsed	FU:26 m	
R + TTP	DLBCL	10	60 %	30 %	NG	[351]
R + ADOX	DLBCL	20	70 %	25 %	Median survival: 11 m	[352]
R-CHOP	DLBCL	108	NG	NG	Significant improvement *vs*. CHOP	[353, 354]
R-CHOP-like	DLBCL	194 (97 GCB *vs*. 97 bob-GCB)	NG	NG	OS (GCB: 70 % *vs*. non-GCB: 47 %)	[104]
					FFS, 59 % *vs*. 30 %,	
					FU: 52 m	
R-TCOP	DLBCL	38	NG	NG	OS and FS both significantly greater compared to TCOP alone	[354]
R-CHOP-like	DLBCL	113	NG	NG	OS: 77 %	[355]
					EFS:59 %	
R-DHAOX	DLBCL	42	42.85 %	26.19 %	OS: 71 %	[102]
					PFS: 44 %	
					FU:2 years	

8.3.3 Targeting CD20 with New Anti-CD20 mAbs

Despite the promising results with rituximab, resistance is expected to be observed in about 60 % of the previously responding FL patients, shedding light on the importance of developing other anti-CD20 mAbs [13]. To stem the tide of resistance to CD20 mAb, a new mind-set has been adopted, leading to the development of second- and third-generation CD20 mAbs. The presence of humanized or completely human CDR in second-generation anti-CD20 mAbs leads to significant reduction in the formation of human anti-chimeric Abs, preventing therapy resistance. Third-generation anti-CD20 mAbs are not only reinforced by a humanized CDR region, but also a modified Fc region is present, leading to

stronger activation of the complement system or effector cells [13].

The development of novel anti-CD20 Abs aims at constructing new Abs which exhibit significant activity in patients with rituximab-resistant lymphoma or possess increased efficacy in comparison with rituximab in head-to-head comparative trials. Nonetheless, available data in rituximab-refractory patients is scarce, and further studies are needed to establish their clinical application. According to FDA guidelines, rituximab resistance is defined as disease on progression during rituximab monotherapy or rituximab chemotherapy. Progressive disease or relapse less than 6 months after the last rituximab infusion or after the last course of rituximab chemotherapy is also considered as rituximab resistance.

Anti-CD20 mAbs are divided into two subgroups, types I and II. Type I anti-CD20 mAbs trigger the complement system through aggregating CD20 molecules into lipid microdomains upon binding [74]. Conversely, type II antibodies do not activate CDC. Instead, they lead to the induction of direct cell death, through homotypic adhesion and actin-dependent lysosome-mediated cell death [105]. In the following, brief description is provided on the new generations of CD20 mAbs applied in lymphoma. Comparison between CD20 mAb variants is provided in Tables 8.2 and 8.3.

8.3.4 First-Generation Anti-CD20 mAb

8.3.4.1 Reengineered Rituximab

A variety of reengineered rituximab variants have been developed recently. The triple variant, constructed by three-point mutations in the CDR region, targets the same epitope as rituximab. Nonetheless, it is more efficient in the induction of apoptosis. In a rituximab-resistant lymphoma mouse model, the triple variant led to prolonged survival [106]. Furthermore, a genetically engineered tetravalent version of rituximab has been constructed which possesses a stronger anti-lymphoma and antiproliferative effect *in vitro* and in a lymphoma mouse model [107].

8.3.4.2 Tositumomab (B1)

Tositumomab, a clinically used type II murine IgG2a mAb with a covalently bound iodine-131 radioisotope, has yielded promising results in the treatment of low-grade lymphoma. It is an efficient caspase-independent mAb with antiproliferative effect. However, it acts through a non-apoptotic manner. The direct effect of antibody binding, as well as the cytotoxic effect of irradiation, has led to its potentiality [117]. Tositumomab proved more efficacious in a mouse model compared to rituximab [118]. Even though tositumomab lacked rituximab's efficiency in CDC induction, it displayed the same ability in the activation of Fc-bearing effector cells, possessing the same binding affinity and half-life *in vivo*. Furthermore, it significantly surpassed rituximab in B-cell depletion from the periphery and from secondary lymphoid organs [118]. Its ability to directly induce nonclassical apoptosis can explain its superiority. In a clinical study, administration of 900 mg of unlabeled mAb prior to therapeutic anti-CD20 radioimmunoconjugate exerted an efficient tumor-reduction effect [117]. It has provided the opportunity to treat lymphoma through CDC-independent pathways [118].

Low-dose type II antibody, GA101 (400 mg/infusion on days 1, 8, and 22 and subsequently every 3 weeks for a total of nine infusions), yielded an ORR of 8 %. High-dose GA101 treatment (1,600 mg on days 1 and 8, followed by 800 mg thereafter) resulted in significant activity (55 % ORR) in rituximab-refractory patients. Therefore, a dose–effect relationship is proposed [119].

8.3.4.3 Veltuzumab (hA20, IMMU-106)

Veltuzumab, a mAb constructed on the framework regions of the humanized anti-CD22 mAb epratuzumab, consists of CDRs taken from the murine A20 anti-CD20 mAb. During preliminary studies, no difference was observed between the effectiveness of rituximab and veltuzumab. Further studies demonstrated a slower off-rate with superior CDC and significantly improved therapeutic outcomes with veltuzumab in different lymphoma models, as well as potent anti-B-cell activity in cynomolgus monkeys [120].

Table 8.2 Comparison between the different properties of different generations of anti-CD20

Anti-CD20	Generation	Localize CD20 to lipid rafts	ADCC activity	CDC activity	Direct induction of cell death	Homotypic aggregation	Number of binding sites on CD20	ORR	Reference
Type I									
Rituximab	I	+	++	+++	±	–	100 %	38 %	[108]
Reengineered rituximab	I	+	++	+++	±	–	100 %	NG	
Ocrelizumab	II	+	++	+++	±	–	100 %	38 %	[109]
Ofatumumab	II	+	++	+++	±	–	100 %	42 %	[111]
Veltuzumab	II	+	++	+++	±	–	100 %	44 %	[110]
AME-133	III	+	++	+++	±	–	100 %	NG	
PRO131921	III	+	++	+++	±	–	100 %	NG	
Type II									
B1 (tositumomab)	I	–	++	–	+++	+	50 %	NG	
GA101	III	–	++	–	+++	+	50 %	(Low dose) 1/13 (8 %) (High dose) 6/11 (55 %)	[119]
Small modular immunopharmaceutical anti-CD20 protein									
TRU-015			++	+	+	–		NG	

Table 8.3 Clinical trials on novel anti-CD20

Monoclonal Ab	Generation	IgG type	Patients	Disease status	Regimen	ORR	Adverse events	Reference
Ofatumumab (OFA)	2nd	IgG1 Kappa	116	Refractory FL	Ofatumumab 500 mg 1,000 mg	13 % 10 %	Infections, rash, urticaria, pruritus, neutropenia, anemia, thrombocytopenia	[356]
			59	(Untreated FL)	O-CHOP 500 mg 1,000 mg	90 % 100 %	Leucopenia, neutropenia	[357]
Veltuzumab	2nd	IgG1	82	Relapsed/refractory B-cell NHL	Veltuzumab 80–750 mg/m^2	44 % (FL) 83 % (MZL) 43 % (DLBL)	Fatigue, pruritus, asthenia, fever, dyspnea, headache, infection	[110]
Ocrelizumab	2nd	IgG1	47	Relapsed/refractory FL	(Ocrelizumab) 750 mg/m^2	38 %	Infusion-related reaction, nasopharyngitis, asthenia, lymphopenia, infection	[109]
Obinutuzumab (GA-101)	3rd	IgG1	21	Refractory B-cell NHL	Obinutuzumab 1,600/800 mg 400/400 mg	60 % 35 %	Infusion-related reaction, neutropenia, anemia, thrombocytopenia, tumor lysis syndrome	[358]
			28	Relapsed or refractory FL	G-CHOP 1,600/800 mg 400/400 mg	94 %	Infusion-related reaction, neutropenia, neuropathy, infection	[358]
			28	Relapsed or refractory FL	G-FC 1,600/800 mg 400/400 mg	93 %	Infusion-related reaction, neutropenia, rash, infection	[358]
PRO131921	3rd	IgG1	24	Relapsed/refractory B-cell NHL	PRO131921 25–800 mg/m^2	27 %	Infusion-related reaction, upper respiratory tract infection, neutropenia	[359]
Ocaratuzumab (AME-133v)	3rd	IgG1	56	Relapsed/refractory FL	Ocaratuzumab 100 mg/m^2 375 mg/m^2	36 %	Infusion-related reaction, nasopharyngitis, asthenia, lymphopenia, infection	[360]

Low doses of veltuzumab at short infusions were well tolerated with no serious adverse events in 55 pretreated patients with FL in a multicenter phase I/II trial [110], which were comparable with the ranges in rituximab re-treatment in patients with relapsing disease [108]. Subcutaneous injection of veltuzumab resulting in a slow release pattern over several days with a rapid depletion of B cells was studied in a phase I/II study, yielding an OR of 55 % and a CR rate of 20 % [121]. A modified hexavalent antibody named hex-hA20 has been developed with interesting properties of both type I and II antibodies, capable of inducing lipid raft formation (type I), in addition to its antiproliferative and apoptotic properties and homotypical adhesion induction (type II); nonetheless, it has a shorter half-life in comparison with its origin [122].

8.3.4.4 Ocrelizumab (PRO70769, rhuH27)

Ocrelizumab, a type I mAb with an IgG1 isotype generated from the murine 2H7 anti-CD20 Ab, predominantly acts through ADCC mechanism, while it has a reduced capacity of CDC compared to rituximab [123]. Its efficient B-cell removal, from the periphery and to a lesser extent in secondary lymphoid organs, has been observed in monkeys achieving results comparable to rituximab [79]. Even though it is postulated to be more effective than rituximab due to its enhanced ADCC capabilities, mild infusion-related toxicity with doses up to 750 mg/m^2 was experienced in a phase I/II dose-escalating trial administered to previously rituximab-treated FL patients [109]. However, decreased intravascular complement activation was proposed to be attributable to the observed toxicity; moreover, the safety profile was similar to rituximab [109].

8.3.5 Second-Generation CD20 mAb

Second-generation type I anti-CD20 mAbs, including ofatumumab, veltuzumab, and ocrelizumab, have been constructed which are effective in patients with FL previously treated with rituximab. Nonetheless, in a comparison between the clinical responses to these mAbs with the responses of patients who received rituximab re-treatment or maintenance therapy, no significantly improved survival was demonstrated. In comparable patient populations from different studies, the ORR for rituximab re-treatment was 38 % [108], 38 % for ocrelizumab [109], 44 % for veltuzumab [110], and 42 % in those receiving ofatumumab [111].

8.3.5.1 Ofatumumab (Arzerra, HuMax-2F2)

Ofatumumab, a completely human anti-CD20 mAb, is constructed in a human transgenic mouse. Remarkable activation of the complement system is its outstanding property. In addition, it is favored by the ability to kill target cells with much lower numbers of CD20 molecules on the cell surface, requiring less human serum, as it binds two to three times more than rituximab to C1q [112]. Binding of ofatumumab to the small 7-mer extracellular loop of the CD20 molecule close to the cell membrane accounts for its exceptional efficiency in complement fixation [112]. Nonetheless, rituximab is significantly more efficient in ADCC activation pathway in patients with NK cells expressing the FcγRIIIa-158V allotype than the low-affinity receptor FcγRIIIa-158F allotype [113]. Promising results were obtained with *in vitro* and *in vivo* administration of ofatumumab against rituximab-resistant, as well as rituximab-sensitive models [114]. Heavily pretreated patients with relapsed/refractory FL in the first phase I/II clinical trial yielded an ORR of 42 %. In addition, it is considered as a safe agent [111]. These results were in the same range with rituximab re-treatment patients [108]. In addition, an 11 % OR with a mean duration of 6 months was achieved with ofatumumab monotherapy in 116 heavily pretreated FL patients refractory to rituximab; patients refractory to rituximab monotherapy achieved a 22 % ORR [115]. Furthermore, promising results have been obtained with ofatumumab in combination with CHOP (cyclophosphamide, adriamycin, vincristine, and prednisone) chemotherapy. An ORR up to 100 % was achieved, as well a 69 % complete response with favorable toxicity profiles [116].

Overall, some studies have demonstrated an ORR of 11–17 % in rituximab-refractory patients with the application of novel type I antibodies, ofatumumab and ocrelizumab, which is quite modest.

8.3.6 Third-Generation CD20 mAb

8.3.6.1 PRO131921 (RhumAb v114)

To overcome the less favorable disease outcome in patients with low-affinity receptor (FcγRIIIa) expressed on NK cells after rituximab monotherapy, a modified version of ocrelizumab (PRO131921) was developed; it achieved a 30-fold better binding to FcγRIIIa (FF or FV) than rituximab. An up to tenfold stronger ADCC was demonstrated in *in vitro* studies [124, 125]. Efficient B-cell depletion in cynomolgus monkeys was observed in a dose-escalating study. However, adverse events including dose-dependent reversible neutropenia and thrombocytopenia were also manifested [125]. In a dose-escalating phase 1 trial on patients with relapsed or refractory indolent lymphoma who were previously treated with rituximab, single-agent administrations of PRO131921 were well tolerated [126].

8.3.6.2 AME-133v (LY2469298)

AME-133v, a third-generation IgG1 anti-CD20 mAb, was developed in an attempt to enhance ADCC of anti-CD20 mAb. It possesses increased affinity to the FcgRIIIa on NK cells, as well as a ten times stronger B-cell killing. Targeted insertion of a synthetic oligonucleotide pool into a human germ line sequence was used for the construction of the CDRs. AME-133v proved as an effective activator of NK cells which induced the same degree of ADCC with lower doses as compared to rituximab in *in vitro* studies [46].

8.3.6.3 GA-101 (RO5072759, Obinutuzumab)

GA-101, derived from the murine IgG1-k antibody B-lyl, is the first Fc-engineered type II anti-CD20 mAb which mediates homotypic adhesion and does not relocate CD20 into lipid rafts after binding to CD20 [42]. In comparison with rituximab, a distinct but overlapping epitope on CD20 is recognized by GA-101; in addition, it binds in a different orientation and on a larger surface area; additionally, it possesses increased induction of direct cell death after binding. GA-101 exhibited more efficient direct cell death induction and nonclassical apoptosis in comparison with tositumomab. Significantly more efficient killing of lymphoma was observed in whole blood sample assays. In addition, GA-101 was more efficient in killing normal and malignant B cells in several murine models and in monkeys, and contrary to rituximab, it proved capable in B-cell elimination from the spleen and lymph nodes [42]. During a dose-escalating phase 1 clinical trial in 25 patients with NHL, receiving 9 infusions of GA-101 starting at 50–2,000 mg per dose, promising results were obtained, with a toxicity profile comparable to rituximab and no dose-limiting toxicities. An ORR of 36 % was achieved [127]. Likewise, doses up to 2,000 mg of GA-101were well tolerated, and an ORR of 22 % was achieved in pretreated patients with NHL in a phase 1 clinical trial [128]. Furthermore, single-agent GA-101 had a high response rate in heavily pretreated patients with indolent NHL, which demonstrated a possible dose–effect relation; an ORR up to 55 % was observed in patients with rituximab-refractory disease [129]. Obinutuzumab in combination with chlorambucil was compared with chlorambucil alone or rituximab and chlorambucil in a phase III randomized study in patients with previously untreated CLL. The median PFS was 23 months in patients treated with obinutuzumab plus chlorambucil and 11.1 months for patients treated with chlorambucil alone. This led to the FDA approval of obinutuzumab in CLL in November 2013. A survival advantage was also demonstrated in the obinutuzumab and chlorambucil arm compared with rituximab and chlorambucil (Goed V et al., Abstrace #6, Blood, ASH conference 2013).

8.3.7 Small Modular Immunopharmaceutical Anti-CD20 Protein

8.3.7.1 TRU-015

Small modular immunopharmaceuticals are encoded by a single-chain protein expressed as homodimers. TRU-015, a very small protein, is

generated from the heavy and light chain variable regions from murine anti-CD20 mAb 2H7, which are linked to HuIgG1 CH2 and CH3 domains [130]. TRU-015 manifests comparable ADCC and a reduced CDC activity compared to rituximab during *in vitro* studies. Noteworthy, it was found superior to rituximab in terms of reduction of tumor mass and prolonged survival of mice with human lymphoma. Moreover, a dose-dependent and durable B-cell depletion was experienced with escalating single-dose injections of TRU-015 in cynomolgus monkeys [130]. Remarkably, TRU-015 proved as a safe B-cell-depleting agent in patients with rheumatoid arthritis in a dose-dependent manner during a dose-escalating phase I/II trial [130]. However, its safety in lymphoma patients remains to be defined.

8.4 CD22

CD22, a 135 kDa molecule, is exclusively expressed on the transmembrane of mature (IgM and IgG) B cells [131]. It plays a crucial role in the regulation of B-cell activation, survival, and BCR and CD19 signaling, after being phosphorylated [132]. Prolonged contact hypersensitivity reactions have been observed in CD22-deficient B cells, implying its inhibitory role within the immune system [133]. Apoptotic pathways are activated upon binding of an antibody or the natural ligands (sialylated glycans) to CD22 and its internalization [134]. CD22 is expressed on more than 90 % of the FLs.

8.4.1 Epratuzumab

Epratuzumab is derived from the murine IgG2a LL2 anti-CD22 mAb, which was previously used for the radioimmunodetection of NHL. LL2 has been humanized by CDR grafting techniques, and the murine IgG2a has been replaced with human IgG1; hence, its immunogenicity is reduced, and an efficient immunotherapeutic mAb is resulted. Epratuzumab and CD22 are rapidly internalized upon binding, possibly leading to its phosphorylation and downstream signaling [135]. *In vitro* studies have demonstrated no complement system activating capability, no clear direct cytotoxic effect, and only a modest ADCC activity. Nonetheless, significant antiproliferative effect has been observed *in vitro*. Noteworthy, a stronger antiproliferative effect has been observed for the combination of epratuzumab and rituximab compared with rituximab alone [135, 136]. Single doses of epratuzumab were well tolerated and resulted in transient B-cell depletion in a dose-escalating phase I trial in patients with different subtypes of NHL. Patients with FL revealed the best clinical response (43 %) among other subtypes at a dose of 360 mg/m^2/week [137]. A multicenter study in patients with various subtypes of refractory/relapsed NHL assessed the effect of the combination of epratuzumab with rituximab; the injection of weekly doses of 360 mg/m^2 epratuzumab and 375 mg/m^2 rituximab yielded promising results in the FL group (ORR, 54 %; CR/CRU, 24 %; median response duration, 13.4 months) [138]. In addition, a phase II clinical trial revealed similar results in patients with indolent lymphoma. Epratuzumab (360 mg/m^2) combined with rituximab (375 mg/m^2) administered weekly for four consecutive weeks yielded comparable toxicities to rituximab alone with an ORR of 64 % (24 % CR/CRu) with a response duration of 14 months in patients with refractory/relapsed FL [139]. The combination of epratuzumab and rituximab with CHOP chemotherapy has been studied in a phase II study in patients with untreated DLBCL; six cycles of treatment obtained an OR of 87 % and CR/Cru of 67 % [140]. In a Raji lymphoma mouse model, the combination of anti-CD20 mAb veltuzumab and epratuzumab obtained no significantly different results compared to veltuzumab alone [141]. On the other hand, an anti-CD20/anti-CD22 bispecific mAb was developed, which obtained remarkable antiproliferative effects in *in vitro*, in contrast to the parental Abs (veltuzumab and epratuzumab or combined) [142]. Even though the cross-linking of CD20 and CD22 does not lead to significant activation of the complement system, marked ADCC activity is obtained. Moreover, the bispecific Ab was

demonstrated to be superior to anti-CD20 in a Daudi lymphoma model [143]. Hexavalent bispecific anti-CD20/anti-CD22 antibody manifested similar results [142]. Due to its rapid internalization after binding to CD22, epratuzumab is considered an ideal immunoconjugate for drug delivery, resulting in increased potency; nonetheless, increased toxicity may be incurred [144].

8.4.2 Inotuzumab Ozogamicin (CMC-544)

Inotuzumab, an IgG4 humanized anti-CD22 mAb which is derived from the murine mG5/44 Ab, resides on to human acceptor frameworks. The antibody–antigen complex is internalized after binding to CD22 [144]. No toxic effect has been reported from CMC-544 [145]. By conjugation of inotuzumab to ozogamicin (calicheamicin), a potent antitumor antibiotic, growth of CD22 B cells has been inhibited *in vitro*. Moreover, dose-dependent significant anti-lymphoma effect has been attributed to this immunoconjugation *in vivo* [145]. In addition, it possessed greater cytotoxicity in comparison with rituximab conjugated to ozogamicin [146]. On the other hand, an even stronger anti-lymphoma effect in similar B-cell lymphoma mouse models was attributed to the combination of rituximab and inotuzumab ozogamicin [147]. The safety and efficacy of inotuzumab ozogamicin were studied in patients with pretreated NHL in a multicenter, dose-escalating (0.2–2.4 mg/m^2) phase I study; a maximum dose of 1.8 mg/m^2 was well tolerated. Moreover, adverse events consisting of thrombocytopenia (90 %), asthenia (67 %), nausea (51 %), and neutropenia (51 %) were all reversible. Subgroup analyses on FL patients demonstrated an objective response rate of 68 % with a 32 % CR/CRu rate and a PFS of 10.4 months [148]. The combination of inotuzumab, ozogamicin, and rituximab in patients with FL resulted in similar reversible adverse events in another clinical study [149]. The immunoconjugate inotuzumab ozogamicin (CMC544) with rituximab obtained an ORR of 87 % with a 23.6 months' response duration in 38 patients with recurrent/refractory lymphoma [149].

8.5 CD19

CD19, a member of the immunoglobulin superfamily, consists of two extracellular immunoglobulin-like domains with an extensive cytoplasmic tail [158]. It is exclusively expressed on B-cell lineage from the very early B cell and is lost upon differentiation to plasma cells [158]. Due to its high, homogeneous expression in nearly all different subtypes of lymphoma, it is considered a potential target for immunotherapy [159]. Even though CD19 was one of the first targets for immunotherapy [160] and its safety and efficacy were approved, its development has been stagnated by the more promising results obtained by anti-CD20 mAbs [160].

8.5.1 XmAb5574

XmAb5574, a novel humanized anti-CD19 mAb, mediates significantly higher ADCC compared with rituximab, owing to its engineered Fc domain [161]. It acts through modest induction of apoptosis and activation of the phagocytic system [161, 162]. This mAb has excellent preclinical features, and further studies should be awaited.

8.5.2 Blinatumomab (MT102/MEDI-538)

Blinatumomab (MT102/MEDI-538), a new anti-CD19-CD3 bispecific Ab, which gathers lymphoma (CD19) and effector T cell (CD3) together, leads to efficient elimination of lymphoma cells. Despite the promising clinical results of previous anti-CD19/CD3 bispecific Abs [163], they proved ineffective in clinical trials [164]. However, blinatumomab yielded promising results in a phase I clinical study [165]. *In vitro*, it demonstrated efficient anti-lymphoma cytotoxicity at extremely low doses (10 pg/ml, 100,000-folds lower than rituximab) and low effector/target cell (2:1) ratio [166]. Clinically, in 38 patients with relapsed NHL (FL, CLL, and MCL), doses ranging from 0.0005 to 0.06 mg/m^2/day were found safe and effective. Complete remission was achieved in four and seven patients with

doses starting at 0.015 mg/m^2/day with 13 months' duration of response in one patient [165].

8.5.3 hu-DM4/SAR3419

Various phase I studies have evaluated the application of anti-CD19 mAbs coupled to immunotoxins. Binding of the tubulin inhibitor maytansinoid derivate DM4 to the humanized IgG1 anti-CD19 mAb, huB4 (huB4-DM4/SAR3419), has yielded promising results. After binding to CD19, SAR3419 undergoes internalization, resulting in intracellular release of DM4, eventually leading to cell death. SAR3419 was found superior to rituximab in preclinical xenograft models [167]. In addition, its safety was proved in a dose-escalating phase I study in patients with different types of lymphoma. However, dose-limiting toxicities including severe transient blurred vision, associated with microcystic epithelial corneal changes, were observed. Finally, 53 % of the patients refractory to rituximab experienced remission [168].

8.6 CD30

CD30, a member of TNF receptor superfamily, is expressed on the cell surface of 10 % of NHLs [23]. It is considered a diagnostic immunomarker and a potential target for immunotherapeutic approaches for anaplastic large cell lymphoma (ALCL) [24]. The prognosis of ALCL is significantly correlated to serum CD30 level [25]. Other NHL subtypes including DLBCL, primary mediastinal large B-cell lymphoma, FL, and Epstein–Barr virus (EBV)-positive lymphomas express lower levels of CD30 expression [26]. A variety of CD30 mAbs are applied in the NHL including M67, SGN-30, Ki-1, M67, and Ber-H2 [27, 28], most of which are effective on ALCL cells. A summary of trials on the application of CD30 mAbs in NHL is summarized in Table 8.4.

8.6.1 M67

M67, a monoclonal anti-CD30 developed in 1994 by Gruss et al., was established as an efficient growth inhibitor of ALCL cell lines *in vitro* [29, 30]. In addition, a significant correlation was observed between its antitumor effects and the differences in the constitutive NF-kB signaling in ALCL and HD cell lines [31]. As evident in *in vitro* studies, ALCL cells undergo apoptosis in the presence of M67 due to their inability to activate the transcription factor NF-kB, whereas HD cell lines (L428, KM-H2, L591) were resistant to M67, attributed to constitutive expression of NF-kB [8].

Table 8.4 Anti-MHC-II monoclonal antibodies

Anti-MHC-II	Origin	Notable comments	Adverse events
Anti-CD74			
Milatuzumab	Murine LL1	Acts through direct growth inhibition, apoptosis	No serious adverse event
		No CDC or ADCC	Reversible T-cell reduction
HLA-DR			
Apolizumab	Murine 1D10	Acts through APC, ADCC, apoptosis	No serious adverse event
			Type I hypersensitivity
IMMU-114	Humanized IgG4	Leads to disease-free survival	No serious adverse event
LYM-1	Murine IgG2a	Acts through CDC	No serious adverse event
			Dose-limiting thrombocytopenia
SHAL		Very rapid blood clearance	Non reported
		Suitable carrier for radio-isotypes	

8.6.2 SGN-30

SGN-30, a chimeric IgG1 mAb derived from the murine AC10 anti-CD30 mAb, was demonstrated an antiproliferative effect *in vitro* and a potent anti-HL effect in xenografts [32]. Macrophages play a critical role in the activity of SGN-30, proven by the abolished effect of SGN-30 in the absence of macrophages in experimental studies [32]. It has proved as a safe and well-tolerated mAb, yielding optimal results in patients with (cutaneous) ALCL [33–35].

8.7 CD37

CD37, a heavily glycosylated 40–52 kDA glycoprotein, is a member of the tetraspan transmembrane family of proteins [169, 170], which internalizes and displays modest shedding in transformed B cells expressing the Ag. It is expressed in cells progressing from pre-B to peripheral mature B cell. Nonetheless, it is lost during B-cell development in terminal differentiation to plasma cells. It is considered an optimal target for immunotherapy in B-cell NHL and other B-cell malignancies, owing to its high selectivity [55].

8.7.1 Tetulomab (HH1)

Tetulomab (HH1), a murine IgG1 Ab, was the first anti-CD37 developed in the 1980s [171]. The binding properties to various NHL subtypes of tetulomab has been compared with the chimeric IgG1 antibody rituximab, and significant therapeutic effect of 177Lu-tetulomab was established with tolerable toxicity [55].

8.8 CD40

CD40, a member of the TNF receptor family, is constitutively expressed on antigen-presenting cells (B cells, dendritic cells, and macrophages), acting as a co-stimulatory molecule, which interacts with CD40L (CD154) expressed by activated T cells. In addition, endothelial cells, smooth muscle cells, fibroblasts, and epithelial cells express CD40 on their membrane. In addition, malignant cells such as NHL, multiple myeloma, and various solid tumors express CD40 in considerable amounts. Stimulation of CD40 leads to immunoglobulin isotype switching and activation of B cells. Expression of CD40L can also be found on activated B cells, NK cells, monocytes, dendritic cells, endothelial cells, and smooth muscle cells. CD40–CD40L interaction plays a general role in the immune regulation (apoptosis and enhancing cell survival) [150]. In addition, soluble CD40L (sCD40L) has been obtained from serum of patients with lymphoma, CLL, MM, and autoimmune diseases. It is recognized as an independent risk factor for some hematological malignancies [151]. Since CD40 and CD40L can be co-expressed on B-cell lymphoma, it is postulated that this system may act as an autocrine–paracrine survival loop of malignant hematopoietic cells [152]. CD40 has the structure of a typical type I transmembrane molecule with a large extracellular part, acting as a binding side for anti-CD40 mAbs which acts as a target for Abs [150].

8.8.1 Dacetuzumab (SGN-40)

Dacetuzumab, a humanized mAb with CDRs of murine S2C6 in the human IgG1 framework sequences, is found to have potent anti-lymphoma effects, including growth arrest upon cross-linking, induction of apoptosis, and ADCC that is Fc dependent *in vitro* assays [153, 154]. Dacetuzumab significantly increased the survival of mice compared to controls in a Daudi mouse model [154]. It has been found to result in transient decrease of T cells and NK cells and a persistent decrease in CD20-positive cells, after injection into cynomolgus monkeys. Dacetuzumab was well tolerated in patients with CLL, MM, and relapsed/refractory NHL, as demonstrated in different phase I studies conducted on different CD40-positive malignancies [155]. Nonetheless, no response was seen in patients with FL in a dose-escalating trial for NHL,

whereas an ORR of 12 % was maintained in patients with pretreated DLBCL [156].

8.8.2 Lucatumumab (HCD122, CHIR-12.12)

Lucatumumab, a fully human anti-CD40 mAb, is generated in a human IgG1 transgenic mouse by immunizing mice with the extracellular domain of recombinant human CD40. Effector cells are more potently activated by Lucatumumab compared to rituximab [157].

8.9 CD52

CD52, a low molecular weight glycoprotein (21–28 kDa) of unknown function [172], is exclusively expressed on mature B and T lymphocytes, NK cells, monocytes, and dendritic cells and is absent on hematopoietic precursors.

8.9.1 Alemtuzumab (CAMPATH-1H)

Alemtuzumab, a humanized rat IgG CD52 mAb, is created by transferring the antigen-specific CDRs of the rat mAb onto a human framework. It is known to act through CDC, ADCC, and apoptosis induction in *in vitro*, yet its exact mechanism for the *in vivo* killing remains to be unveiled [172]. Studies have revealed its efficacy in cutaneous T-cell lymphoma and peripheral T-cell lymphoma [173]. Although CD52 is also expressed on FL cells, no clinical trials have been conducted in this regard [13]. It has proved as a competent mAb in combination with chemotherapy. In a recent study on patients with relapsed or refractory advanced T-cell NHL (age range: 11–65), who had previously received remission induction by cladribine, cytosine, arabinosine, and etoposide combined with granulocyte colony-stimulating factor support (CLAEG), patients received medium doses of alemtuzumab in combination with carmustine, etoposide, cytosine, arabinoside, and melphalan (BEAM) treatment; BEAM and alemtuzumab appeared beneficial in 20 patients from the overall 21 patients receiving CLAEG induction therapy. Nine patients experienced CR, and 50 % did not achieve CR by the time of hematopoietic stem cell transplantation (HSCT). After HSCT, 20 patients reached CR during a median follow-up of 11 months. Overall, this study revealed that reduced-intensity BEAM-alemtuzumab conditioning and allogeneic HSCT proceeding intense reinduction therapy provide curative potential in patients with advanced T-cell lymphomas, even for those not in remission [174].

8.10 CD80

CD80 (B7-1), a protein expressed on NHL cells [175], is normally limited to the cell surface of activated antigen-presenting cells including B cells, dendritic cells, and monocytes [176]. It is considered as a co-stimulatory molecule for CD28 and is expressed on T cells. Since the extracellular part of CD80 contains two Ig-like domains, it is recognized as a suitable target for mAb therapy. CD80 together with CD86 stimulates Cd28 and T-cell receptor, leading to the activation and clonal expansion of T cells. Moreover, the interaction between CD80 (and CD86) and CTLA-4 (CD152) expressed on activated T cells results in decreased T-cell response. Nonetheless, the intrinsic function of CD80 is unclear [176].

8.10.1 Galiximab (IDEC-114)

Galiximab, a chimeric IgG1 anti-CD80 mAb derived from the cynomolgus monkey and man, includes both human constant regions and monkey variable regions. Due to its structure similarity to human Abs, an immune response in patients is less likely. Galiximab has demonstrated antiproliferative and anti-apoptotic properties, as well as a dose-dependent ADCC induction in *in vitro* studies [177]; galiximab prolonged survival in mice compared to controls, to the same degree as rituximab in *in vivo* human lymphoma mouse models. Furthermore, the combination of rituximab and galiximab increased survival of mice

compared to rituximab alone [177]. After binding of galiximab to CD80, CTLA-4 is blocked, which may induce an anti-lymphoma environment [178]. A dose up to 1,200 mg/m^2 galiximab in monkeys for 5 months exhibited no adverse events [177]. In addition, its safety and efficacy were evaluated in a multicenter, dose-escalating phase I clinical trial of 37 patients with relapsed or refractory FL who received four weekly infusions of galiximab. Even though it was well tolerated, it resulted in an ORR as low as 11 % with CR in only two patients. Remarkably, delayed response was observed in some cases. Despite its 2–4 weeks half-life, its efficacy may last for years, conferring to its further immune response induction [179]. Safety and efficacy of the combination of galiximab and rituximab were evaluated in 75 patients with relapsed or refractory FL, in which 500 mg/m^2 of galiximab was recommended in combination with standard doses of rituximab. An ORR of 66 % (33 % CR/CRu; 33 % partial response) with a PFS of 12.1 months was achieved, and no adverse event was observed [85].

8.11 CD74 and HLA-DR

CD74 and HLA-DR are both members of MHC class II. CD74, the cell surface form of the invariant chain, acts as a chaperone molecule for MHC class II. CD74 binds to HLA-DR within the endoplasmic reticulum; the complex is then transferred to the late endosomal compartment, where CD74 is cleaved into peptide fragments and is dissociated from DR. Peptides form complexes with DR and are transported to the cell surface for antigen presentation to T cells [180]. Besides aiding peptide presentation, CD74 functions as a signaling molecule. Anti-CD74 mAbs have led to maturation of B cells through a direct signaling pathway involving NF-kB [181]. It also acts as a high-affinity receptor for the proinflammatory cytokine macrophage migration inhibitory factor [182]. In addition to expression on APCs, CD74 is a marker on various tumor cells, including B-cell lymphomas, gastric cancer, renal cancer, and non-small cell lung cancer. Its expression has been found to contribute to poor prognosis, possibly explained by the suppressive effects on the immune system [180]. It is regarded as an efficient target for mAb therapy, resulting in the development of different anti-CD74 Abs. Nonetheless, clinical experience is lacking. Various CD74 monoclonal antibodies are discussed below and summarized in Table 8.4.

8.11.1 Milatuzumab (IMMU-115, hLL1), Naked and Conjugated

Milatuzumab, an IgG1k anti-CD74 mAb, is derived from the murine LL1 and is humanized by CDR grafting. Rapid internalization is resulted in both CD74 and milatuzumab upon binding and is replaced by newly synthesized CD74. It has exhibited growth inhibition and apoptosis induction in *in vitro*, whereas no CDC or ADCC is exerted by milatuzumab. Significant prolonged survival was observed in human Burkitt lymphoma xenograft mouse model compared to control mice by administrating milatuzumab [141]. In single-dose and multidose experiments, no serious adverse events were experienced with only a reversible decrease in T cells, B cells, NK cells, and monocytes in cynomolgus monkeys [180]. Due to its rapid internalization (106–107 molecules/cell/day), it is a perfect target for conjugation with radioisotopes, drugs, or toxins. *In vitro* studies on murine versions of milatuzumab conjugated to different radioisotopes revealed high efficiency in eliminating B-cell lymphomas. Auger emitters (111In and 67Ga) demonstrated a potent anti-lymphoma effect and prolonged survival compared to unlabeled LL1. No significant toxicity has been observed; however, further clinical trials are warranted to establish its efficacy [180]. Milatuzumab (BR96-Dox, hLL1-Dox, IMMU-110) combined to doxorubicin (dox) manifested as a lethal combination upon binding to CD74-positive cells. A 100 % survival rate was exhibited by the administration of HLL1-Dox to mice bearing Raji lymphoma; in addition, no toxicity was observed in mice models [180, 183]. Doses up to 30 mg/kg were well tolerated in cynomolgus monkeys, and the first signs of bone marrow toxicity

were observed with doses of 30 mg/kg [183]. The combination of milatuzumab and the toxin ranpirnase, a frog RNase that results in the degradation of tRNA, yielded similar results, with respect to protein synthesis, inhibition, and apoptosis [184]. To reduce the effect of Fc-expressing cells, milatuzumab was altered into IgG4 (2L-Rap-hLL1-g4P), which manifested more potentiality *in vitro*. High remission rates were observed in Daudi and Raji mouse models. High doses of the conjugated antibody resulted in hepatotoxicity in mice. While highly efficient anti-lymphoma effects have been observed in mice, clinical trials are needed to study its safety and efficacy in clinic [184].

8.11.2 Apolizumab (Hu1D10, Remitogen)

HLA-DR, a heterodimer comprising of DRa and DRb subunits, presents antigen to the TCR on CD4-positive T cells, hence initiating a humoral immune response. In addition to APCs, most neoplastic cells, including FL, express HLA-DR. Apolizumab is derived from the murine 1D10 mAb and is humanized by CDR grafting. The polymorphic determinant on HLA-DRb is the target of apolizumab; nonetheless, the HLA-DRb is not shed or internalized from the cell surface after binding [185]. Its capable mediation of CDC, ADCC, and apoptosis has been demonstrated in *in vitro* studies [186]. Bolus infusions in rhesus macaques resulted in type I hypersensitivity reactions; nonetheless, no serious adverse event was experienced with slow infusions, except transient decrease in B cells [187]. Doses ranging from 0.15 to 15 mg/kg were found to be safe and were well tolerated in patients with relapsed NHL in a phase I dose-escalating study. Nevertheless, it showed no clinical efficacy in relapsed/refractory FL [188]. In addition, it appeared more effective in combination with rituximab in a phase I study in 35 patients with relapsed/refractory NHL. An ORR of 28 % with 17 % CR/Cru was achieved. Toxicities were mostly minor and reversible, with atypical hemolytic uremic syndrome in some patients [189]. Overall, apolizumab monotherapy seems clinically ineffective, while its efficacy may be enhanced in combination with other mAbs [13].

8.11.3 IMMU-114 (hL243g4P)

IMMU-114 is a novel humanized mAb developed with an IgG4 isotype, which targets the HLA-DRa chain, leads to direct binding, and eventually leads to antiproliferative effect and apoptosis induction [190]. An increased antiproliferative effect was observed with the combination of IMMU-114 and rituximab [191]. It revealed a disease-free survival in mice bearing CD20-resistant lymphoma cells in human lymphoma mouse models [192].

8.11.4 LYM-1

LYM-1, a murine IgG2a mAb generated by immunizing mice with Raji Burkitt lymphoma cells, targets the polymorphic variants on the HLA-DR10 b chain, activates complement, effector cells and induces apoptosis *in vitro* [193]. In a study of ten patients with refractory NHL, limited results were obtained, with only small reduction in lymph node size in some patients but with good safety profiles [194]. LYM-1 conjugated to 131-iodide has been extensively tested in two phase I/II trials in patients with therapy refractory NHL in which unconjugated LYM-1 was injected prior to the administration of escalating doses of 131-I-LYM-1. Thrombocytopenia was the only dose-limiting toxicity. In the low-dose trial, 85 % of patients obtained tumor regression with 10 % CRs. In the maximum tolerated dose trial, 52 % of the patients experienced remission, and 33 % achieved CR. A significant correlation between the levels of human anti-mouse antibodies which developed after the administration of LYM-1 and clinical response was observed [195]. LYM-1 combined with other radioisotopes including yttrium-90 [196] or copper-67 [197] yielded similar results. A synergistic anti-lymphoma effect was induced by adding

LY-M1, yttrium-conjugated Lym-1, and a chimeric form of LYM-1 to rituximab *in vitro* [193, 198].

8.11.5 Selective High-Affinity Ligands (SHALs)

Small molecules, called selective high-affinity ligands (SHALs) or antibodies, which mimic the binding of LYM-1 on HLA-DR, have been generated by linking two ligands (molecules) that recognize the LYM-1 epitope based on computational and experimental methods and are 50 times smaller than mAbs. Despite long residence time in the circulation and toxicities caused by their combination with radioisotopes, SHALs are favored by their very rapid blood clearance, making them suitable carriers for radioisotopes [199]. Studies on mice have demonstrated that radioisotopes coupled to SHAL located and targeted the tumor cells with a rapid blood clearance and no toxicity [200]; yet, no direct anti-lymphoma effect has been attributed to SAH [201]. The production and selection of SHALs need to be optimized, and the anti-lymphoma activity of radioisotopes coupled to SHALs needs to be tested in *in vivo* models before drawing a comprehensive conclusion [13].

8.12 CD1d and NK Cells

8.12.1 CD1d

CD1d, normally expressed on hematopoietic cells of myelomonocytic and B-cell lineages, is a marker for malignancies originating from the corresponding tissues.

B-cell malignancies have also been found to display CD1d. Studies on murine models have demonstrated the expression of CD1d on many leukemia and lymphoma cell lines. Moreover, NKTs have exhibited a protective role in the A20 murine B-cell lymphoma model [202], which is correlated to the level of CD1d expression on lymphoma cells and was lost in NKT-deficient mice. Studies on human lymphomas have revealed that CD1d is expressed on the surface of Reed–Sternberg (RS) cells in half of the cHL cases and in 30 % NHLs [203]. Notably, NKTs were present at high frequencies in primary cHL tumors and reactive lymph nodes irrespective of CD1d expression on tumor cells. However, the functional role of tumor-infiltrating NKTs in cHL biology and disease outcome is yet to be determined. It is postulated that NKTs may co-localize with CD1d-positive tumor-associated monocytes/macrophages (TAMs) in the microenvironment of CD1d-negative tumors [204]. In addition, the increased number of TAMs is significantly correlated to decreased survival rates in patients with cHL [205]. Targeting both RS cells and TAMs for immunotherapy with NKTs and/or their ligands seems a promising approach [206].

8.12.2 Function of NK Cells in NHL

The strongest known risk factor for the development of lymphoma is immunosuppression, predominantly NK cell dysfunction. NK cells are critical effectors in tumor immunology and were usually regarded as effector cells of innate immunity. However, more recently it has been shown that they attribute to both innate and adaptive immunity, playing a regulatory role in shaping antigen-specific T- and B-cell responses [207]. A study evaluating NK cell activity in patients with NHL and HL prior to therapy applied lactate acid dehydrogenase (LDH) release cytotoxicity assay and revealed that decreased NK cell activity in NHL patients is significantly correlated to unfavorable histology, with the lowest activity in very aggressive forms. The clinical stage of the disease also contributed to the degree of NK cell dysfunction. NK cell activity is significantly impaired in HL compared to controls, irrespective of histological type and clinical stage. Notably, the most profound NK cell dysfunction, present and persistent in HL and present in very aggressive NHL, is associated with increased LDH release activity from peripheral blood mononuclear cells. NK cell function is greatly impaired in HL and in very aggressive NHL; in addition, impaired NK cell activity is associated

with increased spontaneous release activity of LDH from patients' PBL, which is indicative of cell membrane damage, followed by the release of cytotoxic proteins, and eventually impaired NK cell activity [7].

8.12.3 Adoptive Transfer of Highly Cytotoxic NK Cells

ADCC is considered one of the major effector functions of mAbs, which is triggered following the binding of the antibody Fc region to the Fcγ receptor (FcγR) on effector cells. Most NK cells express CD16 (FcγRIII), a receptor that binds to the Fc region of IgG1, and are the major effector cells related to ADCC [208]. Given the capability of NK cells in controlling tumor growth and metastatic dissemination [209–213], novel NK cell-based cancer immunotherapies are promising [214]. The adoptive transfer of highly cytotoxic NK cells has emerged as a promising strategy in immunotherapy, which have been expanded from peripheral blood mononuclear cells (PBMC) by a feeder-cell-free expansion method [215]. Given the absence of cancer feeder cells or genetically modified cells, it is considered a safe method [216, 217]; in addition, greater NK cell enrichment and higher expansion fold than other reported methods [218–220] are achieved. T lymphocytes expansion is also accomplished. Lines of evidence suggest that the efficacy of cancer treatment is enhanced by combining mAb drugs with adoptively transferred *ex vivo* expanded NK cells [221]. Cytotoxicity and ADCC functions of expanded NK cells in combination with rituximab against $CD20^+$ lymphoma cell lines were compared with that of freshly isolated NK cells, which revealed that expanded NK cells *ex vivo* are significantly more efficient in the induction of activating receptor expression, production of IFN-γ and TNF-α, as well as cytotoxicity against various cancer cell lines including $CD133^+$ primary cancer cells, as compared to freshly isolated NK cells [215]. The emergence of other therapeutic mAbs including trastuzumab [222–224], cetuximab [225], and alemtuzumab [226] and their potential combinations with expanded NK cell therapy are hypothesized to be broadly applicable to a wide range of malignancies [215].

8.13 Therapeutic Efficacy of Antibody-Targeted Cytokines

Various limitations have led to trivial application of antibody-targeted cytokines in NHL. Some cytokines possess high systemic toxicity, hence limiting their application for specifically targeted tumor tissues. On the other hand, decreased function is observed when fused to other protein domains. Therefore, the potentiality of the antibody-targeted cytokine depends on the binding avidity and the immunomodulatory capacity of the fusion protein [10]. Various cytokines have been applied in the treatment of NHL, as discussed in the following.

8.13.1 Interferon-α (IFN-α)

IFN, a natural glycoprotein, is subdivided into three subgroups of IFN-α, IFN-β, and IFN-γ; IFN-α is produced by leukocytes, whereas fibroblasts secrete IFN-β, and IFN-γ is a product of activated T and NK cells. They are described to have immunoregulatoy activity combined with antiproliferative effects [227]. Monoclonal immunotherapy with IFN-α has yielded promising results in considerable number of patients with low-grade lymphoma [228]. Pilot studies were designed to assess the efficacy of IFN-α in low-grade lymphomas [229]. It yielded partial remission in one patient among four in a clinical trial. Another clinical trial resulted in 4 remissions among 13 patients [230]. A phase II trial studied the safety and efficacy of rituximab and IFN-α-2a in 38 patients with relapsed or refractory low-grade or follicular B-cell NHL. A dose of 2.5 MIU of IFN-α-2a, three times weekly for 12 weeks, combined with 375 mg/m^2 rituximab starting at week 5, yielded an OR of 45 %, with CR in 11 %. No toxicities were observed. Adverse events included asthenia, chills, fever,

headache, nausea, and myalgia [231]. The GELA-GOLELAMS FL2000 study investigated the efficacy of rituximab combined with CHVP (cyclophosphamide, adriamycin, etoposide, and prednisolone) chemotherapy and IFN in patients with FL as the first-line treatment. An EFS of 53 % was achieved which was significantly greater than those receiving the same regimen without rituximab [232]. Moreover, a meta-analysis investigated the effect of adding IFN-α2 to chemotherapy in patients with newly diagnosed FL. The regimen was found to be most efficacious when relatively intensive initial chemotherapy was applied, when doses ≥5 MIU with a cumulative dose ≥36 MIU per month were applied, and when it was applied in combination with chemotherapy rather than as maintenance therapy. In addition, remission duration was significantly greater when IFN-α2 was added to the regimen [233].

8.13.2 Interleukin-2 (IL-2)

The pleiotropic cytokine, interleukin-2, a 15–17 kd glycoprotein, is secreted by T lymphocytes and plays a crucial role in their proliferation. Three types of membrane components, the α, β, and γ chains, comprise the receptor. A variety of receptor types with different binding affinities are formed by different combinations of the α chain (CD25, 55 kd glycoprotein), the β chain (75 kd), and the γ chain (50–64 kd). Remarkably, hematopoietic malignancies have been described to express a high level of IL-2 receptor [234]. Therefore, it was among the initial immunotherapeutic agents. It plays a crucial role in the augmentation NK cell cytotoxicity, induction of lymphokine-activated killer (LAK) cells, and activation of T and B lymphocytes, as well as monocytes. Serum-soluble interleukin-2 (sIL-2R) level has been found to possess a prognostic value in patients with DLBCL [235] and T-cell lymphoma [236]. In addition, its correlation with the tumor burden at diagnosis and during the clinical course of therapies in patients has been recently established [237]. Notably, it has been mostly studied in HL patients as discussed in the previous chapter.

8.13.3 Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL)

TRAIL (Apo-2), a member of the TNF superfamily, consists of 28 receptors and 18 ligands. Similar to all other members of the TNF superfamily, it regulates cell survival and cell death upon infection or malignant transformation. A death-inducing signaling complex is formed, and eventually apoptosis is triggered after binding of TRAIL to its death-containing transmembrane receptor [240]. As explained by differential expression of its receptors and the absence of TRAIL-R1 and TRAIL-R2 on normal cells, they are spared, while cancer cells including lymphoma cells are selectively killed. As demonstrated in *in vitro* studies, triggering death receptors with TRAIL or agonistic antibodies activates both extrinsic and intrinsic intracellular death signaling pathways [241–243]. Various TRAIL mAbs are developed which are discussed below.

8.13.3.1 Mapatumumab (HGS-ETR1, TRM-1)

Mapatumumab, an agonistic mAb directed against TRAIL-R1, is a fully human IgG1 mAb activating both intrinsic and extrinsic apoptotic pathways upon binding. By using a single-chain variable fragment (scFv) human antibody phage library, the domain TRAIL-R1-flag fusion protein is isolated, and its potent apoptosis induction *in vitro* and *in vivo* is established. In addition, it enhances the antitumor effect of chemotherapy in mouse models [241]. It exerts its antitumor effect on different lymphoma cell lines through apoptosis induction, ADCC, and CDC. Notably, significantly increased efficacy was demonstrated when combined with rituximab in mouse models [244]. Doses up to 40 mg/kg every 10 days for 6 months were well tolerated in chimpanzees [245]. During phase I/II trials on different solid tumors, mapatumumab was well tolerated, no patient required maximum tolerated dose, and a clinical response was reported in some cases (8 %) [245].

8.13.3.2 Lexatumumab (HGS-ETR2)

Lexatumumab, a human IgG1 anti-TRAIL-R2 mAb, is constructed under the same conditions as mapatumumab, by making use of a human phage display library [241]. Even though it was found efficient in apoptosis induction and growth inhibition in NHL cell lines after cross-linking, no survival gain was reached with lexatumumab or with the combination of lexatumumab plus rituximab in mice bearing human lymphoma [244]. It yielded no objective response in a phase Ib study in patients with solid tumors; however, stable disease was observed [246]. Overall, lexatumumab has proved less beneficial in the clinical setting, compared with mapatumumab. Further preclinical studies on different tumors need to be conducted to evaluate the effect of targeting TRAIL-R2 receptors [13].

8.13.3.3 Conatumumab (AMG 655)

Conatumumab, a fully human IgG1 monoclonal agonist antibody targeting human TRAIL-R5 upon binding, induces apoptosis via caspase activation [247]. The addition of recombinant TRAIL (rhApo2) to rituximab was found to induce a strong clinical effect; it was well tolerated in patients with low-grade lymphoma who previously failed therapy with rituximab, and a CR of 25 % and PR 13 % were obtained [248]. The combination of rhApo2L/TRAIL with rituximab led to increased survival rates in subcutaneous and disseminated tumors in a xenograft model [249].

8.14 Novel Immunotherapeutic Treatment Strategies

It has been postulated that eradication of human cancers may be accomplished by combining cancer treatment modalities [250]. The lack of specificity is acknowledged as the major shortcoming of conventional cancer therapies [251]. Promising results have been yielded by combining immunotherapy and conventional treatment procedures. Due to their different therapeutic mechanism, side effects differ, and toxicities are manageable [252]. In addition, the combination is proven to yield a synergic effect [54]. Furthermore, antibodies are considered ideal vehicles for drug and radionuclides delivery, due to their high specificity [54]. Multiple clinical trials have been conducted in this regard, yet their clinical applications need to be established.

8.14.1 Molecular Engineered Antibodies

Despite significant improvement in the survival of lymphoma patients by the development of first-generation mAbs, particularly rituximab, various limitations are encountered; hence, attempts have been made to overcome these constraints. The development of humanized or fully human next-generation antibodies demonstrated reduced immunogenicity, which made them more applicable in certain patient populations. More recently, novel technologies of antibody engineering have been developed, which offer the potential to tailor antibody effector functions. Peipp et al. demonstrated that glycoprotein engineering of the Fc region of the antibody yields promising activity in preclinical models. However, these novel molecules are still in their infancy, and further clinical studies are required to determine their efficacy in improvement lymphoma treatment [48].

Antibody engineering [10] is on the progress, and it is hoped to overcome some of these limitations. Various novel techniques are discussed below.

8.14.1.1 Target Antigen and Epitope Selection

Clonal idiotypes which have been developed recently ideally fulfill the abovementioned conditions, and striking results have been obtained by the application of idiotype-specific antibodies in clinical studies. Since each antibody employed in this technique needs to be patient specific, it poses particular challenges. Currently, B-cell idiotype is mainly employed for tumor vaccination strategies [48]. Notably, none of the available antigens fulfill all the requirements for an ideal target antigen. Results from recent preclinical studies demonstrated that the fine specificity of the targeted epitope may critically affect effector mechanisms of particular antibodies.

8.14.2 Radioimmunoconjugates

8.14.2.1 Radioimmunotherapy for Follicular Lymphoma

Complexing radioisotope to a monoclonal anti-CD20 has emerged as a promising treatment approach in patients with advanced FL. The two radioimmunoconjugates currently approved by the US FDA (Food and Drug Administration) are 90Y-ibritumomab tiuxetan and 131I-tositumomab which combine the antitumor activity of rituximab with the cell-killing activity of radioisotopes [253]. High response, OS, and PFS rates have been yielded. Hematologic toxicity (neutropenia, thrombocytopenia) has been observed as the most common adverse event [254]. The efficacy of 90Y-ibritumomab tiuxetan consolidation therapy with no further therapy in patients who at least achieved PR after different induction chemotherapy regimens was studied in phase III trial (FIT trial) which yielded a high PR-to-CR conversion rate and a significantly prolonged median PFS by 2 years. Interestingly, in the subgroup of patients who received rituximab-based induction chemotherapy, PFS was not different between treatment arms [255].

8.14.2.2 CD20-Directed Radioimmunotherapy

Significant antitumor activity has been observed by applying beta-emitting radioimmunoconjugates in patients with relapsed or refractory B-cell lymphoma [256, 257], comprising both patients refractory to mAbs [258, 259] and chemotherapy [260]. To reduce antibody binding to normal B cells by depleting peripheral blood B cells and lymph node B cells, radioimmunotherapy (RIT) is administered with large quantities of unlabeled "cold" antibodies to CD20, 1 week and 4 h prior to the administration of radiolabeled antibodies to CD20 [50, 261]. Therefore, sufficient amounts of radiolabeled antibody bypass these sites and eventually penetrate less accessible compartments including the lymph nodes and target tumor cells. Nonetheless, clinical and experimental studies in mice have revealed that even low blood rituximab concentrations lead to reduction in tumor cell targeting, followed by impairment in the clinical efficacy of CD20-directed RIT [262]. On the other hand, several cycles of "cold" rituximab may lower the effect of subsequent treatment [263, 264]. Due to the competition for the CD20 target, RICs targeting CD20 are not commonly used in medical practice.

CD20-directed RIT treatment in lymphoma patients is challenging in those previously treated with rituximab, as explained by the antigenic drift and possible blockage of the CD20 antigen. Therefore, RIT targeting other antigens seems intriguing.

131I-Tositumomab

131I-tositumomab (Bexxar) is a radioimmunoconjugate consisting of the radioisotope 131I and the murine CD20 mAb, tositumomab. 131I is both a beta and gamma emitter; therefore, it can be used for imaging and dosimetry. Initial clinical trials have been conducted by Kaminski et al. using either non-myeloablative or myeloablative doses [254]. An ORR of 50–70 %, with 20–40 % CR (in a pooled analysis of 250 patients with relapsed indolent or transformed lymphoma treated in five phase I/II trials), and a 5-year PFS of 17 % were achieved when used as monotherapy in the relapsed setting [257]. Surprisingly, durable responses are observed in complete responders. An overall of 32 % of complete responders including heavily pretreated patients with bone marrow infiltration, histologic transformation, and bulky disease yielded a PFS of 1 year or longer; in addition, approximately 17 % of the original treated population was still alive and disease-free in the 5-year follow-up [265]. Overall, 131I-tositumomab has proved effective in rituximab-refractory patients [257] and achieved significant results in terms of OR and CR rates in comparison with the unlabelled parent mAb tositumomab. In addition to experiences in refractory patients, 131I-tositumomab has also been applied in the front-line setting: An ORR of 95 %, CR of 75 %, and a median time to tumor progression (TTP) of >5 years were achieved in a clinical study on 76 previously untreated patients with FL. Nonetheless, the low tumor burden in the patient population might have biased the results [266].

90Y-Ibritumomab Tiuxetan Monotherapy

90Y-ibritumomab tiuxetan consists of the pure beta-emitter, yttrium-90 isotope. Due to its nature, it cannot be applied in imaging; in

addition, densitometry is not routinely required, and dosing is done on the basis of body weight, with dose adjustment in patients with mild thrombocytopenia. It has the appeal of being administered on an outpatient basis, and no isolation measures are required. Promising results in the treatment of NHL, specifically FL, have been obtained, with an ORR of 50–80 % and CR of 20–30 %. It has been effective in rituximab-refractory patients [267]. In comparison with rituximab, 90Y-ibritumomab tiuxetan led to a higher ORR (80 *vs*. 56 %) and CR (30 *vs*. 16 %). However, no remarkable differences were observed in PFS or response duration [257]. A study demonstrated a long-term response exceeding 1 year, with a median duration of 21 months in 25 % of the treated population. Durable responses were mostly observed in complete responders, in patients with stage I/II or non-bulky disease [268].

8.14.2.3 CD37-Directed RIT

CD37-directed 177Lu-tetulomab demonstrated significantly enhanced inhibition of cell growth as compared with CD20-directed 177Lu-rituximab. 177Lu-tetulomab *vs*. 177Lu-rituximab revealed a growth delay factor of 1.6. 177Lu-tetulomab showed lower or similar uptake in lymphoma cells compared to 177Lu-rituximab. Notably, as explained by higher internalization of tetulomab compared to rituximab (almost ten times), the differences in cell growth inhibition were higher for 18-h than for 2-h incubation with the RICs. In SCID mice, the intravenously injection of Daudi cells was more effective when combined with 50 and 100 MBq/kg 177Lu-tetulomab *vs*. unlabeled tetulomab. CD37-targeted RIT has been previously studied with 131I-labeled murine monoclonal antibody (MB-1), both in mouse and human models [269–271]. In comparison with CD20, CD37 yielded a higher grade of internalization and de-halogenation of 131I-labeled RIC [269]. Despite clinical responses observed in that study, CD20 was chosen for further development. No subsequent efforts have been made to target CD37 with RICs. Initial studies with CD37 RIT used the chloramine-T method for 131I labeling [269]. However, the application of 131I-labeled Abs with the iodogen or the chloramine-T method is limited as they lack maintenance in the cells after internalization of the antigen–antibody complex [272]. Remarkably, metallic radionuclides labeled to antibodies with chelators are better preserved intracellularly after internalization [273]. Several metallic nuclides are applied for RIT against CD37. As indicated by clinical studies, NHLs are responsive to low linear energy transfer (LET) β-emitters [256, 274]; therefore, 177Lu has been chosen in clinical studies [55]. In addition, it is favored by its availability, suitable radiochemistry and half-life, and promising radiation properties. In another study conducted by Gethie et al. in [275] 177Lu-tetulomab demonstrated relatively high toxicity in SCID mice. It was postulated that the unusual biodistribution, as well as the high radiosensitivity of these DNA double-strand repair-defective mice (due to the SCID mutation), has led to high toxicity level. Yet, in line with the previous study, therapeutic effect of 177Lu-tetulomab was significantly greater than the unlabeled antibody [276]. 125I-labeled tetulomab and rituximab have also been compared which revealed similar antigen-binding properties for tetulomab and rituximab (Kd: 2.7–12.7 for tetulomab and 4.8–12 for rituximab, depending on the applied cell line). The variance in the obtained Kd for different cell lines could be explained by the possible bias caused by the curve-fitting method, as the parameters measured may influence each other. On the other hand, differences in antigen expression in various cell lines due to mutations or posttranslational changes could be involved [55]. Overall, tetulomab antibody was described as an appropriate candidate for RIT for CD37-expressing lymphoma cells. However, future clinical investigations are warranted [55]. Remarkable results have been obtained with anti-CD20 mAbs conjugated to radioisotopes. Patients refractory to rituximab who received Zevalin–rituximab combination achieved an ORR of 74 % with a duration of response of 8.7 months [257]. As demonstrated in the FIT trial, a single injection of Zevalin in first remission FL led to 3-year increase in PFS with reversible tolerable toxicity [255]. In conclusion, radioimmunotherapy has

emerged as the most effective single agent in the treatment of FL; in addition it has proved beneficial in other lymphoma subtypes. Nonetheless, the need to logistic procedures has limited its application in the clinical setting [13].

8.14.3 Immunotherapy with Genetically Modified T Cells

Adoptive immunotherapy with genetically modified T cells expressing chimeric T-cell receptors, which target lymphoma-associated antigens, has become an interesting approach [38]. It is based on grafting cytotoxic T lymphocyte with chimeric antigen receptors consisting of a tumor-specific single-chain antibody (scFv) and a cellular activation intracellular signaling domain [277]. Evidence shows that genetically modified T cells with integral membrane scFv chimeric signaling receptors react with tumor-associated antigens in a non-MHC-restricted manner, thereby bypassing the MHC–peptide complex loss, which is a significant escape mechanism for most tumors [277–279]. The intracellular signaling domain, which induces cellular activation, is derived from the cytoplasmic part of a membrane-bound receptor and induces cellular activation. The CD3ζ chain has manifested as the most potent and sufficient T-cell activation mediator [280]. The introduction of a chimeric T-cell antigen receptor gene, consisting of an extracellular scFv and an intracellular part of a signaling molecule (CD3ζ), has led to the construction of tumor-specific cytotoxic T lymphocyte [281]. To elicit substantial lymphocyte activation, adequate co-stimulatory signals are required [38]. T cells modified with chimeric antigen receptors incorporating a CD28 signaling domain have been found much more active when tested in *in vitro* and in murine models [277, 279, 280].

8.14.3.1 Engineered CD20-Specific T Cells

Adoptive immunotherapy with T cells expressing CD20-specific chimeric T-cell receptors has led to immense improvement in the treatment of lymphoma patients. However, modification of the cellular signaling pathways in target tumor cells by treatment with engineered CD20-specific T cells has yet to be fully elucidated [282]. Engineered T cells, expressing a single-chain anti-CD20 Ab, are fused to the T-cell receptor complex CD3ζ chain and MHC-unrestricted cytolysis of CD20-specific lymphoma cells [283]. The CD3ζ chain has been shown to result in sufficient T-cell activation signals [284]. In addition, CD3 and CD28 signals have revealed fundamental roles in cellular proliferation and antigen-induced IL-2 secretion of grafted T cells in an anti-CEA scFv-mediated T-cell adoptive immunotherapy study [280]. Therefore, both signals are elucidated by one recombinant receptor [280, 285]. NHL Raji cell lines were co-cultured with genetically modified T cells with anti-CD20scFvFc/CD28/CD3ζ or anti-CD20scFvFc gene, and the cytolytic activity of this engineered CD20-specific T cells was assessed. It was shown that treatment of Raji cells with T cells genetically modified with anti-CD20scFvFc/CD28/CD3ζ chimera (compared to anti-CD20scFvFc) yields a higher cytotoxicity against Raji cells. Additionally, engineered CD20-specific T cells led to a decrease in IL-10 secretion, as well as inhibition of phosphor-STAT3 and Bcl-2 expression in Raji cells, possibly through the downregulation of p38 MAPK and NF-κB activity. Thus, it was concluded that treatment of Raji cells with engineered CD20-specific T cells enhances its antitumor activities against $CD20^+$ tumor cells through the inhibition of cellular p38 MAPK signaling pathways [282]. Furthermore, engineered CD20-specific T cells were shown to particularly lyse $CD20^+$ target tumor cells and secrete IFN-γ and IL-2 after binding to their target cells. A recombinant anti-CD20scFvFc/CD28/CD3ζ gene has provided both primary and co-stimulatory signals to T cells through one chimera. It was revealed that engineered CD20-specific T cells specifically lysed CD20-positive target tumor cells and produced IFN-γ and IL-2 cytokines after binding to their target cells. Additionally, they significantly inhibited IL-10 secretion. Serum IL-10 is elevated in a number of patients with NHL, and a high IL-10 is associated with poor survival rate [286]. In addition exogenous IL-10 significantly increases NHL tumor cell proliferation [287].

It enhances growth progression and aids in the pathogenesis of NHL through autocrine–paracrine loops [287, 288]; hence, its inhibition seems crucial in the treatment of NHL. Engineered CD20-specific T cells were found to inhibit p-Lyn and p38 MAPK activities and decrease Sp1 and IL-10 levels in targeted Raji cells. In addition, genetically modified T cells reduced NF-κB DNA-binding activities and downregulated p-STAT3 and Bcl-2 expression levels.

It has been established that the downregulation of NF-κB activity induced by rituximab is mediated through the p38 MAPK signaling pathway and that phosphor-Lyn and p38 MAPK activities are inhibited by rituximab, resulting in the inhibition of IL-10 transcription via Sp1. Consequently, downregulation of the autocrine–paracrine loop of IL-10/IL-10R signaling leads to partial inhibition of p-STAT3 and Bcl-2 expression. Sp1 transcription factor is activated by p38 MAPK, and Sp1 is involved in the regulation of IL-10 expression in a number of cell lines [289]. Engineered T cells expressing anti-CD20scFvFc/CD28/CD3ζ have displayed stronger inhibition of p38 MAPK activity, downregulation of Bcl-2 expression, and IL-10 secretion, compared to the engineered T cells expressing anti-CD20scFvFc. It confers to increased cytotoxicity via inhibition of p38 MAPK activity and decrease in IL-10 secretion in the target tumor cells. Therefore, it is postulated that modifications of the cellular p38 MAPK signaling pathways in target cells hold potential in the antitumor effect of adoptive T-cell therapy [282].

8.14.4 Genetic Augmentation of Adoptive T Cells

Various challenges are encountered during the development of T-cell cancer immunotherapy; since T-cell therapy targets are mostly self-proteins, developing tolerance and weak antigenic properties, their cytotoxic activity is limited [290]. In addition, the immune system may be antagonized by the expression of inhibitory ligands and secreted factors. Thus, genetic modification of adoptively transferred T cells has been developed to overcome these evasive mechanisms. By redirecting T cells to tumor antigens through the expression of transgenic TCRs or chimeric antigen receptors (CARs), negative selection can be bypassed, and much higher levels of tumor-specific cells, with reduced dependence on co-stimulation and target cell MHC expression, are yielded. Transgenic expression of activating cytokines such as IL-2 and IL-15 can restore lymphocyte activity; in addition, suppressive factors can lead to T-cell resistance through overexpression of dominant-negative receptors [291]. Transgenic expression of receptors for tumor-secreted chemokines is believed to improve the localization of T cells at tumor site [292]. On the other hand, genetic modification may successfully result in T-cell resistance to immunosuppressive drugs [293].

8.14.4.1 Redirecting T-Cell Specificity with Transgenic TCRs

Given that tumor antigens are typically recognized as "self," tumor-specific T cells are negatively selected during development, which have low-affinity TCRs, are often anergic, and consequently have poor tumor-killing activity. However, large numbers of highly active tumor-specific T cells can be generated in a short period of time by expression of transgenic TCRs which are specific for antigens expressed on tumor cells, conferring to tumor specificity on non-tumor-specific T cells. Typically, transgenic TCRs are generated by cloning a and b subunits of class I (HLA)-restricted TCRs from tumor-reactive cytotoxic T-cell clones. Then integrating retroviral or lentiviral vectors or plasmids are used for transferring the cloned TCR into patient T cells, *ex vivo*. Transgenic TCR expression is favored by the ability to optimize affinity between TCRs and their target antigens, hence improving activation. Since cancer-specific T cells are HLA dependent, their treatment scope is limited to MHC-matched tumors, as well as tumors in which HLA antigens haven't been downregulated [294].

8.14.4.2 Redirecting T-Cell Specificity with CARs

Transgenic CARs have been developed to tackle the limitations of TCRs. CARs are synthetic constructs which have the appeal of conferring

target antigen specificity without HLA restriction. It consists of an extracellular antigen-binding domain, a transmembrane region, and a signaling endodomain. The extracellular domain, typically a single-chain variable fragment (scFv), is derived from a tumor-specific monoclonal antibody. The hinge/spacer region between the binding and transmembrane domains provides flexibility and increased access to antigens [295]. The application of an antibody-derived domain for antigen recognition facilitates the recognition of both protein-derived peptides and surface proteins with varying degrees of posttranslational modification [296]. In addition, greater affinity to antigens are observed compared with TCRs, leading to more stable immunological synapse [297]. Three groups of CARs have been developed to date, which maintain progressively increasing co-stimulatory activity. First-generation CARs, which contain a single signaling unit derived from the CD3z chain or Fc1RIg IgG receptor, have yielded modest clinical response when transferred to adoptively transferred lymphocytes for treatment of lymphoma [298]. However, they have been inadequate in achieving full T-cell activation. In an attempt to overcome this limitation, tumor-specific CAR was expressed on Epstein–Barr virus (EBV)-specific T cells [299]. An additional co-stimulation was observed when T-specific cells encountered EBV antigens *in vivo*. EBV-specific cytotoxic T lymphocytes (CTLs) yielded greater response rates and revealed tumor regression or necrosis in four out of eight patients with active disease.

Since full activation and proliferation of T cells require signaling through the CD28 receptor, the CD28 intracellular domain is inserted proximal to the CD3z endodomain in the second generation; thus, its stimulatory effects are enhanced [300]. The combining of two signaling domains results in increased proliferation, decreased activation-induced apoptosis, and increased cytokine secretion [296]. In addition, other signaling sequences such as CD137 (4-1BB) and CD134 (OX40) have been included in third-generation CARs, and T-cell function is improved [301]. Anti-CD19 CAR (FMC63 antibody-CD28-CD3z) yielded complete response in a patient with high-grade progressive follicular lymphoma [302]. Other active clinical trials involving all three generations of CARs have been reviewed, by Cooper and colleagues [303], and second- and third-generation CARs were found to be superior [304], yet their potentially supraphysiological signal is a matter of concern [305]. Events such as acute respiratory distress syndrome have been reported in a patient with metastatic colon cancer rapidly after infusion of autologous T cells transduced with an ERBB2-specific CAR (herceptin-CD28-CD137-CD3z); in addition fever and hypotension with elevated cytokine levels within 24 h have been reported in a patient with bulky chronic lymphocytic leukemia after receiving T cells transduced with a CD19-28z CAR. Notably, more studies are mandated to reveal their adverse events in lymphoma patients [306].

8.14.5 Genetic Modifications of NK Cells

NK cells as targets for cancer immunotherapy have drawn attention in recent years. Contrary to T cells, NK cells are not antigen specific, and their cytotoxicity is directed at a number of targets on cells expressing low levels of MHC class I [307]. Studies on CD20+ lymphoma have revealed that genetic modification with CARs can retarget NK cells specifically to tumor antigens [308]. A recent comparison between the classical CAR endodomain of CD28-CD3z *vs*. 2B4 (CD244), an important regulator of NK cell activation, revealed that the addition of the 2B4 endodomain proximally to CD3z significantly enhances NK cell activation as well as cytokine secretion in a tumor-specific manner [309]. Genetic modifications in the production of cytokines (IL-2, IL-12, and IL-15) can increase survival, as well as the antitumor activity of NK cells *in vivo* [310]. Remarkably, the lower potential of NK cells in GvHD induction compared to T cells renders them more suitable targets for redirecting antigen specificity modification after allogeneic transplant; however, more studies in this regard seem mandatory [303]. Overall, genetic modifications of adoptively transferred cells seem to improve the clinical outcome of

lymphoma patients. However, further clinical studies are required to ensure the long-term safety of adoptively transferred lymphocytes [290].

8.15 Vaccines

Targeting the immune system with inactivated tumor cell vaccines, which provides several tumor-specific and associated antigens as targets for the immune system, has emerged as an attractive therapeutic option [11]. Therapeutic vaccination has been recognized as potential complementary treatment for NHL. Due to the lack of co-stimulatory molecules in B-NHL, it cannot elicit proper antitumor responses. Various approaches have been developed to increase the immunogenicity of the tumor cells to be applied in the construction of cellular vaccines. Gene transfection of co-stimulatory molecules into tumor cells has been proposed to enhance their immunogenicity [311]. Genetic modification of tumor B cells with CD40L has been employed in this regard. Nonetheless, many difficulties are encountered in gene transfer-based immunotherapy, which is explained by the presence of tumor cells refractory to transfection, leading to low transfer efficiency and transgene expression level. Moreover, it is time consuming and has biosafety concerns. Therefore, attempts have been made to develop a simple method that allows the enhanced expression of several co-stimulatory molecules in recent years. It has been reported that a therapeutic whole-cell vaccine formulated with IL-2 adsorbed onto aluminum hydroxide as cytokine-depot formulation exerts potent antitumor-specific immunity, induces delayed tumor growth, controls tumor dissemination, and eventually leads to longer survival in mice with A20-lymphoma [238].

8.15.1 Salmonella Vaccine

A novel approach to design improved whole tumor cell vaccines for B-NHL was developed using salmonella (SL) infection. Salmonella infection is found to upregulate CD80, CD86, CD40, and MHC-II expression in lymphoma cells. In addition, strong antitumor-specific immunity and extended survival in lymphoma-bearing mice is observed with the administration of therapeutic vaccination with infected and then irradiated lymphoma cells combined with IL-2. It is considered to be the basis of an effective immunotherapy against B-NHL [12]. Infection of tumor cells with well-characterized, attenuated bacteria strains is proposed to upregulate the co-stimulatory molecules on the cell surface within a short period of time, hence modifying the immunogenicity of such cells in a simpler and faster way [12]. The therapeutic effect of a vaccine formulated with whole tumor cells combined with IL-2 adsorbed onto aluminum hydroxide as a depot formulation in an aggressive lymphoma murine model has been reported, which resulted in strong antitumor immunity, associated with delayed tumor growth and longer survival in A20-bearing mice [238]. Using salmonella infection improves the immunogenicity of tumor cells by modifying their phenotype. In addition, extended survival in lymphoma-bearing mice was induced, and an additive effect is obtained by combining these cells to the IL-2 depot system [12]. Attenuated strains of salmonella have clearly shown to be safe in preclinical models, as well as in several phase 1 and 2 clinical trials [312–314]. It is well established that B-cell malignancies can function as effective APCs, presenting tumor antigens directly to T cells. Tumor cell transfection with co-stimulatory molecules has been applied to achieve this. However, the presence of CD80 or CD86 on tumor cells is found to induce CD8+ T-cell-mediated rejection in many animal models, in addition to memory response induction. Infection of lymphoma cells results in rapid upregulation of CD80, CD86, and CD40 as well as MHC-II in these cells, thus increasing their immunogenicity and APC function in both mouse lymphoma cell line and primary human lymphoma cells isolated from patients with different B chronic lymphoproliferative disorders [12]. Many pathogen-associated molecular patterns (PAMPs) are recognized in salmonella which are considered as agonist of TLRs and other pattern recognition receptors (PRRs) [315, 316].

They are known to potently induce B-cell activation, increase the production of proinflammatory cytokines, and upregulate co-stimulatory molecule expression [317]. Specifically, TLR-7 and TLR-9 agonists cause an increased expression of MHC-I and co-stimulatory molecules on B cells [318, 319]. In addition, TLR agonists induce the upregulation of co-stimulatory molecules by normal and clonal B cells, which partly explains for the effect of salmonella infection vaccine on lymphoma cells. LPS or flagellin stimulation is also known to increase CD80, CD86, CD40, and MHC-II expression in A20 cells. However, they are less effective than salmonella infection. Since salmonella infection stimulates different PRRs at the same time, a greater intracellular signal is produced compared with those generated by a single TLR agonist. The authors of the study proposed salmonella-modified A20 cells as the only efficient inductor of the recruitment of activated CD8+ T cells to the tumor [12]. As observed in the clinical setting, MHC-II expression on DLBCL cells is correlated with higher numbers of tumor-infiltrating lymphocytes and prolonged survival. Therefore, it is hypothesized that MHC-II loss in B-cell lymphomas leads to tumor immunoevasion followed by decreased patient survival. CD40 expression on DLBCL tumors has been associated with improved prognosis [18]. In addition, a correlation between loss of CD86 expression and decreased tumor-infiltrating lymphocytes in aggressive human B-cell lymphomas has been observed. Nonetheless, further investigations are mandated to establish the relationship between co-stimulatory molecule expression and prognosis of patients with B-cell malignancies expressing MHC-II [19]. A strong Th1-type IFN-mediated tumor antigen-specific cellular response elicited at both local and systemic levels combined with a strong recruitment of neutrophils and NK cells to the tumor as well as activated NKT and CD8+ T cells explains the benefits of combined treatment [320]. Overall, the feasibility of designing a cell-based immunotherapy against human lymphoma using lymphoma cells modified *ex vivo* by salmonella infection in combination with a system for *in vivo* slow release of IL-2 should be taken into consideration for more efficient immunotherapy in lymphomas; in addition further clinical trials are necessitated before its clinical application [12]. However, a few studies on animal models are available. Houghton et al. reported marked increase in tumor-associated neutrophils (TANs) in animals receiving the salmonella-activated lymphoid tumor cells. In addition, they described a pro-tumoral as well as an antitumoral role for neutrophils [321]. In the same line, Fridlender et al. indicated that TAN can possess an antitumorigenic (N1) or a pro-tumorigenic (N2) phenotype. Cytokine and chemokine secretion, download of arginase, and killing tumor cells are the recognized antitumor mechanism; moreover, they are potential producers of reactive oxygen species by which the growth and invasiveness of tumor cells are modified [322]. Marked increase in tumor-associated neutrophils (TANs) was demonstrated in animal models, receiving the salmonella-activated tumor cells [321]. TANs are shown to possess both an antitumorigenic (N1) and a pro-tumorigenic (N2) phenotype [322]. Secretion of cytokines and chemokines, download levels of arginase, and enhanced capability of killing tumor cells *in vitro* are the recognized antitumor mechanisms of N1 TANs [322]. Reactive oxygen species and proteinases capable of modifying tumor growth and invasiveness are known to be produced by killer tumor cells [322, 323].

8.15.2 DNA Vaccines

In an attempt to develop innovative approaches for cancer immunotherapy, DNA vaccines were constructed which offer a great therapeutic potential [324]. Plasmid DNA containing a DNA sequence coding for an antigen and a promoter for gene expression in the mammalian cell is applied. Since plasmid DNA does not require formulation or a viral vector for delivery, it is considered a safe and stable approach which sustains the expression of Ag in cells for longer durations compared to RNA or protein vaccines. Some strategies are used for enhancing the efficacy of DNA vaccines to overcome their weak immunogenicity [325]. DNA vaccines are

novel genetic vaccines which deliver antigens and engage in multiple routes to activate innate immunity as well as adaptive immunity against cancer antigens.

The molecular format of antigen to select the desired effector pathway can be modified by the vaccine design [326]. Small amounts of antigen produced by DNA vaccines lead to effective priming, since amplification of antigen levels is obtained by the "prime/boost" strategies [327]. Electroporation is an efficient technique used during boosting which increases both antigen levels and inflammatory activity [327, 328]. In order to maximize epitope-specific immunity during engineering DNA vaccine design, T-cell epitope-driven vaccine design was developed.

8.15.3 Epitope-Driven Vaccine Design

Antitumor vaccination is favored by its potential in harnessing the full power of the immune system [324]. Induction of antitumor $CD8^+$ T cells, which exhibit cytolytic activity towards tumor cells expressing tumor-specific or tumor-associated Ags, is the mainstay of most immunotherapeutic approaches. Despite the key role of cytotoxic T lymphocytes (CTLs) in the generation of antitumor therapeutic effects, immunization strategies solely emphasizing on CTLs often prove suboptimal. The generation and maintenance of CTLs response are dependent on $CD4^+$ T cells through the supply of cytokines or by the major pathway, including dendritic cell (DC) licensing [329–331]. In addition, $CD4^+$ T helper (Th) lymphocytes promote B-cell activation and proliferation to produce neutralizing antibodies [332]. Considering the critical role of $CD4^+$ T cells, inclusion of efficient $CD4^+$ T-cell epitopes seems an interesting approach to enhance vaccine efficacy. The identification of peptide sequences recognized by $CD8^+$ or $CD4^+$ T cells on antigenic proteins has been feasible by T-cell epitope mapping.

Detection of T-cell epitopes is recognized to benefit the design of prophylactic, diagnostic, and therapeutic vaccines [333]. The aim of therapeutic vaccines is to intensify an existing immune response. Hence, identification of epitope targets in a given antigen capable of eliciting a T-cell response remains the cornerstone. In the T-cell epitope-driven vaccine design immunoinformatics are employed for the identification of potential targets against cancer [334], as well as for the development of "in silico DNA vaccines."

8.15.4 Preclinical Efficacy of Epitope-Driven DNA Vaccines Against B-Cell Lymphoma

Gene-based vaccines and immunoinformatics lead to the exploration of epitope-based DNA vaccines against B-cell lymphoma by providing molecular precision tools offered. Since idiotypic immunoglobulin (Ig)M expressed by B-cell lymphoma is a clonal marker and a tumor-specific antigen, it is considered an ideal target for immunotherapy. The hypervariable regions and mainly the complementarity-determining regions contain the idiotypic antigenic determinants. Therefore, specific immunogenic epitopes identified from these tumor antigens can be used as vaccines to activate an immune response against tumor cells [335]. DNA immunization of outbred mice with different patient-derived epitopes encompassing the variable heavy (VH) CDR3 region has been demonstrated to trigger a specific antibody immune response able to recognize native idiotypic immunoglobulins expressed on the surface of individual patient's tumor B cells [336]. Specific immune response through plasmid-based gene transfer is induced by the "molecular rescue" of the short VHCDR3 region of the idiotypic Ig, expressed on B cells of chronic lymphoproliferative disorders. Therefore, effective specific antibody response was achieved by significant matching potential of individual VHCDR3 peptides. By specific targeting of the individual CDR3 region, xenogenic or allogeneic epitopes contained in the variable and constant regions of the idiotypic Ig are excluded; therefore, the safety margin is enhanced when transferred in a syngeneic context. Rinaldi et al.

investigated the potential *in vivo* immunogenicity and safety of CDR3-based genetic immunization in the murine 38 C13 B-cell lymphoma tumor model. Therefore, epitope-based DNA vaccines were developed and investigated in murine B-cell lymphoma models by Rinaldi et al.; all three epitopes had low dissociation half-life and a low score for ligation strength value range [328]. Lines of evidence also confirm that the majority of the clonal Ig-derived peptides have a low predicted binding affinity [337, 338]. T-cell epitope prediction analysis is routinely performed using currently in progress predictive tools available at the Immune Epitope Database and Analysis Resources (IEDB) website (http://www.immuneepitope.org) [361, 362]. Various methods have been applied to modify the poor immunogenicity of a "self"-tumor-associated epitope, including alteration of the amino acid residues that interact with the MHC class I molecules at the peptide-binding cleft, possibly leading to the generation of potential agonist or "heteroclitic" epitopes resulting in enhanced binding to MHC class I [339]. Substituting suboptimal primary anchors with more optimal amino acids in "heteroclitic" peptides, in which enhanced stability of the peptide–MHC complex compared to native peptides is observed leading to improved immunogenicity [340]. Remarkably, "heteroclitic" peptides are found to trigger T-cell responses against the native peptide which they were originated, in addition to the altered peptides [338]. The potential *in vivo* immunogenicity of CDR3-based genetic immunization in the murine 38C13 B-cell lymphoma tumor model has been investigated, in which the variable light (VL) and heavy (VH) chain amino acid sequences of the idiotypic IgM 38C13 (38C13-Id) were used for the construction of two synthetic mini-genes [328]. The MHC class I-binding epitope, VHCDR3 sequence specified the 8-mer H-2KK "anchor-modified" YEGYFDYI109–116 epitope, was used. Moreover, the VLCDR3 sequence expressed the 11-mer peptide starting with the cysteine (Cys) 88 (i.e., Cys104 in the IMGT unique numbering) and encompassing the CDR3 plus the conserved phenylalanine (Phe), and glycine (Gly) residues of framework (FR) four were employed. To evaluate the potentiality of CDR3-based DNA vaccines in the induction of an immune response and protection against a subsequent lethal tumor challenge, two plasmid constructs independently expressing VH and VL determinants were developed. Finally, the tumor-protective effect recruited by CDR3-based vaccination in the poorly immunogenic, highly aggressive murine 38C13 B-cell lymphoma was observed. Experiments were performed to indicate the optimal immunization regimen, which revealed that injection of two plasmid DNAs at 3-week intervals, combined in a single vaccine formulation, led to immense suppression of tumor growth and yielded a long-term tumor-free survival in 57 % of syngeneic mice C3H/HeN (H-2KK haplotype) [328]. The combined CDR3-based vaccines displayed *in vitro* specific reactivity against peptides encompassing the CDR3 sequences after humoral immune response activation. Moreover, the native idiotypic Ig exposed on malignant lymphoma cells were specifically targeted by the induced antibodies [328]. In the same line, "heteroclitic" peptides were found to elicit significant immune responses against the native peptide from which they were derived from. Lines of evidence have described the prime/boost vaccination protocol as an effective strategy for enhancing the antigen-specific immune response. To further enhance the efficacy of this vaccination platform with the emphasis on immune response and tumor protection, a DNA fusion vaccine design encoding tumor Ags linked to pathogen-derived sequences was developed which demonstrated promising results and confirmed the efficacy of the foreign protein as an effective adjuvant. Once the antigen is fused to the foreign universal T helper epitope, the existing immune tolerance to the self-antigen is demolished, resulting in increased immunogenicity of a weak tumor antigen [341]. Since $CD4^+$ Th cells play a crucial role in coordinating innate and adaptive immune responses [326], the development of fusion vaccine incorporating a pathogen-derived sequence to activate tolerance-breaking $CD4^+$ Th cells plays a predominant role in tumor antigen-specific B- and T-cell activation. It was revealed that by placing

the candidate MHC-I binding "anchor-modified" VHCDR3109–116 tumor epitope at the 3′-terminus of the selected TTFrC 933–1126 sequence, optimal processing and presentation were achieved [342]. Overall, it is observed that by applying the epitope-driven vaccine design, immune response is focused onto the candidate tumor epitope, and $CD4^+$ T helper cells are employed from the antimicrobial repertoire [326]. In this model, gene delivery is accomplished using the nuclear targeting sequence (NTS)-harboring pRC110 vector combined with electroporation [328]. Moreover, plasmid-driven TTVHCDR3 (pTTVHCDR3) immunization is known to trigger IFN-γ-producing $CD8^+$ T-cell precursors immensely, which indicates the activation of $CD8^+$ T lymphocytes by vaccination with the fusion vaccine [327]. Rinaldi et al. demonstrated that intramuscular injection of pTTVHCDR3 DNA vaccine in combination with electroporation strongly affected the onset of highly aggressive 38C13 B-cell lymphoma and led to significant and long-lasting protection from tumor in syngeneic mice with about 85 % surviving, compared to naïve animals or those given the control vaccine. It is well established that the DNA vaccination strategy achieves protective tumor immunity. By using this strategy in which an exogenous protein is fused to tumor-specific epitope, a weak Ag can be converted into a vaccine with considerable activity. The efficacy of the VHCDR3 and VLCDR3 peptides fused to TT933–1126 FrC portion has been further assessed in a therapeutic setting. Tumorigenic dose of 38C13 cells was injected to syngeneic mice followed by electrotransfer with DNA fusion vaccines or with control plasmid after 4 days and repeated 11 days later. DNA vaccine fusion prolonged death to day 35 post tumor challenge, compared to days 18–22 post tumor challenge in control mice. However, differences were not statically significant [327]. Remarkably, the application of the TTFrC933–1126 peptide sequence proved as an effective adjuvant to overcome the intrinsic weakness of the epitope DNA-based vaccines [328]. Moreover, the combined TT933–1126-VL-AAY-VH plasmid-based vaccine demonstrated antitumor effects and conferred a prolonged animal survival rate. The application of FrC933–1126 as a peptide sequence is known to improve the efficacy of epitope-based vaccines [343].

Efforts towards developing improved treatment strategies including chemotherapy followed with radioimmunotherapy are recommended [55].

8.16 Concluding Remarks

Overall, the emergence of immunotherapy for NHL has the potential to replace all other conventional treatment modalities and has led to better outcome of the disease. Significant advances have been made in the past decade with improvement in survival as evidenced by the declining rate of lymphoma-related death. Extensive clinical studies, however, are warranted before applying the novel immunotherapies in clinic.

References

1. Lunning MA, Vose JM. Management of indolent lymphoma: where are we now and where are we going. Blood Rev. 2012;26(6):279–88.
2. Czuczman MS, Weaver R, Alkuzweny B, Berlfein J, Grillo-López AJ. Prolonged clinical and molecular remission in patients with low-grade or follicular non-Hodgkin's lymphoma treated with rituximab plus CHOP chemotherapy: 9-year follow-up. J Clin Oncol. 2004;22(23):4711–6.
3. Robert N, Leyland-Jones B, Asmar L, Belt R, Ilegbodu D, Loesch D, et al. Randomized phase III study of trastuzumab, paclitaxel, and carboplatin compared with trastuzumab and paclitaxel in women with HER-2–overexpressing metastatic breast cancer. J Clin Oncol. 2006;24(18):2786–92.
4. Neuberger M, Williams G, Mitchell E, Jouhal S, Flanagan J, Rabbitts T. A hapten-specific chimaeric IgE antibody with human physiological effector function. Nature. 1985;314(6008):268–70.
5. Foon KA. Immunologic classification of leukemia and lymphoma. Blood. 1986;68(1):1–31.
6. Ghetie V, Ward ES. Multiple roles for the major histocompatibility complex class I-related receptor FcRn. Annus Rev Immunol. 2000;18(1):739–66.
7. Konjevic G, Jurisic V, Jovic V, Vuletic A, Martinovic KM, Radenkovic S, et al. Investigation of NK cell function and their modulation in different malignancies. Immunol Res. 2012;52(1–2):139–56.

8. Gerber H-P. Emerging immunotherapies targeting CD30 in Hodgkin's lymphoma. Biochem Pharmacol. 2010;79(11):1544–52.
9. Carter PJ. Potent antibody therapeutics by design. Nat Rev Immunol. 2006;6(5):343–57.
10. Jahn T, Zuther M, Friedrichs B, Heuser C, Guhlke S, Abken H, et al. An Il12-Il2-antibody fusion protein targeting Hodgkin's lymphoma cells potentiates activation of Nk and T cells for an anti-tumor attack. PLoS One. 2012;7(9):e44482.
11. Brody J, Levy R. Lymphoma immunotherapy: vaccines, adoptive cell transfer and immunotransplant. Immunotherapy. 2009;1(5):809–24.
12. Grille S, Moreno M, Brugninib A, Lensb D, Chabalgoity J. A therapeutic vaccine using Salmonella-modified tumor cells combined with interleukin-2 induces enhanced antitumor immunity in B-cell lymphoma. Leuk Res. 2012;37:341–8.
13. van Meerten T, Hagenbeek A. Novel antibodies against follicular non-Hodgkin's lymphoma. Best Pract Res Clin Haematol. 2011;24(2):231–56.
14. Rimsza LM, Roberts RA, Miller TP, Unger JM, LeBlanc M, Braziel RM, et al. Loss of MHC class II gene and protein expression in diffuse large B-cell lymphoma is related to decreased tumor immunosurveillance and poor patient survival regardless of other prognostic factors: a follow-up study from the Leukemia and Lymphoma Molecular Profiling Project. Blood. 2004;103(11):4251–8.
15. Miller TP, Lippman SM, Spier CM, Slymen DJ, Grogan TM. HLA-DR (Ia) immune phenotype predicts outcome for patients with diffuse large cell lymphoma. J Clin Invest. 1988;82(1):370.
16. Driessens G, Kline J, Gajewski TF. Costimulatory and coinhibitory receptors in anti-tumor immunity. Immunol Rev. 2009;229(1):126–44.
17. Chambers CA. The expanding world of co-stimulation: the two-signal model revisited. Trends Immunol. 2001;22(4):217–23.
18. Linderoth J, Ehinger M, Jerkeman M, Bendahl P-O, Åkerman M, Berglund M, et al. CD40 expression identifies a prognostic ally favourable subgroup of diffuse large B-cell lymphoma. Leuk Lymph. 2007;48(9):1774–9.
19. Stopeck AT, Gessner A, Miller TP, Hersh EM, Johnson CS, Cui H, et al. Loss of B7. 2 (CD86) and intracellular adhesion molecule 1 (CD54) expression is associated with decreased tumor-infiltrating T lymphocytes in diffuse B-cell large-cell lymphoma. Clin Cancer Res. 2000;6(10):3904–9.
20. Tiemessen MM, Baert MR, Schonewille T, Brugman MH, Famili F, Salvatori DC, et al. The nuclear effector of Wnt-signaling, Tcf1, functions as a T-cell–specific tumor suppressor for development of lymphomas. PLoS Biol. 2012;10(11):e1001430.
21. Böckle B, Stanarevic G, Ratzinger G, Sepp N. Analysis of 303 Ro/SS-A antibody-positive patients: is this antibody a possible marker for malignancy? Br J Dermatol. 2012;167(5):1067–75.
22. Vera-Recabarren M, García-Carrasco M, Ramos-Casals M, Herrero C. Comparative analysis of subacute cutaneous lupus erythematosus and chronic cutaneous lupus erythematosus: clinical and immunological study of 270 patients. Br J Dermatol. 2010;162(1):91–101.
23. Chiarle R, Podda A, Prolla G, Gong J, Thorbecke GJ, Inghirami G. CD30 in normal and neoplastic cells. Clin Immunol. 1999;90(2):157–64.
24. Yurchenko M, Sidorenko S. Hodgkin's lymphoma: the role of cell surface receptors in regulation of tumor cell fate. Exp Oncol. 2010;32(4):214–23.
25. Zinzani PL, Bendandi M, Martelli M, Falini B, Sabattini E, Amadori S, et al. Anaplastic large-cell lymphoma: clinical and prognostic evaluation of 90 adult patients. J Clin Oncol. 1996;14(3):955–62.
26. Vega F. Time to look for CD30 expression in diffuse large B cell lymphomas, along the way to immunotherapy. Leuk Lymph. 2013;54:2341–2.
27. Borchmann P, Treml JF, Hansen H, Gottstein C, Schnell R, Staak O, et al. The human anti-CD30 antibody 5F11 shows in vitro and in vivo activity against malignant lymphoma. Blood. 2003;102(10):3737–42.
28. Horn-Lohrens O, Tiemann M, Lange H, Kobarg J, Hafner M, Hansen H, et al. Shedding of the soluble form of CD30 from the Hodgkin-analogous cell line L540 is strongly inhibited by a new CD30-specific antibody (Ki-4). Int J Cancer. 1995; 60(4):539–44.
29. H-J GUSS, DaSilva N, Z-B HU, Uphoff C, Goodwin R, Drexler H. Expression and regulation of CD30 ligand and CD30 in human leukemia-lymphoma cell lines. Leukemia. 1994;8(12):2083–94.
30. Gruss H, Boiani N, Williams D, Armitage R, Smith C, Goodwin R. Pleiotropic effects of the CD30 ligand on CD30-expressing cells and lymphoma cell lines. Blood. 1994;83(8):2045–56.
31. Mir SS, Richter BW, Duckett CS. Differential effects of CD30 activation in anaplastic large cell lymphoma and Hodgkin disease cells. Blood. 2000;96(13):4307–12.
32. Wahl AF, Klussman K, Thompson JD, Chen JH, Francisco LV, Risdon G, et al. The anti-CD30 monoclonal antibody SGN-30 promotes growth arrest and DNA fragmentation in vitro and affects antitumor activity in models of Hodgkin's disease. Cancer Res. 2002;62(13):3736–42.
33. Duvic M, Reddy SA, Pinter-Brown L, Korman NJ, Zic J, Kennedy DA, et al. A phase II study of SGN-30 in cutaneous anaplastic large cell lymphoma and related lymphoproliferative disorders. Clin Cancer Res. 2009;15(19):6217–24.
34. Bartlett NL, Younes A, Carabasi MH, Forero A, Rosenblatt JD, Leonard JP, et al. A phase 1 multidose study of SGN-30 immunotherapy in patients with refractory or recurrent CD30+ hematologic malignancies. Blood. 2008;111(4):1848–54.
35. Forero-Torres A, Leonard JP, Younes A, Rosenblatt JD, Brice P, Bartlett NL, et al. A Phase II study of SGN-30 (anti-CD30 mAb) in Hodgkin lymphoma or

systemic anaplastic large cell lymphoma. Br J Haematol. 2009;146(2):171–9.
36. Tedder TF, Schlossman SF. Phosphorylation of the B1 (CD20) molecule by normal and malignant human B lymphocytes. J Biol Chem. 1988;263(20):10009–15.
37. van Meerten T, Hagenbeek A, editors. CD20-targeted therapy: the next generation of antibodies. Semin Hematol. 2010;47(2):199–210.
38. Till BG, Press OW. Treatment of lymphoma with adoptively transferred T cells. Expert Opin Biol Ther. 2009;9(11):1407–25.
39. Li H, Ayer LM, Lytton J, Deans JP. Store-operated cation entry mediated by CD20 in membrane rafts. J Biol Chem. 2003;278(43):42427–34.
40. Cragg MS, Walshe CA, Ivanov AO, Glennie MJ. The biology of CD20 and its potential as a target for mAb therapy. Curr Dir Autoimmun. 2005;8:140–74.
41. Glennie MJ, French RR, Cragg MS, Taylor RP. Mechanisms of killing by anti-CD20 monoclonal antibodies. Mol Immunol. 2007;44(16):3823–37.
42. Mössner E, Brünker P, Moser S, Püntener U, Schmidt C, Herter S, et al. Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with enhanced direct and immune effector cell–mediated B-cell cytotoxicity. Blood. 2010;115(22):4393–402.
43. Byrd JC, Kitada S, Flinn IW, Aron JL, Pearson M, Lucas D, et al. The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Blood. 2002; 99(3):1038–43.
44. Di Gaetano N, Cittera E, Nota R, Vecchi A, Grieco V, Scanziani E, et al. Complement activation determines the therapeutic activity of rituximab *in vivo*. J Immunol. 2003;171(3):1581–7.
45. Beum PV, Kennedy AD, Williams ME, Lindorfer MA, Taylor RP. The shaving reaction: rituximab/CD20 complexes are removed from mantle cell lymphoma and chronic lymphocytic leukemia cells by THP-1 monocytes. J Immunol. 2006;176(4):2600–9.
46. Bowles JA, Wang S-Y, Link BK, Allan B, Beuerlein G, Campbell M-A, et al. Anti-CD20 monoclonal antibody with enhanced affinity for CD16 activates NK cells at lower concentrations and more effectively than rituximab. Blood. 2006;108(8):2648–54.
47. Koene HR, Kleijer M, Algra J, et al. Fc gammaRIIIa-158V/F polymorphism influences the binding of IgG by natural killer cell Fc gammaRIIIa, independently of the Fc gammaRIIIa-48L/R/H phenotype. Blood. 1997;3(90):1109–14.
48. Peipp M, van de Winkel JG, Valerius T. Molecular engineering to improve antibodies' anti-lymphoma activity. Best Pract Res Clin Haematol. 2011;24(2): 217–29.
49. McLaughlin P, Grillo-López AJ, Link BK, Levy R, Czuczman MS, Williams ME, et al. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol. 1998;16(8):2825–33.
50. Maloney DG, Grillo-López AJ, White CA, Bodkin D, Schilder RJ, Neidhart JA, et al. IDEC-C2B8 (rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. Blood. 1997;90(6):2188–95.
51. Cheson BD, Leonard JP. Monoclonal antibody therapy for B-cell non-Hodgkin's lymphoma. N Engl J Med. 2008;359(6):613–26.
52. Marcus R, Imrie K, Belch A, Cunningham D, Flores E, Catalano J, et al. CVP chemotherapy plus rituximab compared with CVP as first-line treatment for advanced follicular lymphoma. Blood. 2005;105(4):1417–23.
53. Hosono M, Endo K, Sakahara H, Watanabe Y, Saga T, Nakai T, et al. Human/mouse chimeric antibodies show low reactivity with human anti-murine antibodies (HAMA). Br J Cancer. 1992;65(2):197.
54. Chinn P, Braslawsky G, White C, Hanna N. Antibody therapy of non-Hodgkin's B-cell lymphoma. Cancer Immunol Immunother. 2003;52(5):257–80.
55. Dahle J, Repetto-Llamazares AH, Mollatt CS, Melhus KB, Bruland ØS, Kolstad A, et al. Evaluating antigen targeting and anti-tumor activity of a new anti-CD37 radioimmunoconjugate against non-Hodgkin's lymphoma. Anticancer Res. 2013;33(1):85–95.
56. Ratanatharathorn V, Pavletic S, Uberti JP. Clinical applications of rituximab in allogeneic stem cell transplantation: anti-tumor and immunomodulatory effects. Cancer Treat Rev. 2009;35(8):653–61.
57. Johnson P, Glennie M, editors. The mechanisms of action of rituximab in the elimination of tumor cells. Semin Oncol. 2003;30(1 Suppl 2):3–8.
58. Cragg MS, Glennie MJ. Antibody specificity controls in vivo effector mechanisms of anti-CD20 reagents. Blood. 2004;103(7):2738–43.
59. Maloney DG, Grillo-López AJ, Bodkin DJ, White CA, Liles T-M, Royston I, et al. IDEC-C2B8: results of a phase I multiple-dose trial in patients with relapsed non-Hodgkin's lymphoma. J Clin Oncol. 1997;15(10):3266–74.
60. Coiffier B, Lepage E, Brière J, Herbrecht R, Tilly H, Bouabdallah R, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med. 2002;346(4):235–42.
61. Tobinai K, Kobayashi Y, Narabayashi M, Ogura M, Kagami Y, Morishima Y, et al. Feasibility and pharmacokinetic study of a chimeric anti-CD20 monoclonal antibody (IDEC-C2B8, rituximab) in relapsed B-cell lymphoma. Ann Oncol. 1998;9(5):527–34.
62. Hamaguchi Y, Uchida J, Cain DW, Venturi GM, Poe JC, Haas KM, et al. The peritoneal cavity provides a protective niche for B1 and conventional B lymphocytes during anti-CD20 immunotherapy in mice. J Immunol. 2005;174(7):4389–99.
63. Uchida J, Hamaguchi Y, Oliver JA, Ravetch JV, Poe JC, Haas KM, et al. The innate mononuclear phagocyte network depletes B lymphocytes through Fc receptor–

dependent mechanisms during anti-CD20 antibody immunotherapy. J Exp Med. 2004;199(12):1659–69.
64. Gong Q, Ou Q, Ye S, Lee WP, Cornelius J, Diehl L, et al. Importance of cellular microenvironment and circulatory dynamics in B cell immunotherapy. J Immunol. 2005;174(2):817–26.
65. Maloney D, Liles T, Czerwinski D, Waldichuk C, Rosenberg J, Grillo-Lopez A, et al. Phase I clinical trial using escalating single-dose infusion of chimeric anti-CD20 monoclonal antibody (IDEC-C2B8) in patients with recurrent B-cell lymphoma. Blood. 1994;84(8):2457–66.
66. Feuring-Buske M, Kneba M, Unterhalt M, Engert A, Gramatzki M, Hiller E, et al. IDEC-C2B8 (rituximab) anti-CD20 antibody treatment in relapsed advanced-stage follicular lymphomas: results of a phase-II study of the German Low-Grade Lymphoma Study Group. Ann Hematol. 2000;79(9):493–500.
67. Davis T, White C, Grillo-Lopez A, Velasquez W, Link B, Maloney D, et al. Single-agent monoclonal antibody efficacy in bulky non-Hodgkin's lymphoma: results of a phase II trial of rituximab. J Clin Oncol. 1999;17(6):1851.
68. Colombat P, Salles G, Brousse N, Eftekhari P, Soubeyran P, Delwail V, et al. Rituximab (anti-CD20 monoclonal antibody) as single first-line therapy for patients with follicular lymphoma with a low tumor burden: clinical and molecular evaluation. Blood. 2001;97(1):101–6.
69. Hainsworth JD, Litchy S, Burris HA, Scullin DC, Corso SW, Yardley DA, et al. Rituximab as first-line and maintenance therapy for patients with indolent non-Hodgkin's lymphoma. J Clin Oncol. 2002; 20(20):4261–7.
70. Ardeseina K, Qian W, Smith P, Warden J, Stevens L, Pocock CF, et al. An intergroup randomised trial of rituximab versus a watch and wait strategy in patients with stage II, III, IV, asymptomatic, non-bulky follicular lymphoma (grades 1, 2 and 3a) a preliminary analysis. Lancet. 2010;15(4):424–35.
71. Schulz H, Bohlius J, Skoetz N, Trelle S, Kober T, Reiser M, et al. Chemotherapy plus rituximab versus chemotherapy alone for B-cell non-Hodgkin's lymphoma. Cochrane Database Syst Rev. 2007;4:CD003805.
72. Van Oers MH, Van Glabbeke M, Giurgea L, Klasa R, Marcus RE, Wolf M, et al. Rituximab maintenance treatment of relapsed/resistant follicular non-Hodgkin's lymphoma: long-term outcome of the EORTC 20981 phase III randomized intergroup study. J Clin Oncol. 2010;28(17):2853–8.
73. Salles G, Seymour J, Feugier P, Offner F, Lopez-Guillermo A, Bouabdallah R, et al. Rituximab maintenance for 2 years in patients with untreated high tumor burden follicular lymphoma after response to immunochemotherapy. J Clin Oncol. 2010;28(15S): 8004.
74. van Meerten T, van Rijn RS, Hol S, Hagenbeek A, Ebeling SB. Complement-induced cell death by rituximab depends on CD20 expression level and acts complementary to antibody-dependent cellular cytotoxicity. Clin Cancer Res. 2006;12(13):4027–35.
75. Macor P, Tripodo C, Zorzet S, Piovan E, Bossi F, Marzari R, et al. In vivo targeting of human neutralizing antibodies against CD55 and CD59 to lymphoma cells increases the antitumor activity of rituximab. Cancer Res. 2007;67(21):10556–63.
76. Wang S-Y, Racila E, Taylor RP, Weiner GJ. NK-cell activation and antibody-dependent cellular cytotoxicity induced by rituximab-coated target cells is inhibited by the C3b component of complement. Blood. 2008;111(3):1456–63.
77. Davis TA, Czerwinski DK, Levy R. Therapy of B-cell lymphoma with anti-CD20 antibodies can result in the loss of CD20 antigen expression. Clin Cancer Res. 1999;5(3):611–5.
78. Burger JA, Ghia P, Rosenwald A, Caligaris-Cappio F. The microenvironment in mature B-cell malignancies: a target for new treatment strategies. Blood. 2009;114(16):3367–75.
79. Vugmeyster Y, Beyer J, Howell K, Combs D, Fielder P, Yang J, et al. Depletion of B cells by a humanized anti-CD20 antibody PRO70769 in Macaca fascicularis. J Immunother. 2005;28(3):212–9.
80. Briones J. Emerging therapies for B-cell non-Hodgkin lymphoma. Expert Rev Anticancer Ther. 2009;9(9):1305–16.
81. Cartron G, Dacheux L, Salles G, Solal-Celigny P, Bardos P, Colombat P, et al. Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcγRIIIa gene. Blood. 2002;99(3):754–8.
82. Weng W-K, Levy R. Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. J Clin Oncol. 2003;21(21):3940–7.
83. Anolik JH, Campbell D, Felgar RE, Young F, Sanz I, Rosenblatt J, et al. The relationship of FcγRIIIa genotype to degree of B cell depletion by rituximab in the treatment of systemic lupus erythematosus. Arthritis Rheum. 2003;48(2):455–9.
84. Rothe A, Schulz H, Elter T, Engert A, Reiser M. Rituximab monotherapy is effective in patients with poor risk refractory aggressive non-Hodgkin's lymphoma. Haematologica. 2004;89(7):875–6.
85. Leonard J, Friedberg J, Younes A, Fisher D, Gordon L, Moore J, et al. A phase I/II study of galiximab (an anti-CD80 monoclonal antibody) in combination with rituximab for relapsed or refractory, follicular lymphoma. Ann Oncol. 2007;18(7):1216–23.
86. Rule S, Smith P, Johnson PW, Bolam S, Follows GA, Gambell J et al., editors. The addition of rituximab to fludarabine and cyclophosphamide (FC) improves overall survival in newly diagnosed mantle cell lymphoma (MCL): results of the randomised UK National Cancer Research Institute (NCRI) trial. Blood. 2011;118(21):440.
87. Schulz H, Bohlius JF, Trelle S, Skoetz N, Reiser M, Kober T, et al. Immunochemotherapy with rituximab

and overall survival in patients with indolent or mantle cell lymphoma: a systematic review and meta-analysis. J Natl Cancer Inst. 2007;99(9):706–14.
88. Forstpointner R, Dreyling M, Repp R, Hermann S, Hänel A, Metzner B, et al. The addition of rituximab to a combination of fludarabine, cyclophosphamide, mitoxantrone (FCM) significantly increases the response rate and prolongs survival as compared with FCM alone in patients with relapsed and refractory follicular and mantle cell lymphomas: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. Blood. 2004;104(10):3064–71.
89. Griffiths R, Mikhael J, Gleeson M, Danese M, Dreyling M. Addition of rituximab to chemotherapy alone as first-line therapy improves overall survival in elderly patients with mantle cell lymphoma. Blood. 2011;118(18):4808–16.
90. Martin P, Smith M, Till B. Management of mantle cell lymphoma in the elderly. Best Pract Res Clin Haematol. 2012;25(2):221–31.
91. Heinzelmann F, Ottinger H, Engelhard M, Soekler M, Bamberg M, Weinmann M. Advanced-stage III/IV follicular lymphoma. Strahlenther Onkol. 2010;186(5):247–54.
92. Ghielmini M, Schmitz S-FH, Cogliatti SB, Pichert G, Hummerjohann J, Waltzer U, et al. Prolonged treatment with rituximab in patients with follicular lymphoma significantly increases event-free survival and response duration compared with the standard weekly × 4 schedule. Blood. 2004;103(12):4416–23.
93. Herold M, Haas A, Srock S, Neser S, Al-Ali KH, Neubauer A, et al. Rituximab added to first-line mitoxantrone, chlorambucil, and prednisolone chemotherapy followed by interferon maintenance prolongs survival in patients with advanced follicular lymphoma: an East German Study Group Hematology and Oncology Study. J Clin Oncol. 2007;25(15):1986–92.
94. Gisselbrecht C. Use of rituximab in diffuse large B-cell lymphoma in the salvage setting. Br J Haematol. 2008;143(5):607–21.
95. Feugier P, Van Hoof A, Sebban C, Solal-Celigny P, Bouabdallah R, Ferme C, et al. Long-term results of the R-CHOP study in the treatment of elderly patients with diffuse large B-cell lymphoma: a study by the Groupe d'Etude des Lymphomes de l'Adulte. J Clin Oncol. 2005;23(18):4117–26.
96. Coiffier B, Feugier P, Mounier N, Franchi-Rezgui P, Van Den Neste E, Macro M et al., editors. Long-term results of the GELA study comparing R-CHOP and CHOP chemotherapy in older patients with diffuse large B-cell lymphoma show good survival in poor-risk patients. J Clin Oncol (Meeting Abstracts). 2007;25(18_Suppl):8009.
97. Kewalramani T, Zelenetz AD, Nimer SD, Portlock C, Straus D, Noy A, et al. Rituximab and ICE as second-line therapy before autologous stem cell transplantation for relapsed or primary refractory diffuse large B-cell lymphoma. Blood. 2004;103(10):3684–8.
98. Zelenetz A, Hamlin P, Kewalramani T, Yahalom J, Nimer S, Moskowitz C. Ifosfamide, carboplatin, etoposide (ICE)-based second-line chemotherapy for the management of relapsed and refractory aggressive non-Hodgkin's lymphoma. Ann Oncol. 2002;14:i5–10.
99. Mey UJ, Orlopp KS, Flieger D, Strehl JW, Ho AD, Hensel M, et al. Dexamethasone, high-dose cytarabine, and cisplatin in combination with rituximab as salvage treatment for patients with relapsed or refractory aggressive non-Hodgkin's lymphoma. Cancer Invest. 2006;24(6):593–600.
100. Vellenga E, van Putten WL, van't Veer MB, Zijlstra JM, Fibbe WE, van Oers MH, et al. Rituximab improves the treatment results of DHAP-VIM-DHAP and ASCT in relapsed/progressive aggressive CD20+ NHL: a prospective randomized HOVON trial. Blood. 2008;111(2):537–43.
101. Corazzelli G, Capobianco G, Arcamone M, Ballerini PF, Iannitto E, Russo F, et al. Long-term results of gemcitabine plus oxaliplatin with and without rituximab as salvage treatment for transplant-ineligible patients with refractory/relapsing B-cell lymphoma. Cancer Chemother Pharmacol. 2009;64(5):907–16.
102. Rigacci L, Fabbri A, Puccini B, Chitarrelli I, Chiappella A, Vitolo U, et al. Oxaliplatin-based chemotherapy (dexamethasone, high-dose cytarabine, and oxaliplatin)±rituximab is an effective salvage regimen in patients with relapsed or refractory lymphoma. Cancer. 2010;116(19):4573–9.
103. Cabanillas F, Liboy I, Rodriguez-Monge E, Pavia O, Robles N, Maldonado N, et al. A dose dense low toxicity salvage regimen for histologically aggressive non-Hodgkin's lymphoma (NHL): gemcitabine, rituximab, oxaliplatin combination (GROC) plus pegfilgrastim. J Clin Oncol. 2006;24(June20Suppl):17513.
104. Nyman H, Adde M, Karjalainen-Lindsberg M-L, Taskinen M, Berglund M, Amini R-M, et al. Prognostic impact of immunohistochemically defined germinal center phenotype in diffuse large B-cell lymphoma patients treated with immunochemotherapy. Blood. 2007;109(11):4930–5.
105. Chan HC, Hughes D, French RR, Tutt AL, Walshe CA, Teeling JL, et al. CD20-induced lymphoma cell death is independent of both caspases and its redistribution into triton X-100 insoluble membrane rafts. Cancer Res. 2003;63(17):5480–9.
106. Li B, Zhao L, Guo H, Wang C, Zhang X, Wu L, et al. Characterization of a rituximab variant with potent antitumor activity against rituximab-resistant B-cell lymphoma. Blood. 2009;114(24):5007–15.
107. Li B, Shi S, Qian W, Zhao L, Zhang D, Hou S, et al. Development of novel tetravalent anti-CD20 antibodies with potent antitumor activity. Cancer Res. 2008;68(7):2400–8.
108. Davis TA, Grillo-López AJ, White CA, McLaughlin P, Czuczman MS, Link BK, et al. Rituximab anti-CD20 monoclonal antibody therapy in non-Hodgkin's lymphoma: safety and efficacy of re-treatment. J Clin Oncol. 2000;18(17):3135–43.
109. Morschhauser F, Marlton P, Vitolo U, Lindén O, Seymour J, Crump M, et al. Results of a phase I/II study of ocrelizumab, a fully humanized anti-CD20

mAb, in patients with relapsed/refractory follicular lymphoma. Ann Oncol. 2010;21(9):1870–6.
110. Morschhauser F, Leonard JP, Fayad L, Coiffier B, Petillon M-O, Coleman M, et al. Humanized anti-CD20 antibody, veltuzumab, in refractory/recurrent non-Hodgkin's lymphoma: phase I/II results. J Clin Oncol. 2009;27(20):3346–53.
111. Hagenbeek A, Gadeberg O, Johnson P, Pedersen LM, Walewski J, Hellmann A, et al. First clinical use of ofatumumab, a novel fully human anti-CD20 monoclonal antibody in relapsed or refractory follicular lymphoma: results of a phase 1/2 trial. Blood. 2008;111(12):5486–95.
112. Teeling JL, Mackus WJ, Wiegman LJ, van den Brakel JH, Beers SA, French RR, et al. The biological activity of human CD20 monoclonal antibodies is linked to unique epitopes on CD20. J Immunol. 2006;177(1):362–71.
113. Bowles JA, Weiner GJ. CD16 polymorphisms and NK activation induced by monoclonal antibody-coated target cells. J Immunol Methods. 2005;304(1):88–99.
114. Barth M, Hernandez-Ilizaliturri F, Mavis C, Tsai P, Gibbs J, Czuczman M, editors. Activity of ofatumumab (OFA), a fully human monoclonal antibody targeting CD20, against rituximab (RTX)-sensitive (RSCL) and rituximab-resistant cell lines (RRCL), in vivo, and primary tumor cells derived from patients with B-cell lymphoma. J Clin Oncol (Meeting Abstracts). 2010;28(15):8095.
115. Hagenbeek A, Fayad L, Delwail V, Rossi JF, Jacobsen E, Kuliczkowski K et al., editors. Evaluation of ofatumumab, a novel human CD20 monoclonal antibody, as single agent therapy in rituximab-refractory follicular lymphoma. Blood. 2009;114(22):385–6.
116. Czuczman M, Viardot A, Hess G, Gadeberg O, Pedersen L, Gupta I, et al. Ofatumumab combined with CHOP in previously untreated patients with follicular lymphoma (FL). J Clin Oncol. 2010; 28(15):8042.
117. Sharkey RM, Press OW, Goldenberg DM. A re-examination of radioimmunotherapy in the treatment of non-Hodgkin lymphoma: prospects for dual-targeted antibody/radioantibody therapy. Blood. 2009;113(17):3891–5.
118. Beers SA, Chan CH, James S, French RR, Attfield KE, Brennan CM, et al. Type II (tositumomab) anti-CD20 monoclonal antibody out performs type I (rituximab-like) reagents in B-cell depletion regardless of complement activation. Blood. 2008;112(10): 4170–7.
119. Salles G, Seymour JF, Offner F, López-Guillermo A, Belada D, Xerri L, et al. Rituximab maintenance for 2 years in patients with high tumour burden follicular lymphoma responding to rituximab plus chemotherapy (PRIMA): a phase 3, randomised controlled trial. Lancet. 2011;377(9759):42–51.
120. Goldenberg DM, Rossi EA, Stein R, Cardillo TM, Czuczman MS, Hernandez-Ilizaliturri FJ, et al. Properties and structure-function relationships of veltuzumab (hA20), a humanized anti-CD20 monoclonal antibody. Blood. 2009;113(5):1062–70.
121. Negrea OG, Allen SL, Rai KR, Elstrom R, Abassi R, Farber CM et al., editors. Subcutaneous injections of low doses of humanized anti-CD20 veltuzumab for treatment of indolent B-cell malignancies. Blood. 2009;114(22):1446.
122. Rossi EA, Goldenberg DM, Cardillo TM, Stein R, Wang Y, Chang C-H. Novel designs of multivalent anti-CD20 humanized antibodies as improved lymphoma therapeutics. Cancer Res. 2008;68(20):8384–92.
123. Morschhauser F, Marlton P, Vitolo U, Linden O, Seymour J, Crump M et al., editors. Interim results of a phase I/II study of ocrelizumab, a new humanised anti-CD20 antibody in patients with relapsed/refractory follicular non-Hodgkin's lymphoma. Blood. 2007;110(11):199A.
124. Maloney DG. Follicular NHL: from antibodies and vaccines to graft-versus-lymphoma effects. ASH Educ Program Book. 2007;2007(1):226–32.
125. Bello C, Sotomayor EM. Monoclonal antibodies for B-cell lymphomas: rituximab and beyond. ASH Educ Program Book. 2007;2007(1):233–42.
126. Friedberg JW, Vose JM, Kahl BS, Brunvand MW, Goy A, Kasamon YL et al., editors. A phase I study of PRO131921, a novel anti-CD20 monoclonal antibody in patients with relapsed/refractory CD20 (+) indolent NHL: correlation between clinical responses and AUC pharmacokinetics. Blood. 2009;114(22):3742.
127. Salles G, Morschhauser F, Lamy T, Milpied N, Thieblemont C, Tilly H et al., editors. Phase I study of RO5072759 (GA101) in patients with relapsed/refractory CD20+ non-Hodgkin lymphoma (NHL). Blood. 2009;114(22):169.
128. Sehn LH, Assouline SE, Stewart DA, Mangel J, Pisa P, Kothari J et al., editors. A phase I study of GA101 (RO5072759) monotherapy followed by maintenance in patients with multiply relapsed/refractory CD20 (+) malignant disease. Blood. 2009;114(22):285.
129. Salles A, Morschhauser F, Thieblemont C, Solal-Celigny P, Lamy T, Tilly H, et al. Promising efficacy with the new anti-CD20 antibody GA101 in heavily pre-treated patients-first results from a phase II study in patients with relapsed/refractory indolent NHL (INHL). Haematologica. 2010;95 suppl 2:229.
130. Hayden-Ledbetter MS, Cerveny CG, Espling E, Brady WA, Grosmaire LS, Tan P, et al. CD20-directed small modular immunopharmaceutical, TRU-015, depletes normal and malignant B cells. Clin Cancer Res. 2009;15(8):2739–46.
131. Sgroi D, Varki A, Braesch-Andersen S, Stamenkovic I. CD22, a B cell-specific immunoglobulin superfamily member, is a sialic acid-binding lectin. J Biol Chem. 1993;268(10):7011–8.
132. Nitschke L, Carsetti R, Ocker B, Köhler G, Lamers MC. CD22 is a negative regulator of B-cell receptor signalling. Curr Biol. 1997;7(2):133–43.
133. Nakashima H, Hamaguchi Y, Watanabe R, Ishiura N, Kuwano Y, Okochi H, et al. CD22 expression mediates the regulatory functions of peritoneal B-1a cells

during the remission phase of contact hypersensitivity reactions. J Immunol. 2010;184(9):4637–45.
134. Sato S, Tuscano JM, Inaoki M, Tedder TF, editors. CD22 negatively and positively regulates signal transduction through the B lymphocyte antigen receptor. Semin Immunol. 1998;10(4):287–97.
135. Carnahan J, Wang P, Kendall R, Chen C, Hu S, Boone T, et al. Epratuzumab, a humanized monoclonal antibody targeting CD22 characterization of in vitro properties. Clin Cancer Res. 2003;9(10):3982s–90.
136. Carnahan J, Stein R, Qu Z, Hess K, Cesano A, Hansen HJ, et al. Epratuzumab, a CD22-targeting recombinant humanized antibody with a different mode of action from rituximab. Mol Immunol. 2007;44(6):1331–41.
137. Leonard JP, Coleman M, Ketas JC, Chadburn A, Ely S, Furman RR, et al. Phase I/II trial of epratuzumab (humanized anti-CD22 antibody) in indolent non-Hodgkin's lymphoma. J Clin Oncol. 2003;21(16):3051–9.
138. Leonard JP, Schuster SJ, Emmanouilides C, Couture F, Teoh N, Wegener WA, et al. Durable complete responses from therapy with combined epratuzumab and rituximab. Cancer. 2008;113(10):2714–23.
139. Strauss SJ, Morschhauser F, Rech J, Repp R, Solal-Celigny P, Zinzani PL, et al. Multicenter phase II trial of immunotherapy with the humanized anti-CD22 antibody, epratuzumab, in combination with rituximab, in refractory or recurrent non-Hodgkin's lymphoma. J Clin Oncol. 2006;24(24):3880–6.
140. Micallef IN, Kahl BS, Maurer MJ, Dogan A, Ansell SM, Colgan JP, et al. A pilot study of epratuzumab and rituximab in combination with cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy in patients with previously untreated, diffuse large B-cell lymphoma. Cancer. 2006; 107(12):2826–32.
141. Stein R, Qu Z, Chen S, Rosario A, Shi V, Hayes M, et al. Characterization of a new humanized anti-CD20 monoclonal antibody, IMMU-106, and its use in combination with the humanized anti-CD22 antibody, epratuzumab, for the therapy of non-Hodgkin's lymphoma. Clin Cancer Res. 2004;10(8):2868–78.
142. Rossi EA, Goldenberg DM, Cardillo TM, Stein R, Chang C-H. Hexavalent bispecific antibodies represent a new class of anticancer therapeutics: 1. Properties of anti-CD20/CD22 antibodies in lymphoma. Blood. 2009;113(24):6161–71.
143. Qu Z, Goldenberg DM, Cardillo TM, Shi V, Hansen HJ, Chang C-H. Bispecific anti-CD20/22 antibodies inhibit B-cell lymphoma proliferation by a unique mechanism of action. Blood. 2008;111(4):2211–9.
144. DiJoseph JF, Popplewell A, Tickle S, Ladyman H, Lawson A, Kunz A, et al. Antibody-targeted chemotherapy of B-cell lymphoma using calicheamicin conjugated to murine or humanized antibody against CD22. Cancer Immunol Immunother. 2005;54(1):11–24.
145. DiJoseph JF, Armellino DC, Boghaert ER, Khandke K, Dougher MM, Sridharan L, et al. Antibody-targeted chemotherapy with CMC-544: a CD22-targeted immunoconjugate of calicheamicin for the treatment of B-lymphoid malignancies. Blood. 2004; 103(5):1807–14.
146. DiJoseph JF, Goad ME, Dougher MM, Boghaert ER, Kunz A, Hamann PR, et al. Potent and specific antitumor efficacy of CMC-544, a CD22-targeted immunoconjugate of calicheamicin, against systemically disseminated B-cell lymphoma. Clin Cancer Res. 2004;10(24):8620–9.
147. DiJoseph JF, Dougher MM, Kalyandrug LB, Armellino DC, Boghaert ER, Hamann PR, et al. Antitumor efficacy of a combination of CMC-544 (inotuzumab ozogamicin), a CD22-targeted cytotoxic immunoconjugate of calicheamicin, and rituximab against non-Hodgkin's B-cell lymphoma. Clin Cancer Res. 2006;12(1):242–9.
148. Advani A, Coiffier B, Czuczman MS, Dreyling M, Foran J, Gine E, et al. Safety, pharmacokinetics, and preliminary clinical activity of inotuzumab ozogamicin, a novel immunoconjugate for the treatment of B-cell non-Hodgkin's lymphoma: results of a phase I study. J Clin Oncol. 2010;28(12):2085–93.
149. Dang NH, Smith MR, Offner F, Verhoef G, Johnson P, Rohatiner AZ et al., editors. Anti-CD22 immunoconjugate inotuzumab ozogamicin (CMC-544)+ rituximab: clinical activity including survival in patients with recurrent/refractory follicular or 'aggressive' lymphoma. 51st Annual meeting of the American Society of Hematology. 2009;114:242–43.
150. van Kooten C, Banchereau J. CD40-CD40 ligand. J Leukoc Biol. 2000;67(1):2–17.
151. Hock BD, McKenzie JL, Patton NW, Drayson M, Taylor K, Wakeman C, et al. Circulating levels and clinical significance of soluble CD40 in patients with hematologic malignancies. Cancer. 2006;106(10):2148–57.
152. Younes A. The dynamics of life and death of malignant lymphocytes. Curr Opin Oncol. 1999;11(5):364.
153. Oflazoglu E, Stone I, Brown L, Gordon K, van Rooijen N, Jonas M, et al. Macrophages and Fc-receptor interactions contribute to the antitumour activities of the anti-CD40 antibody SGN-40. Br J Cancer. 2008;100(1):113–7.
154. Kelley SK, Gelzleichter T, Xie D, Lee WP, Darbonne WC, Qureshi F, et al. Preclinical pharmacokinetics, pharmacodynamics, and activity of a humanized anti-CD40 antibody (SGN-40) in rodents and nonhuman primates. Br J Pharmacol. 2006;148(8): 1116–23.
155. Hussein M, Berenson JR, Niesvizky R, Munshi N, Matous J, Sobecks R, et al. A phase I multidose study of dacetuzumab (SGN-40; humanized anti-CD40 monoclonal antibody) in patients with multiple myeloma. Haematologica. 2010;95(5):845–8.
156. Advani R, Forero-Torres A, Furman RR, Rosenblatt JD, Younes A, Ren H, et al. Phase I study of the humanized anti-CD40 monoclonal antibody dacetuzumab in refractory or recurrent non-Hodgkin's lymphoma. J Clin Oncol. 2009;27(26):4371–7.
157. Lugman M, Tong X, Niu X. CHIR-12.12, an antagonist anti-CD40 63. antibody, exhibits greater ADCC than rituximab against a variety of malignant

B cells: evaluation of FcyRIIIa polymorphism and ADCC response [abstract no. 1472]. Blood. 2005; 106:424a.
158. Tedder TF, Inaoki M, Sato S. The CD19–CD21 complex regulates signal transduction thresholds governing humoral immunity and autoimmunity. Immunity. 1997;6(2):107–18.
159. Sato S, Steeber DA, Jansen PJ, Tedder TF. CD19 expression levels regulate B lymphocyte development: human CD19 restores normal function in mice lacking endogenous CD19. J Immunol. 1997; 158(10):4662–9.
160. Hooijberg E, van den Berk PC, Sein JJ, Wijdenes J, Hart AA, de Boer RW, et al. Enhanced antitumor effects of CD20 over CD19 monoclonal antibodies in a nude mouse xenograft model. Cancer Res. 1995;55(4):840–6.
161. Horton HM, Bernett MJ, Pong E, Peipp M, Karki S, Chu SY, et al. Potent in vitro and in vivo activity of an Fc-engineered anti-CD19 monoclonal antibody against lymphoma and leukemia. Cancer Res. 2008;68(19):8049–57.
162. Awan FT, Lapalombella R, Trotta R, Butchar JP, Yu B, Benson DM, et al. CD19 targeting of chronic lymphocytic leukemia with a novel Fc-domain–engineered monoclonal antibody. Blood. 2010; 115(6):1204–13.
163. Reusch ULG, Hensel M, et al. Effect of tetravalent bispecific CD19xCD3 recombinant antibody construct and CD28 costimulation on lysis of malignant B cells from patients with chronic lymphocytic leukemia by autologous T cells. Int J Cancer. 2004;112:509–18.
164. Manzke OTH, Borchmann P, et al. Locoregional treatment of low-grade B-cell lymphoma with CD3xCD19 bispecific antibodies and CD28 costimulation I. Clinical phase I evaluation. Int J Cancer. 2001;91(4):508–15.
165. Bargou R, Leo E, Zugmaier G, Klinger M, Goebeler M, Knop S, et al. Tumor regression in cancer patients by very low doses of a T cell–engaging antibody. Science. 2008;321(5891):974–7.
166. Löffler A, Kufer P, Lutterbüse R, Zettl F, Daniel PT, Schwenkenbecher JM, et al. A recombinant bispecific single-chain antibody, CD19× CD3, induces rapid and high lymphoma-directed cytotoxicity by unstimulated T lymphocytes. Blood. 2000;95(6): 2098–103.
167. Al-Katib AM, Aboukameel A, Mohammad R, Bissery M-C, Zuany-Amorim C. Superior antitumor activity of SAR3419 to rituximab in xenograft models for non-Hodgkin's lymphoma. Clin Cancer Res. 2009;15(12):4038–45.
168. Younes A, Kim S, Romaguera J. Copeland AR, de Castro Farial S, et al. Phase I multi-dose escalation study of the anti-CD19 maytansinoid immunoconjugate SAR3419 administered by intravenous(IV) infusion every 3 weeks to patients with relapsed/refractory B-cell non-Hodgkin's lymphoma (NHL). J Clin Oncol. 2012;30(22):2776–82.
169. Classon BJ, Williams AF, Willis AC, Seed B, Stamenkovic I. The primary structure of the human leukocyte antigen CD37, a species homologue of the rat MRC OX-44 antigen. J Exp Med. 1989;169(4):1497–502.
170. Schwartz-Albiez R, Dörken B, Hofmann W, Moldenhauer G. The B cell-associated CD37 antigen (gp40-52). Structure and subcellular expression of an extensively glycosylated glycoprotein. J Immunol. 1988;140(3):905–14.
171. Smeland E, Funderud S, Ruud E, Blomhoff H, Godal T. Characterization of two murine monoclonal antibodies reactive with human B cells. Scand J Immunol. 1985;21(3):205–14.
172. Nuckel H, Frey U, Roth A, et al. Alemtuzumab induces enhanced apoptosis in vitro in B-cells from patients with chronic lymphocytic leukemia by antibody-dependent cellular cytotoxicity. Eur J Pharmacol. 2005;514(2–3):217–24.
173. Gallamini A, Zaja F, Patti C, et al. Alemtuzumab (Campath-1H) and CHOP chemotherapy as first-line treatment of peripheral T-cell lymphoma: results of a GITIL. Blood. 2007;7(110):2316–23.
174. Czajczynska AGA, Repp R, Humpe A, Schub N, Raff T, Nickelsen M, Schrauder A, Schrappe M, Kneba M, Gramatzki M. Allogeneic stem cell transplantation with BEAM and alemtuzumab conditioning immediately after remission induction has curative potential in advanced T-cell non-Hodgkin's lymphoma. Biol Blood Marrow Transplant. 2013;19(11):1632–7.
175. Dakappagari N, Ho SN, Gascoyne RD, Ranuio J, Weng AP, Tangri S. CD80 (B7. 1) is expressed on both malignant B cells and nonmalignant stromal cells in non-Hodgkin lymphoma. Cytom B Clin Cytom. 2012;82(2):112–9.
176. Schultze J, Nadler L, Gribben J. B7-mediated costimulation and the immune response. Blood Rev. 1996;10(2):111–27.
177. Younes A, Hariharan K, Allen RS, Leigh BR. Initial trials of anti-CD80 monoclonal antibody (Galiximab) therapy for patients with relapsed or refractory follicular lymphoma. Clin Lymphoma Myeloma Leuk. 2003;3(4):257–9.
178. Bhat S, Czuczman MS. Galiximab: a review. Expert Opin Biol Ther. 2010;10(3):451–8.
179. Czuczman MS, Thall A, Witzig TE, Vose JM, Younes A, Emmanouilides C, et al. Phase I/II study of galiximab, an anti-CD80 antibody, for relapsed or refractory follicular lymphoma. J Clin Oncol. 2005;23(19):4390–8.
180. Stein R, Mattes MJ, Cardillo TM, Hansen HJ, Chang C-H, Burton J, et al. CD74: a new candidate target for the immunotherapy of B-cell neoplasms. Clin Cancer Res. 2007;13(18):5556s–63.
181. Starlets D, Gore Y, Binsky I, Haran M, Harpaz N, Shvidel L, et al. Cell-surface CD74 initiates a signaling cascade leading to cell proliferation and survival. Blood. 2006;107(12):4807–16.
182. Leng L, Metz CN, Fang Y, Xu J, Donnelly S, Baugh J, et al. MIF signal transduction initiated by binding to CD74. J Exp Med. 2003;197(11):1467–76.

183. Sapra P, Stein R, Pickett J, Qu Z, Govindan SV, Cardillo TM, et al. Anti-CD74 antibody-doxorubicin conjugate, IMMU-110, in a human multiple myeloma xenograft and in monkeys. Clin Cancer Res. 2005;11(14):5257–64.
184. Chang C-H, Sapra P, Vanama SS, Hansen HJ, Horak ID, Goldenberg DM. Effective therapy of human lymphoma xenografts with a novel recombinant ribonuclease/anti-CD74 humanized IgG4 antibody immunotoxin. Blood. 2005;106(13):4308–14.
185. Gingrich RD, Dahle CE, Hoskins KF, Senneff M. Identification and characterization of a new surface membrane antigen found predominantly on malignant B lymphocytes. Blood. 1990;75(12):2375–87.
186. Stockmeyer B, Schiller M, Repp R, Lorenz HM, Kalden JR, Gramatzki M, et al. Enhanced killing of B lymphoma cells by granulocyte colony-stimulating factor-primed effector cells and Hu1D10–a humanized human leucocyte antigen DR antibody. Br J Haematol. 2002;118(4):959–67.
187. Shi JD, Bullock C, Hall WC, Wescott V, Wang H, Levitt DJ, et al. In vivo pharmacodynamic effects of Hu1D10 (remitogen), a humanized antibody reactive against a polymorphic determinant of HLA-DR expressed on B cells. Leuk Lymphoma. 2002;43(6): 1303–12.
188. Tay K, Dunleavy K, Wilson WH. Targeting HLA-DR. Leuk Lymphoma. 2009;50(12):1911–3.
189. Dunleavy K, White T, Grant N, Shovlin M, Stetler-Stevenson M, Pittaluga S et al., editors. Phase 1 study of combination rituximab with apolizumab in relapsed/refractory B-cell lymphoma and chronic lymphocytic leukemia. J Clin Oncol (Meeting Abstracts). 2005;23(Suppl 16):1607.
190. Gupta P, Goldenberg DM, Rossi EA, Chang C-H. Multiple signaling pathways induced by hexavalent, monospecific, anti-CD20 and hexavalent, bispecific, anti-CD20/CD22 humanized antibodies correlate with enhanced toxicity to B-cell lymphomas and leukemias. Blood. 2010;116(17): 3258–67.
191. Stein R, Qu Z, Chen S, Solis D, Hansen HJ, Goldenberg DM. Characterization of a humanized IgG4 anti-HLA-DR monoclonal antibody that lacks effector cell functions but retains direct antilymphoma activity and increases the potency of rituximab. Blood. 2006;108(8):2736–44.
192. Stein R, Gupta P, Chen X, Cardillo TM, Furman RR, Chen S, et al. Therapy of B-cell malignancies by anti–HLA-DR humanized monoclonal antibody, IMMU-114, is mediated through hyperactivation of ERK and JNK MAP kinase signaling pathways. Blood. 2010;115(25):5180–90.
193. Liu C, DeNardo G, Tobin E, DeNardo S. Antilymphoma effects of anti-HLA-DR and CD20 monoclonal antibodies (Lym-1 and Rituximab) on human lymphoma cells. Cancer Biother Radiopharm. 2004;19(5):545–61.
194. Hu E, Epstein AL, Naeve GS, Gill I, Martin S, Sherrod A, et al. A phase 1a clinical trial of LYM-1 monoclonal antibody serotherapy in patients with refractory b cell malignancies. Hematol Oncol. 1989;7(2):155–66.
195. DeNardo GL, O'Donnell RT, Rose LM, Mirick GR, Kroger LA, DeNardo SJ. Milestones in the development of Lym-1 therapy. Hybridoma. 1999;18(1): 1–11.
196. O'Donnell RT, Shen S, Denardo SJ, Wun T, Kukis DL, Goldstein DS, et al. A phase I study of 90Y-2IT-BAD-Lym-1 in patients with non-Hodgkin's lymphoma. Anticancer Res. 1999;20(5C):3647–55.
197. O'Donnell RT, DeNardo GL, Kukis DL, Lamborn KR, Shen S, Yuan A, et al. A clinical trial of radioimmunotherapy with 67Cu-21T.-BAT-Lym-1for non-Hodgkin's lymphoma. J Nucl Med. 1999;40: 2014–20.
198. DeNardo GL, Tobin E, Chan K, Bradt BM, DeNardo SJ. Direct antilymphoma effects on human lymphoma cells of monotherapy and combination therapy with CD20 and HLA-DR antibodies and 90Y-labeled HLA-DR antibodies. Clin Cancer Res. 2005;11(19):7075s–9.
199. DeNardo GL, Hok S, Natarajan A, Cosman M, DeNardo SJ, Lightstone FC, et al. Characteristics of dimeric (bis) bidentate selective high affinity ligands as HLA-DR10 beta antibody mimics targeting non-Hodgkin's lymphoma. Int J Oncol. 2007;31(4): 729–40.
200. DeNardo GL, Natarajan A, Hok S, Perkins J, Cosman M, DeNardo SJ, et al. Pharmacokinetic characterization in xenografted mice of a series of first-generation mimics for HLA-DR antibody, Lym-1, as carrier molecules to image and treat lymphoma. J Nucl Med. 2007;48(8):1338–47.
201. West J, Perkins J, Hok S, Balhorn R, Lightstone FC, Cosman M, et al. Direct antilymphoma activity of novel, first-generation "antibody mimics" that bind HLA-DR10-positive non-Hodgkin's lymphoma cells. Cancer Biother Radiopharm. 2006;21(6): 645–54.
202. Renukaradhya GJ, Khan MA, Vieira M, Du W, Gervay-Hague J, Brutkiewicz RR. Type I NKT cells protect (and type II NKT cells suppress) the host's innate antitumor immune response to a B-cell lymphoma. Blood. 2008;111(12):5637–45.
203. Xu C, de Vries R, Visser L, Diepstra A, Gadola SD, Poppema S, et al. Expression of CD1d and presence of invariant NKT cells in classical Hodgkin lymphoma. Am J Hematol. 2010;85(7):539–41.
204. Song L, Ashgharzadeh S, Salo J, Engell K, Sposto R, Ara T, Silverman AM, DeClerck YA, Seeger RC, Metelitsa LS. Valpha24-invariant NKT cells mediate anti-tumor activity via killing of tumor-associated macrophages. J Clin Invest. 2009;119:1524–36.
205. Steidl C, Lee T, Shah SP, Farinha P, Han G, Nayar T, et al. Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. N Engl J Med. 2010;362(10):875–85.
206. Metelitsa LS. Anti-tumor potential of type-I NKT cells against CD1d-positive and CD1d-negative

tumors in humans. Clin Immunol. 2011;140(2): 119–29.
207. Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, Lanier LL, et al. Innate or adaptive immunity? The example of natural killer cells. Science. 2011;331(6013):44–9.
208. Sulica A, Morel R, Metes D, Herberman R. Ig-binding receptors on human NK cells as effector and regulatory surface molecules. Int Rev Immunol. 2001;20(3–4):371–414.
209. Terunuma H, Deng X, Dewan Z, Fujimoto S, Yamamoto N. Potential role of NK cells in the induction of immune responses: implications for NK cell-based immunotherapy for cancers and viral infections. Int Rev Immunol. 2008;27(3):93–110.
210. Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. Lancet. 2000;356(9244):1795–9.
211. Miller JS, Soignier Y, Panoskaltsis-Mortari A, McNearney SA, Yun GH, Fautsch SK, et al. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. Blood. 2005;105(8):3051–7.
212. Yang Q, Hokland ME, Bryant JL, Zhang Y, Nannmark U, Watkins SC, et al. Tumor-localization by adoptively transferred, interleukin-2-activated NK cells leads to destruction of well-established lung metastases. Int J Cancer. 2003;105(4):512–9.
213. Pegram HJ, Jackson JT, Smyth MJ, Kershaw MH, Darcy PK. Adoptive transfer of gene-modified primary NK cells can specifically inhibit tumor progression in vivo. J Immunol. 2008;181(5):3449–55.
214. Terme M, Ullrich E, Delahaye NF, Chaput N, Zitvogel L. Natural killer cell–directed therapies: moving from unexpected results to successful strategies. Nat Immunol. 2008;9(5):486–94.
215. Deng X, Terunuma H, Nieda M, Xiao W, Nicol A. Synergistic cytotoxicity of ex vivo expanded natural killer cells in combination with monoclonal antibody drugs against cancer cells. Int Immunopharmacol. 2012;14(4):593–605.
216. Fujisaki H, Kakuda H, Shimasaki N, Imai C, Ma J, Lockey T, et al. Expansion of highly cytotoxic human natural killer cells for cancer cell therapy. Cancer Res. 2009;69(9):4010–7.
217. Harada H, Saijo K, Watanabe S, Tsuboi K, Nose T, Ishiwata I, et al. Selective expansion of human natural killer cells from peripheral blood mononuclear cells by the cell line, HFWT. Cancer Sci. 2002; 93(3):313–9.
218. Sutlu T, Stellan B, Gilljam M, Quezada HC, Nahi H, Gahrton G, et al. Clinical-grade, large-scale, feeder-free expansion of highly active human natural killer cells for adoptive immunotherapy using an automated bioreactor. Cytotherapy. 2010;12(8): 1044–55.
219. Luhm J, Brand J-M, Koritke P, Höppner M, Kirchner H, Frohn C. Large-scale generation of natural killer lymphocytes for clinical application. J Hematother Stem Cell Res. 2002;11(4):651–7.
220. Berg M, Lundqvist A, McCoy Jr P, Samsel L, Fan Y, Tawab A, et al. Clinical-grade *ex vivo*-expanded human natural killer cells up-regulate activating receptors and death receptor ligands and have enhanced cytolytic activity against tumor cells. Cytotherapy. 2009;11(3):341–55.
221. Pievani A, Belussi C, Klein C, Rambaldi A, Golay J, Introna M. Enhanced killing of human B-cell lymphoma targets by combined use of cytokine-induced killer cell (CIK) cultures and anti-CD20 antibodies. Blood. 2011;117(2):510–8.
222. Gennari R, Menard S, Fagnoni F, Ponchio L, Scelsi M, Tagliabue E, et al. Pilot study of the mechanism of action of preoperative trastuzumab in patients with primary operable breast tumors overexpressing HER2. Clin Cancer Res. 2004;10(17): 5650–5.
223. Beano A, Signorino E, Evangelista A, Brusa D, Mistrangelo M, Polimeni MA, et al. Correlation between NK function and response to trastuzumab in metastatic breast cancer patients. J Transl Med. 2008;6(1):25.
224. Kute TE, Savage L, Stehle Jr JR, Kim-Shapiro JW, Blanks MJ, Wood J, et al. Breast tumor cells isolated from in vitro resistance to trastuzumab remain sensitive to trastuzumab anti-tumor effects in vivo and to ADCC killing. Cancer Immunol Immunother. 2009;58(11):1887–96.
225. Maréchal R, De Schutter J, Nagy N, Demetter P, Lemmers A, Devière J, et al. Putative contribution of CD56 positive cells in cetuximab treatment efficacy in first-line metastatic colorectal cancer patients. BMC Cancer. 2010;10(1):340.
226. Siders WM, Shields J, Garron C, Hu Y, Boutin P, Shankara S, et al. Involvement of neutrophils and natural killer cells in the anti-tumor activity of alemtuzumab in xenograft tumor models. Leuk Lymphoma. 2010;51(7):1293–304.
227. Baron S, Tyring SK, Fleischmann Jr WR, Coppenhaver DH, Niesel DW, Klimpel GR, et al. The interferons. JAMA. 1991;266(10):1375–83.
228. McLaughlin P. The role of interferon in the therapy of low grade lymphoma. Leuk Lymphoma. 1993; 10(S1):17–20.
229. Janssen JTP, Ludwig H, Scheithauer W, De Pauw B, Keyser A, Van Tol R, et al. Phase I study of recombinant human interferon alpha-2C in patients with chemotherapy-refractory malignancies. Oncology. 1985;42 Suppl 1:3–6.
230. Leavitt R, Ratanatharathorn V, Ozer H, Ultmann J, Portlock C, Myers J et al., editors. Alfa-2b interferon in the treatment of Hodgkin's disease and non-Hodgkin's lymphoma. Semin Oncol. 1987;14(2 Suppl 2):18.
231. Davis TA, Maloney DG, Grillo-López AJ, White CA, Williams ME, Weiner GJ, et al. Combination immunotherapy of relapsed or refractory low-grade or follicular non-Hodgkin's lymphoma with ritux-

imab and interferon-α-2a. Clin Cancer Res. 2000;6(7):2644–52.
232. Salles G, Mounier N, de Guibert S, Morschhauser F, Doyen C, Rossi J-F, et al. Rituximab combined with chemotherapy and interferon in follicular lymphoma patients: results of the GELA-GOELAMS FL2000 study. Blood. 2008;112(13):4824–31.
233. Rohatiner A, Gregory W, Peterson B, Borden E, Solal-Celigny P, Hagenbeek A, et al. Meta-analysis to evaluate the role of interferon in follicular lymphoma. J Clin Oncol. 2005;23(10):2215–23.
234. Kreitman RJ, Pastan I. Recombinant single-chain immunotoxins against T and B cell leukemias. Leuk Lymphoma. 1994;13(1–2):1.
235. Yamauchi T, Matsuda Y, Takai M, Tasaki T, Tai K, Hosono N, et al. Early relapse is associated with high serum soluble onterleukin-2 receptor after the sixth cycle of R-CHOP chemotherapy in patients with advanced diffuse large B-cell lymphoma. Anticancer Res. 2012;32(11):5051–7.
236. Kitagawa J-i, Hara T, Tsurumi H, Goto N, Kanemura N, Yoshikawa T, et al. Serum-soluble interleukin-2 receptor (sIL-2R) is an extremely strong prognostic factor for patients with peripheral T-cell lymphoma, unspecified (PTCL-U). J Cancer Res Clin Oncol. 2009;135(1):53–9.
237. Yoshizato T, Nannya Y, Imai Y, Ichikawa M, Kurokawa M. Clinical significance of serum-soluble interleukin-2 receptor in patients with follicular lymphoma. Clin Lymphoma Myeloma Leuk. 2013;13(4): 410–6.
238. Grille S, Brugnini A, Nese M, Corley E, Falkenberg FW, Lens D, et al. A B-cell lymphoma vaccine using a depot formulation of interleukin-2 induces potent antitumor immunity despite increased numbers of intratumoral regulatory T cells. Cancer Immunol Immunother. 2010;59(4):519–27.
239. Slivnick DJ, Ellis TM, Nawrocki JF, Fisher RI. The impact of Hodgkin's disease on the immune system. Semin Oncol. 1990;17(6):673–82.
240. Ashkenazi A, Dixit VM. Apoptosis control by death and decoy receptors. Curr Opin Cell Biol. 1999;11(2):255–60.
241. Pukac L, Kanakaraj P, Humphreys R, Alderson R, Bloom M, Sung C, et al. HGS-ETR1, a fully human TRAIL-receptor 1 monoclonal antibody, induces cell death in multiple tumour types in vitro and in vivo. Br J Cancer. 2005;92(8):1430–41.
242. Luster TA, Carrell JA, McCormick K, Sun D, Humphreys R. Mapatumumab and lexatumumab induce apoptosis in TRAIL-R1 and TRAIL-R2 antibody-resistant NSCLC cell lines when treated in combination with bortezomib. Mol Cancer Ther. 2009;8(2):292–302.
243. Georgakis GV, Li Y, Humphreys R, Andreeff M, O'Brien S, Younes M, et al. Activity of selective fully human agonistic antibodies to the TRAIL death receptors TRAIL-R1 and TRAIL-R2 in primary and cultured lymphoma cells: induction of apoptosis and enhancement of doxorubicin-and bortezomib-induced cell death. Br J Haematol. 2005;130(4): 501–10.
244. Maddipatla S, Hernandez-Ilizaliturri FJ, Knight J, Czuczman MS. Augmented antitumor activity against B-cell lymphoma by a combination of monoclonal antibodies targeting TRAIL-R1 and CD20. Clin Cancer Res. 2007;13(15):4556–64.
245. Tolcher AW, Mita M, Meropol NJ, von Mehren M, Patnaik A, Padavic K, et al. Phase I pharmacokinetic and biologic correlative study of mapatumumab, a fully human monoclonal antibody with agonist activity to tumor necrosis factor–related apoptosis-inducing ligand receptor-1. J Clin Oncol. 2007; 25(11):1390–6.
246. Wakelee H, Patnaik A, Sikic B, Mita M, Fox N, Miceli R, et al. Phase I and pharmacokinetic study of lexatumumab (HGS-ETR2) given every 2 weeks in patients with advanced solid tumors. Ann Oncol. 2010;21(2):376–81.
247. Kaplan-Lefko PJ, Graves JD, Zoog SJ, Pan Y, Wall J, Branstetter DG, et al. Conatumumab, a fully human agonist antibody to death receptor 5, induces apoptosis via caspase activation in multiple tumor types. Cancer Biol Ther. 2010;9(8):618–31.
248. Yee L, Fanale M, Dimick K, Calvert S, Robins C, Ing J, et al. A phase IB safety and pharmacokinetic (PK) study of recombinant human Apo2L/TRAIL in combination with rituximab in patients with low-grade non-Hodgkin lymphoma. J Clin Oncol. 2007;25(18 Suppl):8078.
249. Daniel D, Yang B, Lawrence DA, Totpal K, Balter I, Lee WP, et al. Cooperation of the proapoptotic receptor agonist rhApo2L/TRAIL with the CD20 antibody rituximab against non-Hodgkin lymphoma xenografts. Blood. 2007;110(12):4037–46.
250. Lundqvist A, Berg M, Smith A, Childs RW. Bortezomib treatment to potentiate the anti-tumor immunity of ex-vivo expanded adoptively infused autologous natural killer cells. J Cancer. 2011;2:383.
251. Schiffer S, Hansen H, Hehmann-Titt G, Huhn M, Fischer R, Barth S, et al. Efficacy of an adapted granzyme B-based anti-CD30 cytolytic fusion protein against PI-9-positive classical Hodgkin lymphoma cells in a murine model. Blood Cancer J. 2013;3(3):e106.
252. Maunch PM, Armitage JO, Diehl V, Hoppe RT, Weiss LM, editors. Hodgkin's disease. Philadelphia: Lippincott Williams & Wilkins; 1999.
253. DeMonaco NA, Wu M, Osborn J, Evans T, Foon KA, Swerdlow SH, et al. Phase II trial of abbreviated CHOP-rituximab followed by 90Y ibritumomab tiuxetan (Zevalin) and rituximab in patients with previously-untreated follicular non-Hodgkin lymphoma (NHL). Blood. 2005;106(11):2449.
254. Kaminski MS, Tuck M, Estes J, Kolstad A, Ross CW, Zasadny K, et al. 131I-tositumomab therapy as initial treatment for follicular lymphoma. N Engl J Med. 2005;352(5):441–9.
255. Morschhauser F, Radford J, Van Hoof A, Vitolo U, Soubeyran P, Tilly H, et al. Phase III trial of consoli-

dation therapy with yttrium-90–ibritumomab tiuxetan compared with no additional therapy after first remission in advanced follicular lymphoma. J Clin Oncol. 2008;26(32):5156–64.
256. DeNardo GL, DeNardo SJ, Goldstein DS, Kroger LA, Lamborn KR, Levy NB, et al. Maximum-tolerated dose, toxicity, and efficacy of (131) I-Lym-1 antibody for fractionated radioimmunotherapy of non-Hodgkin's lymphoma. J Clin Oncol. 1998;16(10):3246–56.
257. Witzig TE, Gordon LI, Cabanillas F, Czuczman MS, Emmanouilides C, Joyce R, et al. Randomized controlled trial of yttrium-90–labeled ibritumomab tiuxetan radioimmunotherapy versus rituximab immunotherapy for patients with relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. J Clin Oncol. 2002;20(10):2453–63.
258. Horning SJ, Younes A, Jain V, Kroll S, Lucas J, Podoloff D, et al. Efficacy and safety of tositumomab and iodine-131 tositumomab (Bexxar) in B-cell lymphoma, progressive after rituximab. J Clin Oncol. 2005;23(4):712–9.
259. Witzig TE, Flinn IW, Gordon LI, Emmanouilides C, Czuczman MS, Saleh MN, et al. Treatment with ibritumomab tiuxetan radioimmunotherapy in patients with rituximab-refractory follicular non-Hodgkin's lymphoma. J Clin Oncol. 2002;20(15):3262–9.
260. Vose JM, Wahl RL, Saleh M, Rohatiner AZ, Knox SJ, Radford JA, et al. Multicenter phase II study of iodine-131 tositumomab for chemotherapy-relapsed/refractory low-grade and transformed low-grade B-cell non-Hodgkin's lymphomas. J Clin Oncol. 2000;18(6):1316–23.
261. Reff ME, Carner K, Chambers K, Chinn P, Leonard J, Raab R, et al. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. Blood. 1994;83(2):435–45.
262. Gopal AK, Press OW, Wilbur SM, Maloney DG, Pagel JM. Rituximab blocks binding of radiolabeled anti-CD20 antibodies (Ab) but not radiolabeled anti-CD45 Ab. Blood. 2008;112(3):830–5.
263. Wada N, Kohara M, Ogawa H, Sugiyama H, Fukuhara S, Tatsumi Y, et al. Change of CD20 expression in diffuse large B-cell lymphoma treated with rituximab, an anti-CD20 monoclonal antibody: a study of the Osaka Lymphoma Study Group. Case Rep Oncol. 2009;2(3):194–202.
264. Sugimoto T, Tomita A, Hiraga J, Shimada K, Kiyoi H, Kinoshita T, et al. Escape mechanisms from antibody therapy to lymphoma cells: downregulation of< i >CD20</i >mRNA by recruitment of the HDAC complex and not by DNA methylation. Biochem Biophys Res Commun. 2009;390(1):48–53.
265. Gopal AK, Rajendran JG, Gooley TA, Pagel JM, Fisher DR, Petersdorf SH, et al. High-dose [131I] tositumomab (anti-CD20) radioimmunotherapy and autologous hematopoietic stem-cell transplantation for adults ≥ 60 years old with relapsed or refractory B-cell lymphoma. J Clin Oncol. 2007;25(11):1396–402.
266. Press OW, Unger JM, Braziel RM, Maloney DG, Miller TP, LeBlanc M, et al. Phase II trial of CHOP chemotherapy followed by tositumomab/iodine I-131 tositumomab for previously untreated follicular non-Hodgkin's lymphoma: five-year follow-up of Southwest Oncology Group Protocol S9911. J Clin Oncol. 2006;24(25):4143–9.
267. Gordon LI, Witzig TE, Wiseman GA, Flinn IW, Spies SS, Silverman DH et al., editors. Yttrium 90 ibritumomab tiuxetan radioimmunotherapy for relapsed or refractory low-grade non-Hodgkin's lymphoma. Semin Oncol. 2002;29(1):87–92.
268. Witzig TE, Molina A, Gordon LI, Emmanouilides C, Schilder RJ, Flinn IW, et al. Long-term responses in patients with recurring or refractory B-cell non-Hodgkin lymphoma treated with yttrium 90 ibritumomab tiuxetan. Cancer. 2007;109(9):1804–10.
269. Press OW, Eary JF, Appelbaum FR, Martin PJ, Badger CC, Nelp WB, et al. Radiolabeled-antibody therapy of B-cell lymphoma with autologous bone marrow support. N Engl J Med. 1993;329(17):1219–24.
270. Kaminski MS, Fig LM, Zasadny KR, Koral KF, DelRosario RB, Francis IR, et al. Imaging, dosimetry, and radioimmunotherapy with iodine 131-labeled anti-CD37 antibody in B-cell lymphoma. J Clin Oncol. 1992;10(11):1696–711.
271. Eary JF, Press OW, Badger CC, Durack LD, Richter KY, Addison SJ, et al. Imaging and treatment of B-cell lymphoma. J Nucl Med Off Publ Soc Nucl Med. 1990;31(8):1257.
272. Sharkey RM, Behr TM, Mattes MJ, Stein R, Griffiths GL, Shih LB, et al. Advantage of residualizing radiolabels for an internalizing antibody against the B-cell lymphoma antigen, CD22. Cancer Immunol Immunother. 1997;44(3):179–88.
273. Lub-de Hooge MN, Kosterink JG, Perik PJ, Nijnuis H, Tran L, Bart J, et al. Preclinical characterisation of 111In-DTPA-trastuzumab. Br J Pharmacol. 2004;143(1):99–106.
274. Goldenberg DM, Horowitz J, Sharkey R, Hall T, Murthy S, Goldenberg H, et al. Targeting, dosimetry, and radioimmunotherapy of B-cell lymphomas with iodine-131-labeled LL2 monoclonal antibody. J Clin Oncol. 1991;9(4):548–64.
275. Ghetie MA, Richardson J, Tucker T, Jones D, Uhr JW, Vitetta ES. Disseminated or localized growth of a human B-cell tumor (Daudi) in scid mice. Int J Cancer. 1990;45(3):481–5.
276. Repetto-Llamazares AH, Larsen RH, Mollatt C, Lassmann M, Dahle J. Biodistribution and dosimetry of 177Lu-tetulomab, a new radioimmunoconjugate for treatment of non-Hodgkin lymphoma. Curr Radiopharm. 2013;6(1):20.
277. Wang H, Wei H, Zhang R, Hou S, Li B, Qian W, et al. Genetically targeted T cells eradicate established breast cancer in syngeneic mice. Clin Cancer Res. 2009;15(3):943–50.
278. Teng MW, Kershaw MH, Moeller M, Smyth MJ, Darcy PK. Immunotherapy of cancer using systemi-

cally delivered gene-modified human T lymphocytes. Hum Gene Ther. 2004;15(7):699–708.
279. Haynes NM, Trapani JA, Teng MW, Jackson JT, Cerruti L, Jane SM, et al. Single-chain antigen recognition receptors that costimulate potent rejection of established experimental tumors. Blood. 2002; 100(9):3155–63.
280. Hombach A, Wieczarkowiecz A, Marquardt T, et al. Tumor-specific T cell activation by recombinant immunoreceptors: CD3 zeta signaling and CD28 costimulation are simultaneously required for efficient IL-2 secretion and can be integrated into one combined CD28/CD3 zeta signaling receptor molecule. J Immunol. 2001;167:6123–31.
281. Rossig C, Brenner MK. Genetic modification of T lymphocytes for adoptive immunotherapy. Mol Ther. 2004;10(1):5–18.
282. Jiang L, Yu K, Du J, Ni W, Han Y, Gao S, et al. Inhibition of p38 MAPK activity in B-NHL Raji cells by treatment with engineered CD20-specific T cells. Oncol Lett. 2011;2(4):753–8.
283. Jensen M, Cooper L, Wu A, Forman S, Raubitschek A. Engineered CD20-specific primary human cytotoxic T lymphocytes for targeting B-cell malignancy. Cytotherapy. 2003;5(2):131–8.
284. Eshhar Z, Waks T, Gross G, Schindler DG. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. Proc Natl Acad Sci. 1993;90(2):720–4.
285. Yu K, Hu Y, Tan Y, et al. Immunotherapy of lymphomas with T cells modified by anti-CD20 scFv/CD28/CD3zeta recombinant gene. Leuk Lymphoma. 2008;49:1368–73.
286. Parkhurst MR, Yang JC, Langan RC, Dudley ME, Nathan D-AN, Feldman SA, et al. T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis. Mol Ther. 2010;19(3):620–6.
287. Bendle GM, Linnemann C, Hooijkaas AI, Bies L, de Witte MA, Jorritsma A, et al. Lethal graft-versus-host disease in mouse models of T cell receptor gene therapy. Nat Med. 2010;16(5):565–70.
288. Rosenberg SA. Of mice, not men: no evidence for graft-versus-host disease in humans receiving T-cell receptor–transduced autologous T cells. Mol Ther. 2010;18(10):1744.
289. Pulè MA, Straathof KC, Dotti G, Heslop HE, Rooney CM, Brenner MK. A chimeric T cell antigen receptor that augments cytokine release and supports clonal expansion of primary human T cells. Mol Ther. 2005;12(5):933–41.
290. Ngo MC, Rooney CM, Howard JM, Heslop HE. Ex vivo gene transfer for improved adoptive immunotherapy of cancer. Hum Mol Genet. 2011;20(R1): R93–9.
291. Westwood JA, Kershaw MH. Genetic redirection of T cells for cancer therapy. J Leukoc Biol. 2010;87(5): 791–803.
292. Di Stasi A, De Angelis B, Rooney CM, Zhang L, Mahendravada A, Foster AE, et al. T lymphocytes coexpressing CCR4 and a chimeric antigen receptor targeting CD30 have improved homing and antitumor activity in a Hodgkin tumor model. Blood. 2009;113(25):6392–402.
293. De Angelis B, Dotti G, Quintarelli C, Huye LE, Zhang L, Zhang M, et al. Generation of Epstein-Barr virus–specific cytotoxic T lymphocytes resistant to the immunosuppressive drug tacrolimus (FK506). Blood. 2009;114(23):4784–91.
294. Johnson LA, Morgan RA, Dudley ME, Cassard L, Yang JC, Hughes MS, et al. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Blood. 2009;114(3): 535–46.
295. Moritz D, Groner B. A spacer region between the single chain antibody-and the CD3 zeta-chain domain of chimeric T cell receptor components is required for efficient ligand binding and signaling activity. Gene Ther. 1995;2(8):539–46.
296. Sadelain M, Brentjens R, Rivière I. The promise and potential pitfalls of chimeric antigen receptors. Curr Opin Immunol. 2009;21(2):215–23.
297. Beckman RA, Weiner LM, Davis HM. Antibody constructs in cancer therapy. Cancer. 2007;109(2): 170–9.
298. Weijtens ME, Willemsen RA, Valerio D, Stam K, Bolhuis R. Single chain Ig/gamma gene-redirected human T lymphocytes produce cytokines, specifically lyse tumor cells, and recycle lytic capacity. J Immunol. 1996;157(2):836–43.
299. Pule MA, Savoldo B, Myers GD, Rossig C, Russell HV, Dotti G, et al. Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. Nat Med. 2008;14(11):1264–70.
300. Geiger TL, Nguyen P, Leitenberg D, Flavell RA. Integrated src kinase and costimulatory activity enhances signal transduction through single-chain chimeric receptors in T lymphocytes. Blood. 2001;98(8):2364–71.
301. Brentjens RJ, Santos E, Nikhamin Y, Yeh R, Matsushita M, La Perle K, et al. Genetically targeted T cells eradicate systemic acute lymphoblastic leukemia xenografts. Clin Cancer Res. 2007;13(18): 5426–35.
302. Kochenderfer JN, Wilson WH, Janik JE, Dudley ME, Stetler-Stevenson M, Feldman SA, et al. Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. Blood. 2010;116(20):4099–102.
303. Jena B, Dotti G, Cooper LJ. Redirecting T-cell specificity by introducing a tumor-specific chimeric antigen receptor. Blood. 2010;116(7):1035–44.
304. Ahmed N, Salsman VS, Yvon E, Louis CU, Perlaky L, Wels WS, et al. Immunotherapy for osteosarcoma: genetic modification of T cells overcomes low

levels of tumor antigen expression. Mol Ther. 2009;17(10):1779–87.
305. Heslop HE. Safer cars. Mol Ther. 2010;18(4):661.
306. Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. Mol Ther. 2010;18(4): 843–51.
307. Smyth MJ, Cretney E, Kelly JM, Westwood JA, Street SE, Yagita H, et al. Activation of NK cell cytotoxicity. Mol Immunol. 2005;42(4):501–10.
308. Müller T, Uherek C, Maki G, Chow KU, Schimpf A, Klingemann H-G, et al. Expression of a CD20-specific chimeric antigen receptor enhances cytotoxic activity of NK cells and overcomes NK-resistance of lymphoma and leukemia cells. Cancer Immunol Immunother. 2008;57(3):411–23.
309. Altvater B, Landmeier S, Pscherer S, Temme J, Schweer K, Kailayangiri S, et al. 2B4 (CD244) signaling by recombinant antigen-specific chimeric receptors costimulates natural killer cell activation to leukemia and neuroblastoma cells. Clin Cancer Res. 2009;15(15):4857–66.
310. Jiang W, Zhang J, Tian Z. Functional characterization of interleukin-15 gene transduction into the human natural killer cell line NKL. Cytotherapy. 2008;10(3):265–74.
311. Pizzoferrato E. B7-2 expression above a threshold elicits anti-tumor immunity as effective as interleukin-12 and prolongs survival in murine B-cell lymphoma. Int J Cancer. 2004;110(1):61–9.
312. Moreno M, Kramer MG, Yim L, Chabalgoity JA. Salmonella as live trojan horse for vaccine development and cancer gene therapy. Current Gene Ther. 2010;10(1):56–76.
313. Toso JF, Gill VJ, Hwu P, Marincola FM, Restifo NP, Schwartzentruber DJ, et al. Phase I study of the intravenous administration of attenuated Salmonella typhimurium to patients with metastatic melanoma. J Clin Oncol. 2002;20(1):142–52.
314. Heimann DM, Rosenberg SA. Continuous intravenous administration of live genetically modified salmonella typhimurium in patients with metastatic melanoma. J Immunother. 2003;26(2):179.
315. Spaner D, Shi Y, White D, Shaha S, He L, Masellis A, et al. A phase I/II trial of TLR-7 agonist immunotherapy in chronic lymphocytic leukemia. Leukemia. 2009;24(1):222–6.
316. Spaner D, Masellis A. Toll-like receptor agonists in the treatment of chronic lymphocytic leukemia. Leukemia. 2006;21(1):53–60.
317. Månsson A, Adner M, Höckerfelt U, Cardell LO. A distinct Toll-like receptor repertoire in human tonsillar B cells, directly activated by Pam3CSK4, R-837 and CpG-2006 stimulation. Immunology. 2006;118(4): 539–48.
318. Kumar H, Kawai T, Akira S. Toll-like receptors and innate immunity. Biochem Biophys Res Commun. 2009;388(4):621–5.
319. Delbridge LM, O'Riordan MX. Innate recognition of intracellular bacteria. Curr Opin Immunol. 2007;19(1):10–6.
320. Andersen MH, Schrama D, Thor Straten P, Becker JC. Cytotoxic T cells. J Invest Dermatol. 2006; 126(1):32–41.
321. Houghton AM. The paradox of tumor-associated neutrophils: fueling tumor growth with cytotoxic substances. Cell Cycle. 2010;9(9):1732–7.
322. Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, et al. Polarization of tumor-associated neutrophil phenotype by TGF-β:"N1" versus "N2" TAN. Cancer Cell. 2009;16(3):183–94.
323. Pohl C, Renner C, Schwonzen M, Schobert I, Liebenberg V, Wolf J, et al. CD30-specific AB1-AB2-AB3 internal image antibody network: Potential use as anti-idiotype vaccine against Hodgkin's lymphoma. Int J Cancer. 1993;54(3):418–25.
324. Iurescia S, Fioretti D, Fazio VM, Rinaldi M. Epitope-driven DNA vaccine design employing immunoinformatics against B-cell lymphoma: a biotech's challenge. Biotechnol Adv. 2012;30(1):372–83.
325. Fioretti D, Iurescia S, Fazio VM, Rinaldi M. DNA vaccines: developing new strategies against cancer. BioMed Res Int. 2010;2010:174378.
326. Rice J, Ottensmeier CH, Stevenson FK. DNA vaccines: precision tools for activating effective immunity against cancer. Nat Rev Cancer. 2008;8(2): 108–20.
327. Iurescia S, Fioretti D, Pierimarchi P, Signori E, Zonfrillo M, Tonon G, et al. Genetic immunization with CDR3-based fusion vaccine confers protection and long-term tumor-free survival in a mouse model of lymphoma. J Biomed Biotechnol. 2010;2010:316069.
328. Rinaldi M, Fioretti D, Iurescia S, Signori E, Pierimarchi P, Seripa D, et al. Anti-tumor immunity induced by CDR3-based DNA vaccination in a murine B-cell lymphoma model. Biochem Biophys Res Commun. 2008;370(2):279–84.
329. Smith CM, Wilson NS, Waithman J, Villadangos JA, Carbone FR, Heath WR, et al. Cognate CD4+ T cell licensing of dendritic cells in CD8+ T cell immunity. Nat Immunol. 2004;5(11):1143–8.
330. Wan YY, Flavell RA. How diverse—CD4 effector T cells and their functions. J Mol Cell Biol. 2009;1(1):20–36.
331. Hung K, Hayashi R, Lafond-Walker A, Lowenstein C, Pardoll D, Levitsky H. The central role of CD4+ T cells in the antitumor immune response. J Exp Med. 1998;188(12):2357–68.
332. Murphy KM, Travers P, Walport M. Janeway's Immunobiology. 7th ed. New York: Garland Science Publishing; 2007.
333. Kim Y, Sette A, Peters B. Applications for T-cell epitope queries and tools in the Immune Epitope Database and Analysis Resource. J Immunol Methods. 2011;374(1):62–9.
334. Rosa DS, Ribeiro SP, Cunha-Neto E. CD4+ T cell epitope discovery and rational vaccine design. Arch Immunol Ther Exp. 2010;58(2):121–30.

335. Houot R, Levy R. Vaccines for lymphomas: idiotype vaccines and beyond. Blood Rev. 2009;23(3): 137–42.
336. Rinaldi M, Ria F, Parrella P, Signori E, Serra A, Ciafrè SA, et al. Antibodies elicited by naked DNA vaccination against the complementary-determining region 3 hypervariable region of immunoglobulin heavy chain idiotypic determinants of B-lymphoproliferative disorders specifically react with patients' tumor cells. Cancer Res. 2001;61(4): 1555–62.
337. Hansson L, Rabbani H, Fagerberg J, Österborg A, Mellstedt H. T-cell epitopes within the complementarity-determining and framework regions of the tumor-derived immunoglobulin heavy chain in multiple myeloma. Blood. 2003;101(12): 4930–6.
338. Harig S, Witzens M, Krackhardt AM, Trojan A, Barrett P, Broderick R, et al. Induction of cytotoxic T-cell responses against immunoglobulin V region–derived peptides modified at human leukocyte antigen–A2 binding residues. Blood. 2001;98(10): 2999–3005.
339. Terasawa H, Tsang K-Y, Gulley J, Arlen P, Schlom J. Identification and characterization of a human agonist cytotoxic T-lymphocyte epitope of human prostate-specific antigen. Clin Cancer Res. 2002; 8(1):41–53.
340. Borbulevych OY, Baxter TK, Yu Z, Restifo NP, Baker BM. Increased immunogenicity of an anchor-modified tumor-associated antigen is due to the enhanced stability of the peptide/MHC complex: implications for vaccine design. J Immunol. 2005;174(8):4812–20.
341. Williams BB, Wall M, Miao RY, Williams B, Bertoncello I, Kershaw MH, et al. Induction of T cell-mediated immunity using a c-Myb DNA vaccine in a mouse model of colon cancer. Cancer Immunol Immunother. 2008;57(11):1635–45.
342. Link Snyder H, Bačík I, Yewdell JW, Behrens TW, Bennink JR. Promiscuous liberation of MHC-class I-binding peptides from the C termini of membrane and soluble proteins in the secretory pathway. Eur J Immunol. 1998;28(4):1339–46.
343. Signori E, Iurescia S, Massi E, Fioretti D, Chiarella P, De Robertis M, et al. DNA vaccination strategies for anti-tumour effective gene therapy protocols. Cancer Immunol Immunother. 2010;59(10): 1583–91.
344. Wenger C, Stern M, Herrmann R, Rochlitz C, Pless M. Rituximab plus gemcitabine: a therapeutic option for elderly or frail patients with aggressive non Hodgkin's lymphoma? Leuk Lymphoma. 2005;46(1):71–5.
345. El Gnaoui T, Dupuis J, Belhadj K, Jais J, Rahmouni A, Copie-Bergman C, et al. Rituximab, gemcitabine and oxaliplatin: an effective salvage regimen for patients with relapsed or refractory B-cell lymphoma not candidates for high-dose therapy. Ann Oncol. 2007;18(8):1363–8.
346. Corazzelli G, Russo F, Capobianco G, Marcacci G, Della Cioppa P, Pinto A. Gemcitabine, ifosfamide, oxaliplatin and rituximab (R-GIFOX), a new effective cytoreductive/mobilizing salvage regimen for relapsed and refractory aggressive non-Hodgkin's lymphoma: results of a pilot study. Ann Oncol. 2006;17 suppl 4:iv18–24.
347. Smith S, Toor A, Klein J, Rodriguez T, Stiff P. The combination of gallium nitrate, rituximab and dexamethasone is effective and safe as a salvage regimen for diffuse large B-cell lymphoma. J Clin Oncol. 2006;24(June20Suppl):17510.
348. Leonard JP, Coleman M, Ketas J, Ashe M, Fiore JM, Furman RR, et al. Combination antibody therapy with epratuzumab and rituximab in relapsed or refractory non-Hodgkin's lymphoma. J Clin Oncol. 2005;23(22):5044–51.
349. Niitsu N, Kohuri M, Higashihara M, Bessho M. Phase II study of the CPT-11, mitoxantrone and dexamethasone regimen in combination with rituximab in elderly patients with relapsed diffuse large B-cell lymphoma. Cancer Sci. 2006;97(9):933–7.
350. Younes A, McLaughlin P, Romaguera J, Hagemeister F, Pro B, Dang N et al., editors. Taxol plus topotecan plus rituximab (TTR) with G-CSF support: an effective salvage program for the treatment of patients with relapsed/refractory aggressive B-cell non-Hodgkin lymphoma (NHL) who failed CHOP-like and platinum-based therapy. Blood. 2003;102(11):142A–3A.
351. Canales M, Sanjurjo M, García-Vela J, De Paz R, Cobo T, de la Guia A et al., editors. Paclitaxel and topotecan in combination with rituximab as effective second-line salvage regimen in resistant aggressive non-Hodgkin's lymphoma. Ann Oncol. 2005;16:181.
352. Woehrer S, Hejna M, Skrabs C, Drach J, Zielinski CC, Jaeger U, et al. Rituximab, Ara-C, dexamethasone and oxaliplatin is safe and active in heavily pretreated patients with diffuse large B-cell lymphoma. Oncology. 2006;69(6):499–502.
353. Xia Z-G, Xu Z-Z, Zhao W-L, Zhao S-Q, Ding F, Chen Y, et al. The prognostic value of immunohistochemical subtyping in Chinese patients with de novo diffuse large B-cell lymphoma undergoing CHOP or R-CHOP treatment. Ann Hematol. 2010;89(2):171–7.
354. Saito B, Shiozawa E, Usui T, Nakashima H, Maeda T, Hattori N, et al. Rituximab with chemotherapy improves survival of non-germinal center type untreated diffuse large B-cell lymphoma. Leukemia. 2007;21(12):2563–6.
355. Fu K, Weisenburger DD, Choi WW, Perry KD, Smith LM, Shi X, et al. Addition of rituximab to standard chemotherapy improves the survival of both the germinal center B-cell–like and non–germinal center B-cell–like subtypes of diffuse large B-cell lymphoma. J Clin Oncol. 2008;26(28): 4587–94.
356. Czuczman MS, Fayad L, Delwail V, Cartron G, Jacobsen E, Kuliczkowski K, et al. Ofatumumab monotherapy in rituximab-refractory follicular lym-

phoma: results from a multicenter study. Blood. 2012;119(16):3698–704.
357. Czuczman MS, Hess G, Gadeberg OV, Pedersen LM, Goldstein N, Gupta I, et al. Chemoimmunotherapy with ofatumumab in combination with CHOP in previously untreated follicular lymphoma. Br J Haematol. 2012;157(4):438–45.
358. Carlile D, Meneses-Lorente G, Wassner-Fritsch E, Hourcade-Potelleret F, Wenger MK, Cartron G et al., editors. Pharmacokinetics of obinutuzumab (GA101) in patients with CD20+ relapsed/refractory malignant disease receiving concomitant chemotherapy (Phase Ib Study BO21000). Blood. 2011;Abs 374.
359. Friedberg JW VJ, Kahl BS, Brunvand M, Goy A, Kasamon Y, Brington B, Li J, Ho W, Cheson BD. A Phase I Study of PRO131921, a novel anti-CD20 monoclonal antibody in patients with relapsed/refractory CD20+ Indolent NHL: correlation between clinical responses and AUC pharmacokinetics. ASH annual meeting abstract. 2009(114):3472.
360. Wayne JL, Ganjoo KN, Pohlman BL, De Vos S, Flinn IW, Dang NH et al., editors. Efficacy of ocaratuzumab (AME-133v) in relapsed follicular lymphoma patients refractory to prior rituximab. J Clin Oncol. 2012;30(15):2318.
361. Kim Y, Ponomarenko J, Zhu Z, Tamang D, Wang P, Greenbaum J, et al. Immune epitope database analysis resource. 2012(40):525–30.
362. Vita R, Zarebski L, Greenbaum JA, Emami H, Hoof I, Salimi N, et al. The immune epitope database 2.0. 2010;38:854–62.

Tumor Immunotherapy of Esophageal and Gastric Cancers

9

Uhi Toh, Tetsuro Sasada, Ryuji Takahashi, Kyogo Itoh, and Yoshito Akagi

Contents

U. Toh, MD, PhD (✉)
Department of Surgery,
Kurume University School of Medicine,
67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan
e-mail: utoh@med.kurume-u.ac.jp

R. Takahashi, MD, PhD • Y. Akagi
Department of Surgery, Kurume University School of Medicine, Kurume, Fukuoka 830-0011, Japan
e-mail: takahashi_ryuuji@med.kurume-u.ac.jp

T. Sasada, MD, PhD • K. Itoh, MD, PhD
Department of Immunology and Immunotherapy,
Kurume University School of Medicine,
Kurume, Fukuoka 830-0011, Japan
e-mail: tsasada@med.kurume-u.ac.jp

9.1 Introduction

Esophageal cancer affects more than 450,000 people worldwide and its incidence is rapidly increasing [1–5]. The 5-year survival rate for patients with advanced esophageal cancer ranges from 15 to 25 %. Although early diagnosis is associated with favorable clinical outcomes [1, 6], esophageal cancer is often diagnosed at late stages of the disease, usually following the onset of metastasis [7]. Therefore, these patients are subjected to a myriad of treatments that help to prolong their lives and maximize their quality of life. Depending on the condition of the disease, patients with advanced or recurrent esophageal cancer can undergo chemotherapy [8–14], chemoradiotherapy [15], or endoscopic therapy [16–19].

Gastric cancer affects more than 980,000 people worldwide, with higher incidence in developing countries [20]. Despite significant progress in diagnosing and treating gastric cancer, the 5-year survival rate is only 20 % [20]. Treatments such as salvage surgery [21, 22] and chemotherapy [23–26] can improve the outcome. In particular, trastuzumab in combination with chemotherapy improved the clinical outcome for patients with HER2-positive gastric or gastroesophageal junction cancer [27].

Infiltration of distinct immune cells, including lymphocytes, macrophages, dendritic cells (DCs), and granulocytes, as well as immune-related microenvironments can foster or inhibit tumor progression and/or metastatic potential in various

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_9, © Springer-Verlag Berlin Heidelberg 2015

cancers [28, 29]. In gastrointestinal cancers, including esophageal and gastric cancers, the presence of tumor-infiltrating $CD8^+$ cytotoxic T lymphocyte and/or $CD4^+$ T cells has been associated with favorable patient prognoses [30, 31]. These findings have provided the rationale for the development of novel immune-based therapeutics. Here, we summarize the current status of immunotherapies against esophageal and gastric cancers.

9.2 Current Immunotherapeutic Strategies for Esophageal and Gastric Malignancies

9.2.1 Monoclonal Antibody Therapy

The strategy of using monoclonal antibodies (mAbs) to target cancer cells has been well tested over the last several decades. The mechanisms of action of mAb therapy include blocking growth factor/receptor interactions, downregulating proteins required for tumor growth, and activating effector mechanisms of the immune system (including complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC)) [32]. While conventional chemotherapeutic agents destroy neoplastic cells, they can also target normal cells which are mitotically active. In contrast, mAbs have the distinct advantage of being highly specific and therefore have fewer and less severe adverse effects. Antibodies can be used in all patients who express the specific antigen on their tumor. Their ability to mediate target-specific inhibition and immune-mediated tumor suppression confers significantly improved efficacy over standard chemotherapy regimens.

Despite the long-standing promise of mAb therapies, esophageal and gastric cancers (EGCs) are only recently being explored in the context of immunotherapy. Since 1997, the US Food and Drug Administration (FDA) has approved 12 mAbs for clinical use including four that target EGC: bevacizumab, cetuximab, panitumumab, and trastuzumab [32]. However, only the humanized IgG1 trastuzumab has been approved for treating human epidermal growth factor receptor 2 (HER2)/ERBB2-positive gastric cancer or gastroesophageal junction carcinoma in combination with chemotherapies.

The frequency of HER2/neu-positive esophageal squamous cell carcinoma (SCC: mean 23 %, range 0–52 %) and GE junction adenocarcinoma (mean 22 %, range 0–43 %) is varied [33, 34]. In esophageal SCC, HER2/neu overexpression has been correlated with extramural invasion and poor response to neoadjuvant chemotherapy [35]. Similarly, HER2/neu overexpression in gastric and gastroesophageal junction adenocarcinoma has been correlated with increasing invasion, distant organ metastasis, and poor overall survival [36]. Early-phase studies recently demonstrated that trastuzumab combined with chemotherapy, radiation, and standard surgery improves clinical responses and overall survival rates for patients with grade 2+ or 3+ HER2/neu-positive tumors [27, 37]. Another randomized phase III trial (Trastuzumab for Gastric Cancer [ToGA] trial) showed that trastuzumab in combination with chemotherapy significantly improved overall survival in patients with HER2/neu-positive advanced gastric or gastroesophageal cancers, being particularly efficacious in patients with high HER2/neu expression [38]. Moreover, trastuzumab co-treatment did not exacerbate the adverse effects of chemotherapy, including symptomatic heart failure.

In EGCs, EGFR overexpression occurs in 30–90 % of tumors and is correlated with increased invasion and poor prognosis [39]. In general, EGFR overexpression is more common in SCC than gastric adenocarcinomas [40–42]. Currently, mAbs targeting EGFR signaling in gastroesophageal cancers are being developed. Notably, the efficacy of the EGFR inhibitor cetuximab has yet to be explored in esophageal adenocarcinoma or SCC. Other trials examining cetuximab and trastuzumab alone or in combination with radiation or chemotherapy have been performed (Table 9.1) [43–53].

VEGF (vascular endothelial growth factor) specifically induces division and proliferation of angiogenic endothelial cells. In esophageal cancer, VEGF overexpression occurs in 30–60 % of patients and has been correlated with advanced stages of cancer (occurrence of nodal and distant metastases) and poor survival rates [54–57]. Similarly, increased

Table 9.1 Clinical trials using monoclonal antibodies for patients with esophageal and gastric cancer in neoadjuvant and metastatic setting

Treatment	Disease of EGC	No. of enrolled patients	Clinical response (CR/PR)	Median survival (mos)	References
Cetuximab + FOLFOX/RT + surgery	E, ESCC	41	8/12	17	[43]
Cetuximab + cisplatin/docetaxel/RT + surgery	E, ESCC	28	9/10	NA	[44]
Cetuximab + carboplatin/paclitaxel/RT ± surgery	E, G, ESCC	60	13/NA	NA	[45]
Trastuzumab + paclitaxel/cisplatin/RT + surgery	E (HER2 positive)	19	3/1	24	[46]
Cetuximab	E, GEJ, G	35	0/1	3.1	[47]
Cetuximab	E, GEJ	55	0/3	4.0	[48]
Cetuximab + FOLFOX	GEJ, G	52	4/26	9.5	[49]
Cetuximab + cisplatin/docetaxel	GEJ, G	72	1/27	9.0	[50]
Cetuximab + FOLFOX	G	40	0/21	9.9	[51]
Cetuximab + 5-FU/cisplatin *vs.* 5-FU/cisplatin	ESCC	32 *vs.* 30	0/11 *vs.* 1/8	9.5 *vs.* 5.5	[52]

E esophageal adenocarcinoma, *ESCC* esophageal squamous cell carcinoma, *G* gastric adenocarcinoma, *HER2* human epidermal growth factor receptor 2, *FOLFOX* 5-fluorouracil, leucovorin, oxaliplatin, *FOLFIRI* 5-fluorouracil, leucovorin, irinotecan, *5-FU* 5-fluorouracil, *NA* not available, *RT* radiation therapy

VEGF levels in tumors and sera have been correlated with poor prognoses in gastric cancer [54, 58].

Therapies directed against VEGF have been effective in many types of cancers including EGCs. Bevacizumab (Avastin), a humanized IgG1 mAb against VEGF, has been tested in various solid tumors. Early- and late-phase II clinical studies have indicated that bevacizumab in combination with chemotherapy can significantly improve Time to tumor progression (TTP) and overall survival [59–61].

Although novel mAb therapies are currently being explored for esophageal and gastric malignancies, administration of mAbs carries the risk of undesirable immune reactions such as acute anaphylaxis, serum sickness, and production of neutralizing antibodies. Chimerization and humanization of mAbs help to overcome some of these problems. Other adverse effects related to mAb therapy include infections, tumorigenesis, autoimmune disease, and organ-specific toxicity such as cardiotoxicity [62].

9.2.2 Adoptive Cell Therapy

Adoptive cell therapy (ACT) involves the transfer of antitumor lymphocytes into a tumor-bearing host. It is a potent and feasible immunotherapy for certain advanced or relapsed malignancies, although it requires significant front-end "personalization" for each patient [63]. ACT was initially developed to generate lymphokine-activated killer (LAK) cells, which could directly lyse tumor cells [64, 65]. Then, strategies to isolate and expand tumor antigen-specific T cells were developed. Specifically, tumor-infiltrating lymphocytes (TILs) were isolated from resected tumors and expanded *ex vivo* by coculturing them with patient tumors and the IL-2 cytokine. TILs in combination with IL-2 had about a 50 % objective tumor response in patients with metastatic melanoma [66–69]. Moreover, TILs expanded from EGCs may provide a new and promising approach for patients with metastatic esophageal and gastric cancers [38].

The authors conducted a phase I/II trial for esophageal SCC with adoptive cell therapy [70]. Peripheral blood mononuclear cells were stimulated *in vitro* with autologous tumor cells. T cells were directly injected into primary tumors, metastatic lymph nodes, pleural spaces, or ascites in combination with IL-2. The objective tumor responses were achieved in half of the patients. Four of 11 patients (36 %) had confirmed

complete or partial response. Furthermore, one patient with recurrent esophageal SCC had a partial response to the therapy [71].

Adoptive cell therapy has also had some success in patients with gastric cancer. Expanded T cells, called cytokine-induced killer (CIK) cells, were found to have appreciable antitumor activity against human gastric cancer [72]. At an effector to target cell ratio of 30:1, CIK cells were able to destroy 58 % of MKN74 human gastric cancer cells, suggesting that CIK cells can be developed for ACT. CIK cells combined with chemotherapy in postoperative stage III–IV gastric cancer patients significantly improved overall survival time and disease-free survival time compared to conventional chemotherapy alone [73]. In other nonrandomized or randomized trials, patients treated with chemotherapy combined with CIK cells had increased survival rates compared to those who received chemotherapy alone [74, 75]. In addition to CIK cells, *ex vivo* expanded human NK cells can acquire cytolytic activity against gastric tumor cells [76]. Currently, there is no FDA-approved ACT protocol for the treatment of cancer; however, the recent explosion of data regarding ACT should usher these novel strategies into daily clinical practice. To bolster this transition from the bench to the clinic, future trials need to address the barriers raised by Tregs, the use of engineered culture systems, and the genetic modification of T cells [77–80]. Moreover, clinical data concerning the efficacy of ACT in EGCs are insufficient and additional trials are required.

9.2.3 Dendritic Cell (DC) Vaccination for Esophageal and Gastric Cancers

DCs are antigen-presenting cells that most effectively activate the adoptive immune response. Antigen presentation by DCs is critical for the induction of antitumor T-cell immunity. Gastric cancer patients with high levels of infiltrated DCs had a lower frequency of lymphatic invasion and had increased 5-year survival rates. Therefore, DC-based vaccinations could provide a novel immunotherapeutic approach for advanced gastrointestinal cancer patients [81, 82].

Several clinical studies have investigated DC-based vaccinations in patients with esophageal and gastric cancers (Table 9.2). In a clinical study of 12 patients with advanced gastrointestinal carcinoma (6 stomach, 3 esophagus, and 3 colon), Sadanaga et al. reported that *ex vivo* generated autologous DCs pulsed with MAGE-3 peptide were an effective and safe antitumor vaccine [83]. In this study, patients were immunized every 3 weeks for 3 months without experiencing toxic side effects. Peptide-specific CTL responses were detected in four of eight patients. Tumor markers decreased in seven patients, and tumors regressed (evidenced by imaging studies) in three patients, suggesting that DCs are safe and promising components for vaccine development. Kono et al. published a phase I vaccination trial in nine gastric cancer patients using DC pulsed with immunodominant HLA-A2-restricted HER2/neu (p369) peptides [84]. There were no adverse effects noted in the immunized patients. HER2/neu peptide-specific immune responses were detected in six of nine immunized patients (67 %), and peptide-specific hypersensitivity responses occurred in three of nine patients (33 %). One of the patients underwent PR response concurrent with a decrease in tumor markers, and another patient demonstrated SD for a period of 3 months.

Homma et al. generated a vaccine with fused autologous DCs and tumor cells (DC/tumor-fusion vaccine) [85]. The study consisted of 22 patients with advanced cancer, including 3 with gastric cancer. One gastric cancer patient had significantly elevated levels of serum antinuclear antibodies following treatment, which might have resulted from the immune response induced by the vaccine. Malignant ascitic effusion eventually was resolved in this patient, and their serum levels of tumor markers decreased. Fujiwara et al. performed a pilot study involving the intratumoral administration of ^{111}In-labeled DC in combination with chemotherapy (Adriamycin, cisplatin, and 5-FU) before surgical treatment in five esophageal cancer patients [86]. No adverse effects directly related to the intratumoral DC administration

Table 9.2 List of clinical trials of DC therapy for esophageal and gastric cancer

Type of vaccine	Disease condition	Phase of trial	Combined treatment	No. of patients	Clinical response	Median OS	Grade 3/4 toxicities (%)	Humoral response (%)	Cellular response (%)	Reference
MAGE-3 peptide-loaded DCs	Advanced, stomach ($n=6$), esophagus ($n=3$), colon ($n=3$)	I	(−)	12	MR 25 % PD 75 %	NA	0	NA	50	[83]
HER2/neu peptide-loaded DCs	Advanced, stomach	I	(−)	9	PR 11 % SD 11 % PD 78 %	NA	0	NA	67	[84]
DC/tumor-fusion vaccine	Advanced, stomach	–	(−)	3	NA	NA	0	33	33	[85]
Intratumoral administration of DC	Stage III (adjuvant), esophagus	–	Adriamycin, cisplatin, 5-FU	5	SD 80 % PD 20 %	NA	0	0	0	[86]

DCs dendritic cells, *OS* overall survival, *MR* minor response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *M* months, *NA* not available

were observed. None of the antibodies against the 28 tumor antigens were upregulated. Moreover, enhancement of NY-ESO-1-specific cellular immune response was not observed. According to scintigraphic images obtained after treating each patient, DCs remained at the injection sites and did not drain in lymph nodes, suggesting that intratumoral DC administration does not elicit an optimal clinical response.

9.2.4 Protein or Peptide Vaccination for Esophageal and Gastric Cancer

The field of cancer immunotherapy has significantly progressed ever since Boon and his colleagues made the observation in 1991 that a tumor-associated antigen (TAA) can be targeted by cytotoxic T lymphocytes [87–90]. Since then, technical advances have facilitated the identification of many TAAs and peptide epitopes that can be targeted for cancer immunotherapy [91]. For example, esophageal and gastric cancers express a variety of TAAs as potential targets for immunotherapies, and several clinical trials involving these TAAs have had promising results (Table 9.3) [92–99, 100, 101].

HLA-A24-binding peptides derived from testicular cancer-specific antigens have been employed in vaccines against esophageal cancer [94, 96, 97]. A phase II clinical trial of a vaccine comprised of three HLA-A24-binding peptides, TTK protein kinase (TTK), lymphocyte antigen-6 complex locus K (LY6K), and insulin-like growth factor-II mRNA binding protein (IMP3), in combination with incomplete Freund's adjuvant (Montanide ISA51) which were administered in 60 advanced esophageal cancer patients who were either HLA-A24$^+$ ($n=35$) or HLA-A24$^-$ ($n=25$) [96, 97]. The study showed that HLA-A24$^+$ patients had better progression-free survival (PFS) ($p=0.032$). In particular, patients having specific CTL responses to the vaccine peptides had better overall survival. In a similar study, Iwahashi et al. performed a phase I clinical trial of a vaccine derived from two HLA-A24-binding peptides, TTK and LY6K, in combination with the Toll-like receptor 9 agonist, CpG-7909, in 9 HLA-A24$^+$ patients with advanced esophageal cancer [94]. The result of this study underscored the safety and feasibility of this vaccine and found that a strong immune response to tumor-specific antigens was achieved.

Vaccination against another testicular cancer antigen, NY-ESO-1, was tested in esophageal cancer patients [92, 95, 99]. In one study, a vaccine was formulated with a NY-ESO-1 recombinant protein and a cholesterol-bearing hydrophobized pullulan (CHP) (CHP-NY-ESO-1) and tested in eight patients with advanced esophageal cancer [99]. The induction of antibody and CD4 and CD8 T-cell responses were observed in seven, seven, and six patients, respectively, and one partial regression (PR) and four stable diseases (SDs) were observed in six evaluable patients. Similarly, another study using a vaccine made from CHP-NY-ESO-1 and the truncated 146HER2 protein complexed with CHP (CHP-HER2) in combination with immune-adjuvant OK-432 found that 6 (75 %) and 5 (63 %) patients responded to NY-ESO-1 and HER2, respectively [92]. After six rounds of vaccinations, 3 patients (38 %) maintained SD and 5 (63 %) developed progressive disease (PD) with a median PFS of 1.5 months (range, 1–5 months). Another phase I clinical trial tested a vaccine that is comprised of a 20-mer NY-ESO-1f peptide (NY-ESO-1 91-110) in combination with OK-432 and incomplete Freund's adjuvant (Montanide ISA51) in ten patients with advanced esophageal ($n=6$), lung ($n=3$), or gastric ($n=1$) cancer [95]. In this case, vaccination induced antibody production and CD4/CD8$^+$ T-cell responses to NY-ESO-1 in nine of ten patients. Moreover, two lung cancer patients and one esophageal cancer patient showed SD.

For gastric cancer, two clinical trials using either protein- or peptide-based vaccines have been conducted. The first is a phase II clinical trial testing a gastrin-17-diphtheria toxoid (G17DT), which was shown to induce antibodies that block gastrin-stimulated tumor growth [93]. This study included 52 gastric cancer patients (stage I–III, $n=36$; stage IV, $n=16$). The vaccine induced functional antibodies in 6 of 12 (50 %), 7 of 11

Table 9.3 List of clinical trials of protein and peptide vaccination for esophageal and gastric cancer

Vaccine antigens	Disease condition	Phase of trial	Combined treatment	No. of patients	Clinical response	Median OS	Grade 3/4 toxicities (%)	Humoral response (%)	Cellular response (%)	Reference
Peptide (TTK, LY6K, IMP-3)	Advanced, esophagus, HLA-A24(+)	I	(–)	10	CR 10 % SD 30 % PD 60 %	6.6 M	0	NA	90 %	[97]
Peptide (TTK, LY6K, IMP-3)	Advanced, esophagus, HLA-A24(+) ($n=35$) *vs.* HLA-A24(-) ($n=25$)	II	(–)	60	PFS: HLA-A24(+) > A24(-) ($p=0.032$)	4.6 M *vs.* 2.6 M ($p=0.121$)	0	NA	45–63 % *vs.* 12 %	[96]
Peptide (LY6K, TTK), CpG-7909	Advanced, esophagus, HLA-A24 (+)	I	(–)	9	SD 56 % PD 44 %	3.7 M	0	NA	67 %	[94]
CHP-NY-ESYO-1 protein	Advanced, esophagus	I	(–)	8	PR 17 % SD 33 % PD 50 %	NA	0	88 %	CD4, 88 %; CD8, 75 %	[99]
CHP-NY-ESYO-1 protein, CHP-HER2 protein, OK-432	Advanced, esophagus	I	(–)	8	SD 38 % PD 63 %	NA	0	NE-ESO1, 75 %; HER2, 63 %	NA	[92]
NY-ESO-1f peptide	Advanced, esophagus ($n=6$), lung ($n=3$), stomach ($n=1$)	I	(–)	10	SD 20 % PD 80 %	NA	0	90 %	90 %	[95]
G17DT	Stomach, stage I–III ($n=36$) Stage IV ($n=16$)	II	(–)	52	NA	NA	4 %	NA	65 %	[93]
VEGF-1, VEGF-2	Advanced, stomach	I/II	S-1, cisplatin	22	PR 55 % SD 45 %	14.2 M	0	NA	82%	[98]
Personalized peptide vaccine (PPV)	Advanced, stomach	I	(–)	13	SD 45 % PD 55 %	212 days	0	50 %	80 %	[100]
Personalized peptide vaccine (PPV)	Advanced, stomach ($n=4$), colon ($n=7$)	I/II	S-1	11	SD 36 % PD 64 %	NA	18 %	64 %	82 %	[101]

OS overall survival, *PFS* progression-free survival, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *M* months, *NA* not available, *CHP* cholesterol-bearing hydrophobized pullulan

(64 %), and 11 of 12 (92 %) patients at doses of 10, 100, and 250 μg, respectively, in stage I–III gastric cancer patients. Furthermore, 8 of 14 (57 %) stage IV patients dosed at 250 μg responded. G17DT was well tolerated in 47 of 52 patients, but 2 patients suffered significant adverse reactions including injection site pain and abscess. The other study was a highly effective and well-tolerated phase I/II trial to evaluate the safety and efficacy of a vaccine made from HLA-A24-restricted peptides derived from human vascular endothelial growth factor receptor 1 (VEGFR1) and VEGFR2 in combination with chemotherapy (S-1 and cisplatin) [98]. Of the 22 patients with advanced gastric cancer, 12 (55 %) experienced PR and 10 (45 %) experienced SD, with a disease control rate of 100 % after two cycles. The median PFS and OS were 9.6 and 14.2 months, respectively. CTL responses specific to the VEGFR1- and VEGFR2-derived peptides were apparent in 18 (82 %) patients, suggesting that significant antigen-specific CTL responses can be induced under chemotherapy. Based on this study, cancer vaccination combined with standard chemotherapy should be further developed for the treatment of advanced gastric cancer.

9.2.5 Personalized Peptide Vaccination (PPV) for Gastric Cancer

The antitumor immune response might differ widely among vaccinated cancer patients. This is due to the heterogeneity of tumor cells and host immune systems, even in patients with identical HLA or disease states [102–105]. The multiplicity of immune responses and tumor cell characteristics necessitates tailored vaccinations for individual patients. To aid in the rational design of patient-specific vaccines, the authors developed a novel immunotherapeutic approach called personalized peptide vaccine (PPV). This approach helps select patient-specific HLA-matched vaccine peptides and factors in preexisting host immunity [106, 107].

A series of phase I and phase II clinical trials have corroborated the utility of PPV in patients with various types of advanced cancers [100]. The authors conducted a phase I clinical trial of PPV in 13 HLA-A2$^+$ or HLA-A24$^+$ patients with advanced gastric cancer (non-scirrhous, $n=9$; scirrhous types, $n=4$) [108]. A maximum of four peptides were selected and screened for immunoreactivity to each of 14 HLA-A24-restricted peptides or 16 HLA-A2-restricted peptides. The selected peptides (3 mg/each peptide) were emulsified in incomplete Freund's adjuvant (Montanide ISA51) and subcutaneously administrated every 2 weeks. This regimen was generally well tolerated. Increased cellular and humoral immune responses to the vaccinated peptides were observed in peripheral blood mononuclear cells in four of eight patients and in the sera of 8 (80 %) patients. These treatments led to increased survival, particularly in the four patients with scirrhous-type gastric cancer. These results reinforce the need for further development of personalized peptide-based immunotherapy for gastric cancer patients.

In addition, a phase I/II clinical trial of PPV was performed by the authors to investigate the safety and efficacy of combined treatment with varying doses (20, 40, or 80 mg/m^2/day) of orally administered S-1, a 5-fluorouracil derivative. There were 11 HLA-A2$^+$ or HLA-A24$^+$ advanced gastrointestinal cancer patients (4 gastric and 7 colon cancer) who were refractory to S-1-based chemotherapies [101]. A maximum of four peptides were selected and injected biweekly in combination with S-1. Although some patients experienced grade 3 toxicity, including anemia (one patient) and neutropenia (one patient), this therapy was generally well tolerated. An increase in peptide-specific IgG after vaccination was observed in 9 of 11 patients (82 %) irrespective of the dose of S-1. Notably, an increase in peptide-specific IFN-γ production by CTL was most evident in patients taking the highest dose of S-1. This study suggests that the combined administration of PPV with the standard dose (80 mg/m^2/day) of S-1 did not interfere with the immune response to vaccination.

9.3 Concluding Remarks

Several clinical trials involving immunotherapies for esophageal and gastric cancers have been conducted with promising results. Further

randomized trials are essential to prove the clinical benefits of these novel therapies. Therapies that combine vaccinations with immune checkpoint-blocking agents can activate coordinated immune mechanisms, including the removal of suppressor cells, and enhance the therapeutic effects of cancer immunotherapies [109, 110]. Additional chemotherapies in combination with immunotherapies could produce synergistic effects in the treatment of advanced esophageal and gastric cancers.

References

1. Enzinger PC, Mayer RJ. Esophageal cancer. N Engl J Med. 2003;349(23):2241–52.
2. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010;127(12):2893–917.
3. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61(2):69–90.
4. Lepage C, Rachet B, Jooste V, Faivre J, Coleman MP. Continuing rapid increase in esophageal adenocarcinoma in England and Wales. Am J Gastroenterol. 2008;103(11):2694–9.
5. Pohl H, Welch HG. The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence. J Natl Cancer Inst. 2005; 97(2):142–6.
6. Pennathur A, Farkas A, Krasinskas AM, Ferson PF, Gooding WE, Gibson MK, et al. Esophagectomy for T1 esophageal cancer: outcomes in 100 patients and implications for endoscopic therapy. Ann Thorac Surg. 2009;87(4):1048–54.
7. Polednak AP. Trends in survival for both histologic types of esophageal cancer in US surveillance, epidemiology and end results areas. Int J Cancer. 2003;105(1):98–100.
8. Cunningham D, Starling N, Rao S, Iveson T, Nicolson M, Coxon F, et al. Capecitabine and oxaliplatin for advanced esophagogastric cancer. N Engl J Med. 2008;358(1):36–46.
9. Enzinger PC, Ilson DH. Irinotecan in esophageal cancer. Oncology. 2000;14(12 Suppl 14):26–30.
10. Enzinger PC, Ilson DH, Kelsen DP. Chemotherapy in esophageal cancer. Semin Oncol. 1999;26(5 Suppl 15):12–20.
11. Enzinger PC, Ilson DH, Saltz LB, O'Reilly EM, Kelsen DP. Irinotecan and cisplatin in upper gastrointestinal malignancies. Oncology. 1998;12 (8 Suppl 6):110–3.
12. Homs MY, vd Gaast A, Siersema PD, Steyerberg EW, Kuipers EJ. Chemotherapy for metastatic carcinoma of the esophagus and gastro-esophageal junction. Cochrane Database Syst Rev. 2006(4):CD004063
13. Shah MA, Ramanathan RK, Ilson DH, Levnor A, D'Adamo D, O'Reilly E, et al. Multicenter phase II study of irinotecan, cisplatin, and bevacizumab in patients with metastatic gastric or gastroesophageal junction adenocarcinoma. J Clin Oncol. 2006;24(33):5201–6.
14. van Meerten E, Eskens FA, van Gameren EC, Doorn L, van der Gaast A. First-line treatment with oxaliplatin and capecitabine in patients with advanced or metastatic oesophageal cancer: a phase II study. Br J Cancer. 2007;96(9):1348–52.
15. Herskovic A, Martz K, al-Sarraf M, Leichman L, Brindle J, Vaitkevicius V, et al. Combined chemotherapy and radiotherapy compared with radiotherapy alone in patients with cancer of the esophagus. N Engl J Med. 1992;326(24):1593–8.
16. Christie NA, Buenaventura PO, Fernando HC, Nguyen NT, Weigel TL, Ferson PF, et al. Results of expandable metal stents for malignant esophageal obstruction in 100 patients: short-term and long-term follow-up. Ann Thorac Surg. 2001;71(6):1797–801.
17. Christie NA, Patel AN, Landreneau RJ. Esophageal palliation–photodynamic therapy/stents/brachytherapy. Surg Clin North Am. 2005;85(3):569–82.
18. Lightdale CJ, Heier SK, Marcon NE, McCaughan Jr JS, Gerdes H, Overholt BF, et al. Photodynamic therapy with porfimer sodium versus thermal ablation therapy with Nd:YAG laser for palliation of esophageal cancer: a multicenter randomized trial. Gastrointest Endosc. 1995;42(6):507–12.
19. Litle VR, Luketich JD, Christie NA, Buenaventura PO, Alvelo-Rivera M, McCaughan JS, et al. Photodynamic therapy as palliation for esophageal cancer: experience in 215 patients. Ann Thorac Surg. 2003;76(5):1687–92.
20. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer Statistics, 2009. CA Cancer J Clin. 2009;59(4):225–49.
21. Chang YR, Han DS, Kong SH, Lee HJ, Kim SH, Kim WH, et al. The value of palliative gastrectomy in gastric cancer with distant metastasis. Ann Surg Oncol. 2012;19(4):1231–9.
22. Tokunaga M, Terashima M, Tanizawa Y, Bando E, Kawamura T, Yasui H, et al. Survival benefit of palliative gastrectomy in gastric cancer patients with peritoneal metastasis. World J Surg. 2012;36(11):2637–43.
23. Chen XL, Chen XZ, Yang C, Liao YB, Li H, Wang L, et al. Docetaxel, cisplatin and fluorouracil (DCF) regimen compared with non-taxane-containing palliative chemotherapy for gastric carcinoma: a systematic review and meta-analysis. PLoS One. 2013;8(4):e60320.
24. Kang JH, Lee SI, Lim do H, Park KW, Oh SY, Kwon HC, et al. Salvage chemotherapy for pretreated gastric cancer: a randomized phase III trial comparing chemotherapy plus best supportive care with best supportive care alone. J Clin Oncol. 2012;30(13):1513–8.
25. Koizumi W. Chemotherapy for advanced gastric cancer: review of global and Japanese status. Gastrointest Cancer Res. 2007;1(5):197–203.
26. Van Cutsem E, Moiseyenko VM, Tjulandin S, Majlis A, Constenla M, Boni C, et al. Phase III study of docetaxel and cisplatin plus fluorouracil compared

with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: a report of the V325 Study Group. J Clin Oncol. 2006;24(31):4991–7.
27. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet. 2010;376(9742):687–97.
28. Sasada T, Suekane S. Variation of tumor-infiltrating lymphocytes in human cancers: controversy on clinical significance. Immunotherapy. 2011;3(10):1235–51.
29. Senovilla L, Vacchelli E, Galon J, Adjemian S, Eggermont A, Fridman WH, et al. Trial watch: prognostic and predictive value of the immune infiltrate in cancer. OncoImmunology. 2012;1(8): 1323–43.
30. Peng LS, Zhuang Y, Shi Y, Zhao YL, Wang TT, Chen N, et al. Increased tumor-infiltrating CD8(+) Foxp3(+) T lymphocytes are associated with tumor progression in human gastric cancer. Cancer Immunol Immunother. 2012;61(11):2183–92.
31. Zingg U, Montani M, Frey DM, Dirnhofer S, Esterman AJ, Went P, et al. Tumour-infiltrating lymphocytes and survival in patients with adenocarcinoma of the oesophagus. Eur J Surg Oncol. 2010; 36(7):670–7.
32. Scott AM, Allison JP, Wolchok JD. Monoclonal antibodies in cancer therapy. Cancer Immun. 2012; 12:14.
33. Al-Kasspooles M, Moore JH, Orringer MB, Beer DG. Amplification and over-expression of the EGFR and erbB-2 genes in human esophageal adenocarcinomas. Int J Cancer. 1993;54(2):213–9.
34. Ross JS, McKenna BJ. The HER-2/neu oncogene in tumors of the gastrointestinal tract. Cancer Invest. 2001;19(5):554–68.
35. Brien TP, Odze RD, Sheehan CE, McKenna BJ, Ross JS. HER-2/neu gene amplification by FISH predicts poor survival in Barrett's esophagus-associated adenocarcinoma. Hum Pathol. 2000;31(1):35–9.
36. Hudis CA. Trastuzumab–mechanism of action and use in clinical practice. N Engl J Med. 2007;357(1): 39–51.
37. Safran H, Dipetrillo T, Akerman P, Ng T, Evans D, Steinhoff M, et al. Phase I/II study of trastuzumab, paclitaxel, cisplatin and radiation for locally advanced, HER2 overexpressing, esophageal adenocarcinoma. Int J Radiat Oncol Biol Phys. 2007;67(2): 405–9.
38. Amedei A, Niccolai E, D'Elios MM. T cells and adoptive immunotherapy: recent developments and future prospects in gastrointestinal oncology. Clin Dev Immunol. 2011;2011:320571.
39. Kitagawa Y, Ueda M, Ando N, Ozawa S, Shimizu N, Kitajima M. Further evidence for prognostic significance of epidermal growth factor receptor gene amplification in patients with esophageal squamous cell carcinoma. Clin Cancer Res. 1996;2(5):909–14.
40. Gibault L, Metges JP, Conan-Charlet V, Lozac'h P, Robaszkiewicz M, Bessaguet C, et al. Diffuse EGFR staining is associated with reduced overall survival in locally advanced oesophageal squamous cell cancer. Br J Cancer. 2005;93(1):107–15.
41. Itakura Y, Sasano H, Shiga C, Furukawa Y, Shiga K, Mori S, et al. Epidermal growth factor receptor overexpression in esophageal carcinoma. An immunohistochemical study correlated with clinicopathologic findings and DNA amplification. Cancer. 1994;74(3):795–804.
42. Ruhstaller T, Pless M, Dietrich D, Kranzbuehler H, von Moos R, Moosmann P, et al. Cetuximab in combination with chemoradiotherapy before surgery in patients with resectable, locally advanced esophageal carcinoma: a prospective, multicenter phase IB/II Trial (SAKK 75/06). J Clin Oncol. 2011;29(6):626–31.
43. Chan JA, Blaszkowsky LS, Enzinger PC, Ryan DP, Abrams TA, Zhu AX, et al. A multicenter phase II trial of single-agent cetuximab in advanced esophageal and gastric adenocarcinoma. Ann Oncol. 2011;22(6):1367–73.
44. Gold PJ, Goldman B, Iqbal S, Leichman LP, Zhang W, Lenz HJ, et al. Cetuximab as second-line therapy in patients with metastatic esophageal adenocarcinoma: a phase II Southwest Oncology Group Study (S0415). J Thorac Oncol. 2010;5(9):1472–6.
45. Han SW, Kim HP, Shin JY, Jeong EG, Lee WC, Lee KH, et al. Targeted sequencing of cancer-related genes in colorectal cancer using next-generation sequencing. PLoS One. 2013;8(5):e64271.
46. Inoue K, Ozeki Y, Suganuma T, Sugiura Y, Tanaka S. Vascular endothelial growth factor expression in primary esophageal squamous cell carcinoma. Association with angiogenesis and tumor progression. Cancer. 1997;79(2):206–13.
47. Kitadai Y, Haruma K, Tokutomi T, Tanaka S, Sumii K, Carvalho M, et al. Significance of vessel count and vascular endothelial growth factor in human esophageal carcinomas. Clin Cancer Res. 1998;4(9):2195–200.
48. Kleespies A, Guba M, Jauch KW, Bruns CJ. Vascular endothelial growth factor in esophageal cancer. J Surg Oncol. 2004;87(2):95–104.
49. Lordick F, Luber B, Lorenzen S, Hegewisch-Becker S, Folprecht G, Woll E, et al. Cetuximab plus oxaliplatin/leucovorin/5-fluorouracil in first-line metastatic gastric cancer: a phase II study of the Arbeitsgemeinschaft Internistische Onkologie (AIO). Br J Cancer. 2010;102(3):500–5.
50. Lorenzen S, Schuster T, Porschen R, Al-Batran SE, Hofheinz R, Thuss-Patience P, et al. Cetuximab plus cisplatin-5-fluorouracil versus cisplatin-5-fluorouracil alone in first-line metastatic squamous cell carcinoma of the esophagus: a randomized phase II study of the Arbeitsgemeinschaft Internistische Onkologie. Ann Oncol. 2009;20(10):1667–73.
51. Pinto C, Di Fabio F, Barone C, Siena S, Falcone A, Cascinu S, et al. Phase II study of cetuximab in combination with cisplatin and docetaxel in patients with untreated advanced gastric or gastro-oesophageal

junction adenocarcinoma (DOCETUX study). Br J Cancer. 2009;101(8):1261–8.
52. Pinto C, Di Fabio F, Siena S, Cascinu S, Rojas Llimpe FL, Ceccarelli C, et al. Phase II study of cetuximab in combination with FOLFIRI in patients with untreated advanced gastric or gastroesophageal junction adenocarcinoma (FOLCETUX study). Ann Oncol. 2007;18(3):510–7.
53. Safran H, Suntharalingam M, Dipetrillo T, Ng T, Doyle LA, Krasna M, et al. Cetuximab with concurrent chemoradiation for esophagogastric cancer: assessment of toxicity. Int J Radiat Oncol Biol Phys. 2008;70(2):391–5.
54. Maeda K, Chung YS, Takatsuka S, Ogawa Y, Sawada T, Yamashita Y, et al. Tumor angiogenesis as a predictor of recurrence in gastric carcinoma. J Clin Oncol. 1995;13(2):477–81.
55. Shah MA, Jhawer M, Ilson DH, Lefkowitz RA, Robinson E, Capanu M, et al. Phase II study of modified docetaxel, cisplatin, and fluorouracil with bevacizumab in patients with metastatic gastroesophageal adenocarcinoma. J Clin Oncol. 2011;29(7):868–74.
56. Shih CH, Ozawa S, Ando N, Ueda M, Kitajima M. Vascular endothelial growth factor expression predicts outcome and lymph node metastasis in squamous cell carcinoma of the esophagus. Clin Cancer Res. 2000;6(3):1161–8.
57. Shimada H, Hoshino T, Okazumi S, Matsubara H, Funami Y, Nabeya Y, et al. Expression of angiogenic factors predicts response to chemoradiotherapy and prognosis of oesophageal squamous cell carcinoma. Br J Cancer. 2002;86(4):552–7.
58. Enzinger PC, Ryan DP, Clark JW, Muzikansky A, Earle CC, Kulke MH, et al. Weekly docetaxel, cisplatin, and irinotecan (TPC): results of a multicenter phase II trial in patients with metastatic esophagogastric cancer. Ann Oncol. 2009;20(3):475–80.
59. Einzig AI, Neuberg D, Remick SC, Karp DD, O'Dwyer PJ, Stewart JA, et al. Phase II trial of docetaxel (Taxotere) in patients with adenocarcinoma of the upper gastrointestinal tract previously untreated with cytotoxic chemotherapy: the Eastern Cooperative Oncology Group (ECOG) results of protocol E1293. Med Oncol. 1996;13(2):87–93.
60. El-Rayes BF, Zalupski M, Bekai-Saab T, Heilbrun LK, Hammad N, Patel B, et al. A phase II study of bevacizumab, oxaliplatin, and docetaxel in locally advanced and metastatic gastric and gastroesophageal junction cancers. Ann Oncol. 2010;21(10):1999–2004.
61. Heath EI, Urba S, Marshall J, Piantadosi S, Forastiere AA. Phase II trial of docetaxel chemotherapy in patients with incurable adenocarcinoma of the esophagus. Invest New Drugs. 2002;20(1):95–9.
62. Hansel TT, Kropshofer H, Singer T, Mitchell JA, George AJ. The safety and side effects of monoclonal antibodies. Nat Rev Drug Discov. 2010; 9(4):325–38.
63. Restifo NP, Dudley ME, Rosenberg SA. Adoptive immunotherapy for cancer: harnessing the T cell response. Nat Rev Immunol. 2012;12(4):269–81.
64. Rosenberg SA, Lotze MT, Muul LM, Chang AE, Avis FP, Leitman S, et al. A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. N Engl J Med. 1987;316(15): 889–97.
65. Rosenberg SA, Lotze MT, Muul LM, Leitman S, Chang AE, Vetto JT, et al. A new approach to the therapy of cancer based on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2. Surgery. 1986;100(2):262–72.
66. Besser MJ, Shapira-Frommer R, Treves AJ, Zippel D, Itzhaki O, Hershkovitz L, et al. Clinical responses in a phase II study using adoptive transfer of short-term cultured tumor infiltration lymphocytes in metastatic melanoma patients. Clin Cancer Res. 2010;16(9):2646–55.
67. Dudley ME, Rosenberg SA. Adoptive cell transfer therapy. Semin Oncol. 2007;34(6):524–31.
68. Dudley ME, Yang JC, Sherry R, Hughes MS, Royal R, Kammula U, et al. Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. J Clin Oncol. 2008;26(32):5233–9.
69. Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res. 2011;17(13):4550–7.
70. Toh U, Yamana H, Sueyoshi S, Tanaka T, Niiya F, Katagiri K, et al. Locoregional cellular immunotherapy for patients with advanced esophageal cancer. Clin Cancer Res. 2000;6(12):4663–73.
71. Toh U, Sudo T, Kido K, Matono S, Sasahara H, Mine T, et al. Locoregional adoptive immunotherapy resulted in regression in distant metastases of a recurrent esophageal cancer. Int J Clin Oncol. 2002;7(6):372–5.
72. Kim YJ, Lim J, Kang JS, Kim HM, Lee HK, Ryu HS, et al. Adoptive immunotherapy of human gastric cancer with ex vivo expanded T cells. Arch Pharm Res. 2010;33(11):1789–95.
73. Shi L, Zhou Q, Wu J, Ji M, Li G, Jiang J, et al. Efficacy of adjuvant immunotherapy with cytokine-induced killer cells in patients with locally advanced gastric cancer. Cancer Immunol Immunother. 2012;61(12):2251–9.
74. Jiang JT, Shen YP, Wu CP, Zhu YB, Wei WX, Chen LJ, et al. Increasing the frequency of CIK cells adoptive immunotherapy may decrease risk of death in gastric cancer patients. World J Gastroenterol. 2010;16(48):6155–62.
75. Kono K, Takahashi A, Ichihara F, Amemiya H, Iizuka H, Fujii H, et al. Prognostic significance of adoptive immunotherapy with tumor-associated lymphocytes in patients with advanced gastric cancer: a randomized trial. Clin Cancer Res. 2002;8(6):1767–71.
76. Voskens CJ, Watanabe R, Rollins S, Campana D, Hasumi K, Mann DL. Ex-vivo expanded human NK cells express activating receptors that mediate cytotoxicity of allogeneic and autologous cancer cell lines

by direct recognition and antibody directed cellular cytotoxicity. J Exp Clin Cancer Res. 2010;29:134.
77. Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, Schwartzentruber DJ, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science. 2002;298(5594):850–4.
78. Gross G, Waks T, Eshhar Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. Proc Natl Acad Sci U S A. 1989;86(24):10024–8.
79. Klapper JA, Thomasian AA, Smith DM, Gorgas GC, Wunderlich JR, Smith FO, et al. Single-pass, closed-system rapid expansion of lymphocyte cultures for adoptive cell therapy. J Immunol Methods. 2009; 345(1–2):90–9.
80. Woo EY, Chu CS, Goletz TJ, Schlienger K, Yeh H, Coukos G, et al. Regulatory CD4(+)CD25(+) T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. Cancer Res. 2001;61(12):4766–72.
81. Ishigami S, Natsugoe S, Tokuda K, Nakajo A, Xiangming C, Iwashige H, et al. Clinical impact of intratumoral natural killer cell and dendritic cell infiltration in gastric cancer. Cancer Lett. 2000; 159(1):103–8.
82. Tewari M, Sahai S, Mishra RR, Shukla SK, Shukla HS. Dendritic cell therapy in advanced gastric cancer: a promising new hope? Surg Oncol. 2012;21(3):164–71.
83. Sadanaga N, Nagashima H, Mashino K, Tahara K, Yamaguchi H, Ohta M, et al. Dendritic cell vaccination with MAGE peptide is a novel therapeutic approach for gastrointestinal carcinomas. Clin Cancer Res. 2001;7(8):2277–84.
84. Kono K, Takahashi A, Sugai H, Fujii H, Choudhury AR, Kiessling R, et al. Dendritic cells pulsed with HER-2/neu-derived peptides can induce specific T-cell responses in patients with gastric cancer. Clin Cancer Res. 2002;8(11):3394–400.
85. Homma S, Sagawa Y, Ito M, Ohno T, Toda G. Cancer immunotherapy using dendritic/tumour-fusion vaccine induces elevation of serum anti-nuclear antibody with better clinical responses. Clin Exp Immunol. 2006;144(1):41–7.
86. Fujiwara S, Wada H, Miyata H, Kawada J, Kawabata R, Nishikawa H, et al. Clinical trial of the intratumoral administration of labeled DC combined with systemic chemotherapy for esophageal cancer. J Immunother. 2012;35(6):513–21.
87. Finn OJ. Cancer immunology. N Engl J Med. 2008;358(25):2704–15.
88. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. Nature. 2011;480(7378):480–9.
89. Rosenberg SA. Cell transfer immunotherapy for metastatic solid cancer–what clinicians need to know. Nat Rev Clin Oncol. 2011;8(10):577–85.
90. Sasada T, Komatsu N, Suekane S, Yamada A, Noguchi M, Itoh K. Overcoming the hurdles of randomised clinical trials of therapeutic cancer vaccines. Eur J Cancer. 2010;46(9):1514–9.
91. Cheever MA, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT, et al. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. Clin Cancer Res. 2009;15(17):5323–37.
92. Aoki M, Ueda S, Nishikawa H, Kitano S, Hirayama M, Ikeda H, et al. Antibody responses against NY-ESO-1 and HER2 antigens in patients vaccinated with combinations of cholesteryl pullulan (CHP)-NY-ESO-1 and CHP-HER2 with OK-432. Vaccine. 2009;27(49):6854–61.
93. Gilliam AD, Watson SA, Henwood M, McKenzie AJ, Humphreys JE, Elder J, et al. A phase II study of G17DT in gastric carcinoma. Eur J Surg Oncol. 2004;30(5):536–43.
94. Iwahashi M, Katsuda M, Nakamori M, Nakamura M, Naka T, Ojima T, et al. Vaccination with peptides derived from cancer-testis antigens in combination with CpG-7909 elicits strong specific CD8+ T cell response in patients with metastatic esophageal squamous cell carcinoma. Cancer Sci. 2010;101(12): 2510–7.
95. Kakimi K, Isobe M, Uenaka A, Wada H, Sato E, Doki Y, et al. A phase I study of vaccination with NY-ESO-1f peptide mixed with Picibanil OK-432 and Montanide ISA-51 in patients with cancers expressing the NY-ESO-1 antigen. Int J Cancer. 2011;129(12):2836–46.
96. Kono K, Iinuma H, Akutsu Y, Tanaka H, Hayashi N, Uchikado Y, et al. Multicenter, phase II clinical trial of cancer vaccination for advanced esophageal cancer with three peptides derived from novel cancer-testis antigens. J Transl Med. 2012;10:141.
97. Kono K, Mizukami Y, Daigo Y, Takano A, Masuda K, Yoshida K, et al. Vaccination with multiple peptides derived from novel cancer-testis antigens can induce specific T-cell responses and clinical responses in advanced esophageal cancer. Cancer Sci. 2009;100(8):1502–9.
98. Masuzawa T, Fujiwara Y, Okada K, Nakamura A, Takiguchi S, Nakajima K, et al. Phase I/II study of S-1 plus cisplatin combined with peptide vaccines for human vascular endothelial growth factor receptor 1 and 2 in patients with advanced gastric cancer. Int J Oncol. 2012;41(4):1297–304.
99. Wada H, Sato E, Uenaka A, Isobe M, Kawabata R, Nakamura Y, et al. Analysis of peripheral and local anti-tumor immune response in esophageal cancer patients after NY-ESO-1 protein vaccination. Int J Cancer. 2008;123(10):2362–9.
100. Noguchi M, Sasada T, Itoh K. Personalized peptide vaccination: a new approach for advanced cancer as therapeutic cancer vaccine. Cancer Immunol Immunother. 2013;62(5):919–29.
101. Sato Y, Fujiwara T, Mine T, Shomura H, Homma S, Maeda Y, et al. Immunological evaluation of personalized peptide vaccination in combination with a 5-fluorouracil derivative (TS-1) for advanced gastric or colorectal carcinoma patients. Cancer Sci. 2007;98(7):1113–9.

102. Bei R, Scardino A. TAA polyepitope DNA-based vaccines: a potential tool for cancer therapy. J Biomed Biotechnol. 2010;2010:102758.
103. Chen W, McCluskey J. Immunodominance and immunodomination: critical factors in developing effective CD8+ T-cell-based cancer vaccines. Adv Cancer Res. 2006;95:203–47.
104. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science. 2011;331(6024):1565–70.
105. Yewdell JW, Bennink JR. Immunodominance in major histocompatibility complex class I-restricted T lymphocyte responses. Annu Rev Immunol. 1999;17:51–88.
106. Itoh K, Yamada A. Personalized peptide vaccines: a new therapeutic modality for cancer. Cancer Sci. 2006;97(10):970–6.
107. Sasada T, Noguchi M, Yamada A, Itoh K. Personalized peptide vaccination: a novel immunotherapeutic approach for advanced cancer. Hum Vaccine Immunother. 2012;8(9):1309–13.
108. Sato Y, Shomura H, Maeda Y, Mine T, Une Y, Akasaka Y, et al. Immunological evaluation of peptide vaccination for patients with gastric cancer based on pre-existing cellular response to peptide. Cancer Sci. 2003;94(9):802–8.
109. Vanneman M, Dranoff G. Combining immunotherapy and targeted therapies in cancer treatment. Nat Rev Cancer. 2012;12(4):237–51.
110. Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. Nat Rev Immunol. 2008;8(1):59–73.

10 Immunopathology of Hepatobiliary Tumors and Immunotherapy of Liver Cancers

Zhen-Yu Ding and Yu-Quan Wei

Contents

10.1 Introduction

Hepatobiliary tumors are a diverse group of tumors that arise from the hepatobiliary tract including the liver, gallbladder, and bile tract. Accordingly, based on the sites of origin, hepatobiliary tumors can be categorized into liver tumors and bile duct tumors.

Liver cancer or hepatocellular carcinoma (HCC) is the most common (80–90 %) primary tumor in the liver. The most common cause of this disease is cirrhosis (irreversible scarring) of the liver, due to alcohol abuse, hepatitis B or C, a variety of autoimmune diseases, or in rare instance, from iron overload. The mainstay treatment modality includes surgery, interventional therapy, and chemotherapy. Patients with HCC have a poor prognosis, even exacerbated by the background liver disease in the majority of patients.

The biliary tract includes the gallbladder and both the intra- and extrahepatic bile ducts. The biliary tract cancers are generally rare diseases. They are difficult to diagnose, and have overall poor prognosis. Even more confusing is the anatomic segmentation of the bile duct cancer (cholangiocarcinoma). Cholangiocarcinoma should be differentiated as intrahepatic, hilar, and distal cholangiocarcinoma, among which the hilar cholangiocarcinoma is the most common type. Biliary tract cancer is usually worsened by complications such as obstruction of bile drainage and the subsequent risk of cholangitis and liver

Z.-Y. Ding, MD • Y.-Q. Wei, MD, PhD (✉)
State Key Laboratory of Biotherapy,
Cancer Center, West China Hospital,
West China Medical School, Sichuan University,
Guoxue Lane 37, Chengdu 610041, China
e-mail: dingzy333@163.com;
yuquawei@vip.sina.com

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_10, © Springer-Verlag Berlin Heidelberg 2015

failure; therefore, the management of the disease is complex and a skilled multidisciplinary medical team with ample experience is needed for effective management.

Histologically, gallbladder cancer was grouped together with cholangiocarcinoma and was considered as the same disease. Nevertheless, today it is understood that this is an oversimplification of the situation. Gallbladder cancer has a distinct epidemiology, clinical presentation, staging, and surgical treatment, which separate it from cholangiocarcinoma. Gallbladder cancer tends to spread both by lymphatic or hematogenous metastasis, direct invasion into the liver and seed on the peritoneal surfaces. The 5-year survival is less than 5 %, and the median survival is less than 6 months [1].

10.2 Epidemiology

Hepatocellular carcinoma is the third most common cause of death from cancers, with estimated 700,000 deaths each year. Male takes a preponderant role with a male-to-female ratio of 2–4:1. In China, the most common predisposing factor for the development of HCC is chronic hepatitis B virus (HBV) infection. The HBV integrates into the genome of host and induces carcinogenesis of HCC even without evidence of cirrhosis. Additionally, long-term exposure to fungal aflatoxins significantly increases the incidence of HCC. HCC is closely related to hepatitis C virus (HCV) infection outside China.

Gallbladder cancer is not so common in clinical practice, and it is estimated that gallbladder cancer comprises about 0.8–1.2 % of all cancers [2]. The incidence varies geographically, and the high prevalence is reported in areas including South America and middle Europe. However, it is less common in areas such as west Europe, North America, and Oceania. In China, the estimated incidence is about three to five cases per million [3]. Gallbladder cancer is also related to racial-ethnic groups. Data from the National Cancer Institute of America showed that the disease is mainly related to the Mexican origin. Although low in incidence, gallbladder cancer has a high mortality, which almost equals its incidence.

Although the incidence of cholangiocarcinoma is relatively low, the disease is on a rapid rise nowadays. In America, cholangiocarcinoma constitutes about 15–20 % of all hepatobiliary cancers [4]. Likewise, the incidence of cholangiocarcinoma is increasing in China. Most cases of cholangiocarcinoma (about two-thirds) are located in the hilar area, about one-fourth is in the distal segment, and the rest occur in the intrahepatic bile tract. Cholangiocarcinoma is the most common cancer in the hepatobiliary tract secondary to HCC. The etiology of cholangiocarcinoma remains largely unknown, with recognized risk factors such as sclerosing cholangitis, liver fluke infections, and intrahepatic biliary stones.

10.3 Histology

Tumors in the liver could be those that arise spontaneously in the liver (primary tumors) or metastases from other types of tumors including colon cancer, lung cancer, breast cancer, etc. This chapter is mainly focused on the primary tumor of the liver. Either benign or malignant tumor is observed in the liver, although most primary tumors belong to the malignant type. Histologically, liver tumors develop from mesenchymal tissue, or more frequently from epithelial tissue. Histological classification of malignant liver tumors is listed as follows:

1. Epithelial:
   - Hepatocellular carcinoma (HCC)
   - Intrahepatic cholangiocarcinoma
   - Bile duct cystadenocarcinoma
   - Combined hepatocellular and cholangiocarcinoma
   - Hepatoblastoma
   - Undifferential carcinoma
2. Nonepithelial:
   - Epithelioid hemangioendothelioma
   - Hemangiosarcoma
   - Embryonic sarcoma
   - Rhabdomyosarcoma
   - Others

The most frequently seen liver tumor is the malignant epithelial tumor (HCC), which constitutes 85–95 % of all tumors of the liver. Only

1–3 % of liver tumors are malignant mesenchymal tumors. HCC is either macroscopically solitary massive, or appears as multiple nodes in the background of cirrhosis. The tumor nodules are usually round in shape, with gray or green color, sometimes infiltrating into portal veins as thrombus. Microscopically, the HCC could be classified as fibrolamellar, pseudoglandular (adenoid), pleomorphic (giant cell), and clear cell.

The most common type of cholangiocarcinoma is the adenocarcinoma (more than 90 %); other types such as squamous cell carcinoma, sarcoma, and small cell cancer are less common [5]. The histological classification of commonly seen malignant tumors of the gallbladder and extrahepatic bile duct is listed as follows:

1. Epithelial:
   - Adenocarcinoma
   - Papillary adenocarcinoma
   - Adenocarcinoma, intestinal type
   - Adenocarcinoma, gastric foveolar type
   - Mucinous carcinoma
   - Clear cell adenocarcinoma
   - Signet ring cell carcinoma
   - Adenosquamous carcinoma
   - Squamous carcinoma
   - Small cell carcinoma
   - Large cell neuroendocrine carcinoma
   - Undifferentiated carcinoma
   - Carcinoid
   - Others
2. Nonepithelial:
   - Granulous cell tumor
   - Leiomyoma
   - Leiomyosarcoma
   - Kaposi sarcoma
   - Lymphoma
   - Rhabdomyosarcoma

Cholangiocarcinoma is further divided into three types including sclerosing, nodular, and papillary. Similar to cholangiocarcinoma, the most common histological type of gallbladder cancer is adenocarcinoma. But other types of malignant tumors have been described in the literature such as rhabdomyosarcoma, leiomyosarcoma, malignant fibrous histiocytoma, and angiosarcoma.

10.4 Immunopathology

The enigma of cancer has been elucidated to a great extent, and several hallmarks of cancer have been described [6]. In addition to the classical characteristics such as sustained proliferative signaling, cell death resistance, angiogenesis, replicative immortality, activating invasion and metastasis, and growth suppressor evasion, nowadays the abnormal disruption of the interaction of cancer cells and its surrounding microenvironment has been especially emphasized [7]. The cancer microenvironment is constituted of nontransformed host stromal cells such as endothelial cells, fibroblasts, a complex extracellular matrix secreted by both the normal and neoplastic cells, and especially various immune cells that are believed to have complex interactions with cancer cells in the process of carcinogenesis and progression (Fig. 10.1). The significant role played by the cancer microenvironment in cancer biology and cancer therapy is just to be understood.

In the setting of hepatocarcinogenesis, the most important predisposing factor is chronic HBV infection. The genome of HBV alters the expression profile of the host liver, resulting in the discordant proliferation of cells, and finally contributing to the onset of HCC. Recently, the paradigm is shifting and the role played by its immunopathology is being taken into account. Accumulating data indicate that chronic HBV infection induces long-lasting inflammation, which is considered as both innate and adaptive immunoresponses against the virus from the host [8]. The inflammation alone could induce the formation of HCC, even without evidence of productive viral infection [9]. Immune cells, cytokines, and growth factors in the inflammation lead to rapid turnover of liver cells and finally malignant transformation. Relevant participants have been extensively studied. Factors such as lymphotoxins (LT) are known to be related to the hepatocarcinogenesis [10]. Lymphotoxins and their receptors are up-regulated in hepatitis and HCC, and LT expression induces liver inflammation and HCC. Therefore, a causal link exists between LT overexpression to hepatitis and

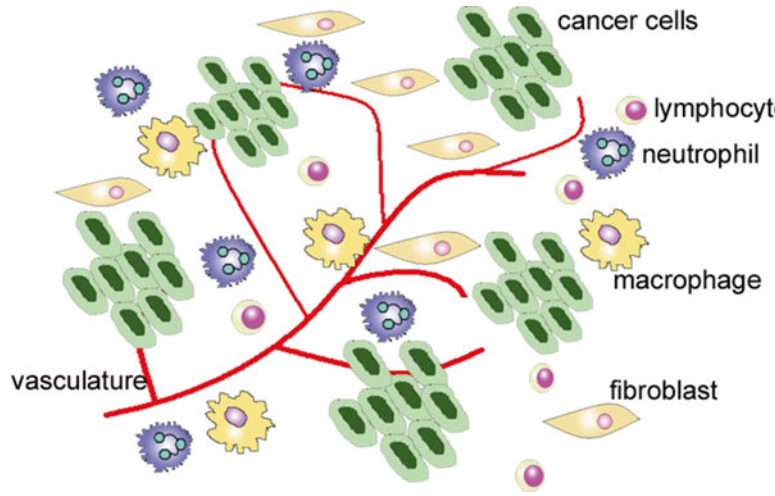


Fig. 10.1 Schematic presentation of immune cells in the cancer microenvironment

HCC. Sustained LT signaling represents a pathway involved in hepatitis-induced HCC. Toll-like receptors (TLR) are a broad family of proteins that recognize a broad spectrum of molecules shared among pathogens and referred to pattern recognition receptor. They play a key role in the pathogenesis of antimicrobe inflammation [11], supporting a role for chronic inflammation in hepatocarcinogenesis [12]. Besides, interleukin-6 (IL-6) is believed to contribute to the HCC formation. IL-6 binds to its specific receptor complex including the ligand-binding protein (gp80) and signal transduction protein (gp130) and regulates important signals such as JAK/STAT, ras/MAPK, and PI3K/AKT pathways [13]. Increased serum IL-6 level is reported in HCC patients compared to healthy controls, and positively related to larger tumor mass, more advanced stages, and more aggressiveness [14–16]. STAT3 is the most important mediator for the carcinogenesis activity of IL-6. STAT3 regulates the expression of important factors in the apoptosis, senescence, cell cycle, and angiogenesis [17]. Recently it was reported the STAT3 was constitutively activated in the HCC, but not in adjacent normal tissue [18]. The most direct link between inflammation and malignant transformation results from the activation of NF-κB pathway. It is well documented that NF-κB plays a critical role in inflammation, and recent lines of evidence show that it contributes to tumorigenesis, invasion, metastasis, and angiogenesis [19]. Overexpression of NF-κB is frequently observed in liver cancer tissues and HCC cell lines [20, 21]. NF-κB acts as a central link between hepatic injury, fibrosis, and HCC, and it may represent a target for the prevention or treatment of liver fibrosis and HCC.

Besides cytokines, immune cells involved in the inflammatory process contribute to carcinogenesis. Neutrophils are important in the inflammation response. They play paradoxical roles of both promoting cancer destruction and inducing the growth of cancer cells [22]. Cancer cells are known to produce chemokines, which act on CXCR2 receptors on neutrophils, and this ligand-receptor interaction leads to the release of VEGF-A by neutrophils that promotes tumor angiogenesis [22, 23]. Then, the neutrophils are recruited and induced to release VEGF or MMP, and both chemokines contribute to the invasion of endothelial cells and vessel formation. Then, angiogenesis follows and cancer progression is enhanced. The same principle applies to the HCC. Neutrophils in the HCC samples predict a shorter recurrence-free survival for HCC patients after liver resection [24, 25]. The number of neutrophils among the tumor margin strongly correlates with tumor angiogenesis and tumor progression [26].

Tumor-associated macrophage (TAM) is gaining focus in the immunopathology of cancer [27]. Growing evidence suggests that TAM promotes tumor growth and progression, instead of fighting

against tumor as considered previously [28]. In a certain instance such as hypoxia, the TAM polarizes to a type 2 macrophage (M2)-like type [29]. M2-like TAM exerts a profound immunosuppressive effect by secreting and releasing cytokines such as CCL17, CCL22, or CCL24; then regulatory T (Treg) cells are recruited to the local milieu by these cytokines. In HCC, the intratumoral prevalence of regulatory T cells was correlated with the density of TAM [30]. TAM expresses programmed cell death (PD-1) ligand, which is considered as one of the most critical suppressive factors for immunity [31]. Additionally, M2-like TAM promotes angiogenesis through the production of VEGF or EGF [32]. In HCC, the TAM count was significantly correlated with microvessel density [33]. TAM was believed to be involved in the different prevalence of HCC between genders, where estrogen (E2) suppressed the macrophage alternative activation by inhibiting the JAK-STAT pathway [34]. TLR on the surface of HCC cancer cells recognizes and interacts with TAM, leading to recruitment of regulatory T cells in the microenvironment [35]. Recent studies suggested that the TAMs, together with regulatory T cells and hepatic stellate cells provided an immunosuppressive environment closely related to HCC recurrence [36].

Regulatory T cells (Tregs) are a unique subset of T cells with characteristic phenotype of $CD4^+$, $CD25^+$, and $FOXP3^+$. Accumulating evidences suggest that Tregs play a prominent role in immune tolerance with the aim to prevent autoimmunity [37]. However, the shut-down of autoimmunity is at a price, and the tumor formation is facilitated at the same time. Recently, efforts have been put to elucidate the relationship between Tregs and hepatocarcinogenesis. Direct evidence comes from clinical observations. A high concentration of tumor-infiltrating Foxp3 Tregs in HCC is associated with high-grade and poorly differentiated tumors and signifies an unfavorable prognosis [38]. Tregs were reported to be associated with poor post-cryoablation prognosis in patients with hepatitis-B-virus-related HCC [39]. The number of Treg cells in HCC tissues could be used as a potential poor prognostic indicator for HCC patients after resection [40]. The prevalence of Treg cell was significantly higher in the peripheral blood and in tumor tissue compared with those in normal donors. The increased prevalence and expanded function of Treg cells in the tumor microenvironment of HCC were correlated to the cancer stage [41]. In order to provide the mechanistic explanation for the tumor-promoting activity of Tregs, one paper reported Tregs induced by HBV infection could suppress the antitumor immune response to HCC tumor antigen. This report therefore suggested that Tregs were involved in the immunopathogenesis of HCC [42]. Another report found that Tregs in the peripheral blood, pritumor, or intratumor sites of HCC patients exhibited different functional status. A higher prevalence and more suppressive phenotype suggested a critical role for intratumoral Tregs in the formation of multicellular immunosuppressive networks [43]. Yet another report examined the relation between γδ T cells and Tregs. The effector function of γδ T cells was substantially impaired in HCC, which is partially mediated by Treg cells [44]. Interestingly, not only classic $CD25^+FoxP3^+$ Tregs, but also noncanonical $CD25^-FoxP3^-$ Tregs were found to have suppressive activities and were believed to take part in the liver cancer formation [45].

Compared to HCC, the immunopathology of cholangiocarcinoma and gallbladder cancer is less extensively studied. IgG4-related diseases consist of a broad spectrum of diseases with characteristics of marked infiltration by immunoglobulin G4 (IgG4)-positive plasma cells in affected organs [46]. Extrahepatic cholangiocarcinoma may be one of them. One study detected high prevalence (43 %) of IgG4 abundance in a total number of 54 cases with cholangiocarcinoma and gallbladder cancer [47]. The same study also suggested that cholangiocarcinoma cells could play the role of nonprofessional APCs and $Foxp3^+$ regulatory cells. Another study examined the infiltration of IgG4-positive cells in 68 surgical specimens from patients with extrahepatic cholangiocarcinoma. Their results showed that ≥10 and ≥50 IgG4-positive cells per high-power field were found in 37 and 6 % of cases, respectively. In addition, the IgG4-positive cells showed a positive and negative

correlation with FoxP3$^+$ and CD8$^+$ cells, respectively. Therefore, the study provided evidence that IgG4-positive cells in extrahepatic cholangiocarcinoma induced the evasion of immune surveillance associated with CD8$^+$ cytotoxic T lymphocyte via the regulatory function of regulatory T cells [48]. B7-H1/PD-1 axis has been intensively studied as an important negative regulator for various cancers including HCC [49]. In the case of cholangiocarcinoma, although not so many, preliminary data showed that this axis played a role in its immunopathology. Totally, 31 intrahepatic cholangiocarcinoma specimens were examined by immunohistochemistry. Expression of B7-H1 and PD-1 was found to be up-regulated in cancer tissues compared with cancer adjacent tissues. Tumor-related B7-H1 expression was significantly correlated with both tumor differentiation and pTNM stage and was inversely correlated to CD8$^+$ T cells [50].

The role played by TAM in cholangiocarcinoma was also explored. CD68$^+$ and CD163$^+$ macrophage infiltration was analyzed in paraffin-embedded tissue samples from 39 patients with intrahepatic cholangiocarcinoma where CD163 was used as a marker of M2 macrophages. The number of CD68$^+$ and CD163$^+$ macrophages was positively correlated with the numbers of vessels and regulatory T cells. Patients with high counts of CD163$^+$ macrophages showed poor disease-free survival ($p=0.0426$). The *in vitro* study suggested that STAT3 pathway was important for TAMs to facilitate tumor progression [51].

10.5 Current Therapies

The clinical management of HCC is the state of the art, especially given the insensitive nature of HCC to conventional chemotherapy or radiotherapy, with complicating underlying liver disease. A multidisciplinary medical team including experts of surgeons, pathologists, radiologists, and medical oncologists is required to provide care to patients with HCC efficiently and effectively.

Small, localized tumors are potentially curable. Patients with early-stage HCC (tumor size ≤5 cm, or ≤3 tumor with each ≤3 cm in size and without evidence of gross vasculature involvement) should be considered as potential candidates to receive curative partial hepatectomy [52, 53]. According to Milan criteria proposed in 1996, patients with small (tumor size ≤5 cm, or ≤3 tumor with each ≤3 cm in size and without evidence of gross vasculature involvement), unresectable HCC should be considered for liver transplantation [54]. Liver transplantation gives a 4-year overall survival and recurrence-free survival of 85 and 92 %, respectively.

For those who are not amenable to surgery, locoregional therapies should be considered. The latter modality contains two categories: ablation and embolization. For ablation, tumor control is achieved by exposure of the tumor to chemical substances (ethanol, acetic acid, etc.) or alteration of temperature (radiofrequency ablation, microwave ablation, etc.). Embolization refers to selective catheter-based infusion of particles to the arterials feeding the tumor, and this is achieved either by transarterial embolization, chemoembolization, or radioembolization. The expert panel from the American National Comprehensive Cancer Network of America recommends ablation alone for the small solitary tumor with tumor size ≤3 cm, and combination of ablation and embolization, embolization for lesions between 3 and 5 cm (www.nccn.org). With the rapid progress in radiotherapy techniques, adaptive external beam radiotherapy has been available for the treatment of HCC. The current radiotherapy such as stereotactic radiotherapy is capable of providing radiation beam to the tumor bed, while sparing the surrounding normal liver tissue. Radiotherapy has become an additional modality in the locoregional therapy of HCC. For the majority of patients with advanced disease, curative therapies are currently unavailable, and a palliative systemic therapy is preferred. Despite ample reports in the literature, the efficacy of cytotoxic chemotherapy is still in a state of debate. Sorafenib, an oral multi-kinase inhibitor, is the only agent for HCC which was evaluated in randomized control phase III trials [55, 56].

10.6 Progress in Immunotherapy

Although much effort has been devoted, the progress in therapy for HCC and biliary tract cancer remains limited and unfortunately, patients still have a disappointing prognosis. Only a minor proportion (approximately 20 %) of patients with HCC have the opportunity to get definite surgery, and others with advanced disease have to receive palliative therapy. With sorafenib, the only confirmed systemic agent, the overall survival in patients with advanced HCC improves by 2.3–2.8 months [55, 56]. The overall survival for patients with advanced-stage HCC is less than 1 year [57]. The prognosis of patients with advanced biliary tract cancer remains poor and the median survival time for those undergoing supportive care alone is short [58]. Given the disappointing efficacy of the currently available therapies, new therapeutic strategies are highly needed.

Cancer immunotherapy aims to treat cancer by eliciting anticancer immunity of the hosts to reject the cancer. Cancer immunotherapy is achieved by either cancer vaccine (active specific immunotherapy) or adoptive transfer of antibodies (antibody therapy) or immune cells (cell therapy). Serious doubts existed for many years as to whether the immune system is capable of eliminating human cancer. The effort was mostly in vain until the elucidation of mechanisms behind the immune recognition of tumor cells at the molecular level. T cells, through their T-cell receptors, specifically recognize tumor antigens that are processed and presented as small peptides in the groove of surface human leukocyte antigen molecules [59, 60]. Later came the milestone discovery when the first tumor-specific antigen MAGE-1 was discovered in the 1990s. Now, cancer immunotherapy is considered to specifically target tumor antigens and therefore is both efficient and safe. Since the report by Rosenberg and colleagues [61], our knowledge in the field of cancer immunotherapy has been rapidly increasing, and cancer immunotherapy will surely expand our armamentarium against cancer.

Although HCC was not considered "immunogenic," lines of evidence indicates that the immune system plays a role in the formation and progression of the tumor (please refer to the immunopathology section). Also, preliminary observations suggest that the immune factor may help to suppress the tumor *in vivo*. For example, one preliminary study showed that lymphocyte infiltration in tumors was a favorite prognostic factor for patients with HCC [62]. Also, diminished frequency and impaired function of natural killer (NK) cells were described in HCC patients compared with healthy controls [63]. Not surprisingly, immunotherapy has been tested for HCC, and some of the protocols have been conducted in clinical trials. The following sections will discuss the immunotherapy at both preclinical and clinical levels.

10.6.1 Cancer Vaccines

Cancer vaccine aims at eliciting specific antitumor humoral and cellular immunity to eradicate the tumor or prevent their progression and spread. Promising progress has been achieved. The list of tumor vaccines includes protein/peptide vaccine, nucleic acid vaccine, anti-idiotype vaccine, recombinant virus vaccine, genetically modified tumor cell vaccine, and dendritic cell (DC) vaccine. Moreover, other novel types of tumor vaccines are emerging. They are mainly designed for specific antigens on the tumor cells or tumor-associated microenvironment. Accordingly, numerous studies have been performed with different cancer vaccines targeting different antigens on HCC.

The transmembrane 4 superfamily member 5 protein (TM4SF5) induces growth of HCC cells through the loss of contact inhibition and has been recognized as a potential antigen for HCC. Researchers formed a complex consisting of TM4SF5R2-3 epitope peptide and a special liposome complex as a vaccine [64]. Immunization with this vaccine in mice HCC models reveals both prophylactic and therapeutic effects. The results of this study suggested that this vaccine technology might be promising for future HCC patients with M4SF5-positive HCC.

Stem cells have received a great deal of attention for their clinical and therapeutic potential in

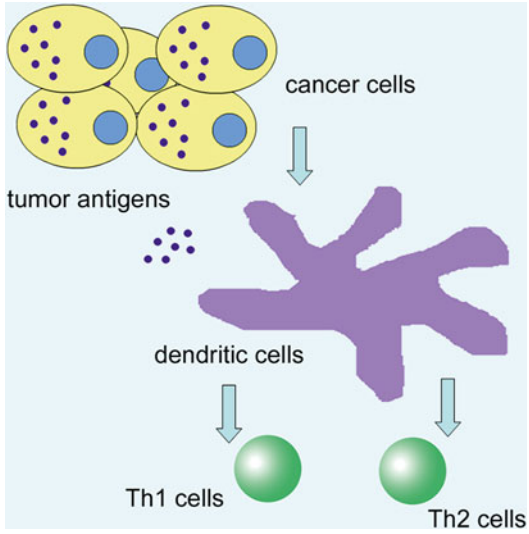


Fig. 10.2 Tumor antigen presented by DC and activation of T cells

cancer treatment. Researchers genetically modified stem cells to express cytosine deaminase (CD) and interferon-β and tested these stem cells as a vaccine to treat mice with HCC tumor burden. In the presence of the prodrug 5-fluorocytosine, the vaccine significantly inhibited the growth of the tumor mass [65].

The field of cancer immunotherapy has been strengthened by the discovery of vaccination with DC pulsed with tumor antigens is a potent strategy to elicit antitumor immunity (Fig. 10.2). DC is recognized as the most potent and efficient professional antigen-presenting cell identified so far, capable of activating both resting and naïve T cells. The ability to isolate and expand DC *in vitro* overcomes the previous obstacles in production. DC-based cancer vaccine has been considered as an attractive therapeutic approach. However, clinical trials often failed to confirm the efficacy of DC vaccine in patients, and this implied more dedicated modification needed to improve current strategy. Gao J et al. prepared a DC vaccine by pulsing DC with heat shock protein 70 from Mycobacterium tuberculosis with H22 tumor-peptide complexes and soluble CD40L [66]. Up-regulation of CD40, CD80, CD86, and HLA-DR expression was found, with higher level of T-helper type 1 cytokine secretion, such as IL-12p70, and resulted in the induction of H22-specific CTLs. Therapeutic administration of the vaccine significantly reduced progression of HCC tumors in mice.

Table 10.1 Potential tumor antigens for immunotherapy of HCC

Tumor antigens	Expression frequency (%)	Detection methods
AFP	~80	ELISPOT and tetramer
GPC3	~70	Cytotoxicity assay
NY-ESO-1	~50	ELISPOT
MAGE-A	~80	Tetramer
TERT	~80	ELISPOT

For the design and development of cancer vaccine, the most important issue is the selection of a suitable tumor antigen as the therapeutic target. After decades of exploration, several tumor antigens have been proposed as potential targets for the development of cancer vaccines. These antigens are listed in Table 10.1. Among the antigens, Alpha-fetoprotein (AFP) may be one of the most well studied and promising. AFP is expressed during fetal development, but disappears shortly after birth. The relative unique distribution of AFP in HCC in an adult has long been harnessed for diagnosis of HCC. T cell epitopes were described in AFP. In a pioneer study, DC genetically modified to express AFP was capable of generating AFP-specific T cell response in peripheral blood mononuclear cells and transgenic mice [67]. Importantly, a 9-mer peptide (542–550) derived from AFP was identified as a potential A2.1-restricted peptide epitope. Later on, more epitopes from AFP were proved to induce AFP-specific T response in patients suffering from HCC [68]; these evidences strongly argue that AFP may serve as a therapeutic tumor antigen.

Another potential antigen with promising prospects is Glypican-3 (GPC3). GPC3 belongs to a family of heparan sulfate proteoglycans, and it functions to bind growth factor and promote tumor growth [69]. GPC3 was found to be specifically expressed in HCC, and may serve as a diagnostic marker [70]. It indicated poor prognosis in HCC patients [71]. Later, the HLA-A24-restricted CTL epitopes were reported from GPC3 and indi-

cated GPC3 was a possible tumor antigen for immunotherapy [72]. Based on these preclinical studies, a phase I clinical trial was conducted in Japan [73]. This registered trial (UMIN-CTR-000001395) recruited an overall of 33 patients with advanced HCC and escalating doses of GPC3 vaccines were administered. The GPC3 peptide vaccine was well tolerated. One patient achieved partial response and 19 patients had stable disease. Given the resistant nature of HCC and the small sample size in the phase I trial, results were promising. And shortly later, a phase II trial (UMIN-CTR-000002614) was performed. In this trial, the GPC3-derived peptide vaccine was used in the adjuvant setting. The primary endpoints were 1- and 2-year recurrence rate [74]. The authors are awaiting the results of this trial.

MUC-1 is another potential antigen which was proposed for cancer vaccine. In a study performed by Japanese researchers, a 100-mer MUC1 peptide consisting of the extracellular tandem repeat domain and incomplete Freund's adjuvant were administered to several patients including three bile duct cancer patients [75]. The study showed that the vaccine was safe and well tolerated; however, no other conclusion was drawn due to the small sample of the phase I trial. However, no sequelae study was reported for this vaccine.

DC vaccines were tested in clinical trials. In one recently published report, DC vaccine prepared by pulsing DC with a liver cancer cell line lysate was administered to 15 patients with advanced HCC [76]. The vaccine achieved two cases of partial response and nine cases of stable disease. Both cellular and humoral immunity were elevated after DC vaccine inoculation. Another similar study was performed in China. In this trial, DC was pulsed with synthesized α1,3-galactosyl epitope modified tumor cells, and totally nine patients received the DC vaccine [77]. The vaccine significantly prolonged the survival of patients as compared with the controls (17.1 ± 2.01 months vs. 10.1 ± 4.5 months, $P = 0.00121$). Elevated level of interferon was detected after DC vaccine inoculation. These pilot studies indicated the possibility of translating the success of DC vaccine in preclinical studies to human subjects.

10.6.2 Cell Therapy

Adoptive cell therapy involves the transfer of immune cells with antitumor reactivity. Adoptive cell therapy aims at tumor elimination through direct or indirect effects of repairing or enhancing the immune function. Early studies involved the transfer of lymphokine-activated killer (LAK) cells with nonspecific ability to recognize and lyse tumor cells *in vitro* to cancer patients [78]. The use of T cells for adoptive therapy may be more attractive, because of their ability to specifically target tumor cells, besides long clonal life span. This strategy of adoptive cell therapy achieved some success in pilot studies where patients with melanoma or renal carcinoma were treated [79]. Further trials concentrated on isolation, propagation, and activation of highly active and avid tumor-specific T cell clones and adoptive transfer for cancer patients. T cell expression of inhibitory proteins can be a critical component for the regulation of immunopathology, but it may also limit T cell responses to malignancies. In a recent report, researchers abrogated the expression of the Src homology region 2 domain-containing phosphatase-1 (SHP-1) in tumor-reactive $CD8^+$ T cells [80]. Following *in vivo* transfer, the SHP-1(−/−) effector T cells exhibited enhanced short-term accumulation, followed by greater contraction, and ultimately formed similar numbers of long-lived, functional memory cells. The increased therapeutic effectiveness of SHP-1(−/−) effector cells was also observed in recipients that expressed the tumor Ag as a self-antigen in the liver. In another report, it was attempted to improve the efficacy of cytokine activated cells (CAK) in HCC by combining them with the chemotherapy agent gemcitabine [81]. In this study, gemitabine treatment led to increased expression of MHC class I chain-related A and B on the surface of HepG2 HCC tumor cells, both of which were recognized as ligands for activating receptors on NK cells. Pretreatment with gemcitabine and CAK cells induced greater cytotoxicity than either treatment alone.

An important randomized control study performed in Japan strongly argued for adoptive

immunotherapy for postsurgical HCC patients [82]. In this trial, a total 150 patients who underwent curative surgery for HCC were enrolled. Half of them ($n=76$) were assigned to receive adoptive transfer of IL-12 and anti-CD-3 antibody-activated lymphocytes, and the other patients received no adjuvant therapy. Adoptive immunotherapy decreased the frequency of recurrence by 18 % compared to the control group. The immunotherapy group had significantly longer recurrence-free survival ($p=0.01$) and disease-specific survival ($p=0.04$) than the control group. In support of this study, a systematic review was recently published where the authors evaluated the efficacy of adjuvant adoptive immunotherapy for postsurgical HCC patients [83] in which four randomized control trials with 423 patients were eligible. All trials reported significantly improved disease-free survival rate or reduced recurrence rate after treating with adjuvant adoptive transfer of cells ($p<0.05$). This study adds to the evidence that postoperative HCC patients treated with adjuvant cell therapy show improvement in disease-free survival rate or recurrence rate. In another study, CAK cell therapy was combined with microwave ablation therapy for HCC [84]. In a phase I study, adoptive transfer of DC, CAK, and CTL cells was conducted together with microwave ablation. The aim of this study was to observe the viral load before and after the combination therapy. After therapy, the viral load was significantly lower, the number of Tregs decreased, and effector T cells increased. However, this study did not report the clinical efficacy of the combinatory regimen.

10.6.3 Antibody Therapy

An important aspect of immunotherapy is antibody therapy. Antibody therapy is in the rapid boosting stage these days, and the list of novel antibody therapies is rapidly increasing. Among the antibodies available for cancer immunotherapy, bevacizumab is recognized as one of the most important for its unique inhibitory effects on tumor vasculature but not on tumor cells. Tumor angiogenesis has been proved to be critical for a panel of tumors, including HCC [6]. HCC is among the highly angiogenic cancers; disorganized and tortuous vasculature was reported in HCC [85]. The poor vasculature led to insufficient infiltration of nutrition, oxygen, therapeutic drugs, as well as lack of immune active cells. The concept of anti-angiogenic therapy was tested in HCC. In a preliminary study, a segment of oligodeoxynucleotides with cytosine-guanine-rich (CpG) motifs was used according to a classical vaccination protocol [86]. This kind of vaccine led to vasculature remodeling, rendered tumor permissive for infiltration of immune cells, and demonstrated antitumor efficacy. Given that the small molecular anti-angiogenic agent, sorafenib, is confirmed to possess antitumor efficacy for HCC, then how about bevacizumab? Several studies tried the bevacizumab for HCC patients. In a recently published phase II trial, 43 patients received bevacizumab [87]. Of the patients, six (14 %) achieved a partial response. The disease control rate at the time point of 16 week was 42 % (95 % confidence interval, 27–57 %). In addition, circulating endothelial cells were found to be associated with response, while interleukin-8 and -6 were negatively related to the therapeutic efficacy. A systematic review analyzed the efficacy of bevacizumab for HCC, although all trials eligible were phase II trials [88]. Eight trials involving 300 patients were included. The results favored the use of becizumab either alone or in combination with other agents. Phase III trials are warranted to comprehensively examine the efficacy and safety of bevacizumab for treatment of advanced HCC.

Recently, the immune regulatory mechanism becomes the focus of cancer immunotherapy. Tumor harnesses numerous regulatory pathways to evade immune surveillance, but these negative regulatory mechanisms provide additional therapeutic targets. The proof-of-concept evidence comes from the anti-Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) antibody. CTLA-4 is a cell surface molecule expressed almost exclusively on $CD4^+$ and $CD8^+$ T cells, and is important for the maintenance of T cell homeostasis [89]. CTLA4 on the surface of T cells binds to CD80 and CD86 on antigen-presenting cells, and transmits an

inhibitory signal to T cells. The CTLA-4 antibody ipilimumab is proved to be capable of inducing objective response in variant tumors, especially melanoma [90]. In one study, the effects of CTLA-4 blocking antibody were tested *in vitro* [91]. Along with a panel of tumor-associated antigen peptide vaccine, ipilimumab resulted in unmasking of immune responses by changing cytokine or chemokine profiles in peripheral blood mononuclear cells. These results suggested that ipilimumab had a role in the immunotherapy of HCC, but further studies are needed to confirm this hypothesis.

The antibody induces the glomerization of series of complements. The complex formed by complements has a direct tumoricidal effect by attacking the cell membrane or releasing a signal for other effector cells (opsonizing). Fc fragment also binds specific receptors on the surface of effector cells like NK cells or T cells, and this antibody-dependent cytotoxicity (ADCC) effect plays an important role in the antitumor effect of the antibody. Equally promising is the use of conjugate antibody (Fig. 10.3). Conjugated antibody comprises two parts: the conjugates and the antibody. The antibody itself does not possess antitumor activity, but rather performs to carry the conjugate ("magic bullet") such as isotope (for instance, Tuxuetan), toxin, or chemotherapy agent to the tumor site.

Because of its great therapeutic and economical potential, efforts have been devoted to the research and design of novel antibody agents, and a radio-labeled antibody-^{131}I labeled Metuximab injection (Licartin) for treatment of HCC has been developed and become commercially available in China.

In a pilot study, the researcher recruited 24 HCC patients and randomly divided them into three groups to receive 18.5, 27.75, and 37 MBq/kg of Licartin per kilogram of body weight, respectively. Licartin was injected by hepatic artery intubation. The positive imaging result of monoclonal antibody (mAb) scanning in 24 patients showed that Licartin was apparently accumulated more in hepatoma. These data supported that ^{131}I-labeled Metuximab could deliver relatively selective radiation to tumor tissues [92]. They also carried clinical trials to demonstrate that Licartin was safe and active for HCC patients. In the phase I trial, 28 patients were randomly assigned to receive the injection in 9.25-, 18.5-, 27.75-, or 37-MBq/kg doses by hepatic artery infusion. In a multicenter phase II trial, 106 patients received the injection (27.75 MBq/kg) on Day 1 of a 28-day cycle. Response rate and survival rate were the endpoints. No life-threatening toxic effects were found. The safe dosage was 27.75 MBq/kg. The blood clearance fitted a biphasic model, and its half-life was 90.56–63.93 h. In the phase II trial, the injection was found to be targeted and concentrated to tumor tissues. Of the 73 patients completing two cycles, 6 (8.22 %) had a partial response, 14 (19.18 %) minor response, and 43 (58.90 %) had stable disease (SD). The 21-month survival rate

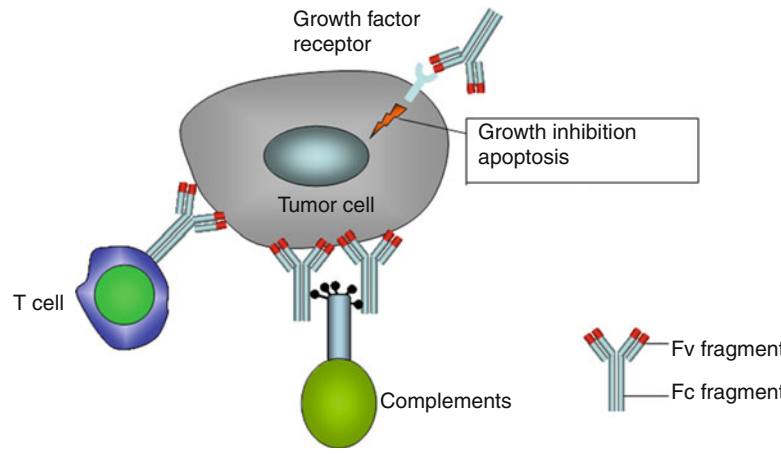


Fig. 10.3 Mechanisms of antitumor efficacy of antibodies

was 44.54 %. The survival rate of progression-free patients was significantly higher than that of patients with progressive disease after either one or two cycles ($p<0.0001$ or $p=0.0019$) [93].

The antibody Licartin was also shown to be effective in preventing hepatoma recurrence after liver transplantation in a randomized controlled trial. This trial was to assess the post-orthotopic liver transplantation (OLT) anti-recurrence efficacy of Licartin in advanced HCC patients. Sixty post-OLT patients with HCC, who were at tumor stage three-fourth and outside the Milan criteria before OLT, were randomized into two groups. Three weeks after OLT, the treatment group received 15.4 MBq/kg of Licartin, while the control group received placebo intravenously for three times with 28-day intervals. At 1-year follow-up, the recurrence rate significantly decreased by 30.4 % ($p=0.0174$) and the survival rate increased by 20.6 % ($p=0.0289$) in the treatment group, compared with those in the control group. For the control group versus the treatment group, the hazard ratio for recurrence was 3.60 (95 % confidence interval [CI], 1.50–8.60) and that for death was 3.87 (95 % CI, 1.23–12.21). Licartin treatment also resulted in an earlier decreased AFP level and a longer time of normal AFP level than placebo ($p=0.0016$). No Licartin-related toxic effects were observed. The authors concluded that Licartin is a promising drug for preventing post-OLT tumor recurrence in advanced HCC patients excluded by the currently strict criteria for OLT [94]. However, human anti-mouse antibody (HAMA) response in some patients after administration limited its clinical use of Licartin. Therefore, attempts were made to develop a more efficient antibody fragment with less immunogenicity. To reduce the immunogenicity of murine antibody, they attempted to humanize HAb18 by variable domain resurfacing based on the three-dimensional structure of Fv fragment. They fabricated a humanized version of HAb18scFv, HAb18-huscFv, to the human IgG1Fc fragment to form (HAb18-huscFv)(2)-Fc. The reactivity of (HAb18-huscFv)(2)-Fc to the serum of patients with HAMA response was decreased, while its specificity and similar binding activity remained intact [95].

10.7 Prospect

The past century has witnessed rapid progress in the therapy of hepatobiliary tract cancer. Surgery, ablation, embolization, and radiotherapy have become mainstays for the treatment of locoregional disease, followed by increasingly effective targeted therapy or chemotherapy for disseminated disease. However, despite the great effort devoted, these cancers continue to exert a great threat to humans. The prognosis is still discouraging for most patients. Therefore, there is a high but unmet need to develop and implement innovative approaches for hepatobiliary cancers. Fortunately, in recent years, our treatment armamentarium has expanded beyond conventional treatment modalities to include immunotherapy, which acts against cancer in a more specific way [96].

A great increase of our knowledge about the cancer immunogenicity and the immune response of our body has been achieved, and the leap has been translated into the development of new cancer immune therapies. Great progress has been achieved in cancer immunotherapy [97]. For example, Trastuzumab, the mAb against the tumor antigen HER2 overexpressed in breast cancer or gastric cancer has been widely used in clinical practice. Additional therapeutic antibodies include Rituximab against CD20 in B-cell lymphoma and Cetuximab against overexpressed EGFR in colon cancer, head and neck cancer, or lung cancer. Also numerous clinical trials began to evaluate the efficacy of adoptive T cell transfer for a panel of tumors [98, 99]. Maybe in the future not so far, some of the immune cell therapies will become a part of the bed-side practice. Not to mention the numerous cancer vaccines that are being developed and some are in the late stages of clinical trial or already available for the clinicians (Table 10.2).

Although decades have passed, cancer immunotherapy is still generally at its early stages. Most studies have been conducted at the preclinical stage, with limited number of clinical trials. It seems a long way to go for immunotherapy. We believe this process can be accelerated if the following issues are improved.

Table 10.2 Vaccines in the late stage of development

Vaccine	Component	Cancer type	Clinical trial phase
Vitespen (Oncophage)	Autologous, tumor-derived heat shock protein gp96 peptide complexes	Melanoma	Phase 3
–	gp1: 209–217(210M)	Melanoma	Phase 3
TroVax	Modified vaccinia Ankara encoding the tumor antigen 5T4 (MVA-5T4)	Renal cell cancer	Phase 3
–	Bec2/bacille Calmette-Guerin (BCG)	Small cell lung cancer	Phase 3
Provenge (Sipuleucel-T)	Antigen-presenting cells pulsed with a fusion protein which consists of PAP and GM-CSF	Prostate cancer	On the market
GVAX	GM-CSF transduced allogeneic prostate cancer cells	Prostate cancer	Phase 3

The immune capabilities of biliary tract cancer patients especially those with advanced cancer are often compromised by the cancer or poor nutritious status (cachexia). It is optimal for the immunotherapy to be administered on patients at earlier stage. For instance, the current ongoing trials that target antigen MAGE (MAGRIT) or MUC1 (INSPIRE) on lung cancer are both performed in patients with postoperative or local advanced lung cancer. We suggest that the earlier stages of hepatobiliary cancer should be put at priority for immunotherapy.

The real problem is the lacking of an efficient and easy way to measure the specific antitumor immunity *in vivo*. Currently, the methods most widely accepted include the ELISPOT to measure the humoral response or the MHC-peptide tetramer analysis to measure the T cell response. These techniques are difficult, time-consuming, and hard to be performed in everyday practice. Detection methods need to be expanded, and new methods need to be developed to detect these immune changes. For example, now we use the Positron Emission Tomography to scan the whole body for the foci of abnormal metabolism, which usually stands for cancer. Can new ways such as functional imaging be adopted to monitor the real-time antitumor immunity? The concept will come into reality for hepatobiliary cancer, given a suitable tracer is available.

Another hurdle is to look for suitable surrogate markers or biomarkers that predict the efficacy of the immunotherapy. From the authors past experience, the success of targeted therapies is known to always depend on the existence of suitable targets. For instance, without the guidance of epithelial growth factor receptor (*EGFR*) gene mutation status, clinical trials with EGFR tyrosine kinase inhibitors (TKIs) most often ended up with negative results. Now lung cancer patients harboring the EGFR mutation are selected for the administration of TKIs. In this population, the TKIs far outperform chemotherapy. But for the most of the immunotherapies, we still do not have appropriate biomakers to select suitable patients. The defects in our knowledge will undoubtedly exert impacts on the development of immunotherapy. Clinical trials conducted in unselected patients will probably veil the success in certain subgroups of patients and mislead us to the wrong conclusion.

10.8 Concluding Remarks

In summary, hepatobiliary tumors are a diverse group of tumors that arise from the hepatobiliary tract including the liver, gallbladder, and bile tract. Cancer from the hepatobiliary tract has poor prognosis and high mortality rate. Even worse, the treatment modality is very limited for this disease. Therefore, a novel effective and specific therapy is highly required for hepatobiliary tract cancer. In this sense, immunotherapy may provide an additional therapeutic opportunity for this notorious disease. Immunotherapy of cancer has been gradually established as one of the treatment modalities. Adoptive transfer of mAbs has achieved definite effects in clinics and it is believed that the booming of mAbs will persist. To some extent, cell therapy

has unique mechanisms of action and has achieved amazing effects in some cases. As far as the cancer vaccine is considered, it is in the stage of rapid rise. Several cancer vaccines have been approved, especially for patients with melanoma or prostate cancer. Given the rapid progress in the field of cancer immunotherapy, it is believed to provide an additional opportunity to the therapy of liver and biliary tract cancer.

Acknowledgments The work is partially supported by grants from National Major Project of China (No.2011ZX09302-001-01), and National Natural Science Fund of China (81272684).

References

1. Kiran RP, Pokala N, Dudrick SJ. Incidence pattern and survival for gallbladder cancer over three decades – an analysis of 10301 patients. Ann Surg Oncol. 2007;14(2):827–32.
2. Eslick GD. Epidemiology of gallbladder cancer. Gastroenterol Clin N Am. 2010;39(2):307–30.
3. Wang JH JX (2010) Epidemiology of gallbladder cancer in China. Lit Chin Gen Surg. 6(2).
4. Patel T. Cholangiocarcinoma. Nat Clin Pract Gastroenterol Hepatol. 2006;3(1):33–42.
5. Lim JH. Cholangiocarcinoma: morphologic classification according to growth pattern and imaging findings. AJR Am J Roentgenol. 2003;181(3):819–27.
6. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74.
7. Ding ZY, Zou XL, Wei YQ. Cancer microenvironment and cancer vaccine. Cancer Microenviron. 2012;5(3):333–44.
8. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008;454(7203):436–44.
9. Nakamoto Y, Guidotti LG, Kuhlen CV, Fowler P, Chisari FV. Immune pathogenesis of hepatocellular carcinoma. J Exp Med. 1998;188(2):341–50.
10. Haybaeck J, Zeller N, Wolf MJ, Weber A, Wagner U, Kurrer MO, et al. A lymphotoxin-driven pathway to hepatocellular carcinoma. Cancer Cell. 2009;16(4):295–308.
11. Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, et al. A toll-like receptor recognizes bacterial DNA. Nature. 2000;408(6813):740–5.
12. Tanaka J, Sugimoto K, Shiraki K, Tameda M, Kusagawa S, Nojiri K, et al. Functional cell surface expression of toll-like receptor 9 promotes cell proliferation and survival in human hepatocellular carcinomas. Int J Oncol. 2010;37(4):805–14.
13. Naugler WE, Karin M. The wolf in sheep's clothing: the role of interleukin-6 in immunity, inflammation and cancer. Trends Mol Med. 2008;14(3):109–19.
14. Goydos JS, Brumfield AM, Frezza E, Booth A, Lotze MT, Carty SE. Marked elevation of serum interleukin-6 in patients with cholangiocarcinoma: validation of utility as a clinical marker. Ann Surg. 1998;227(3):398–404.
15. Giannitrapani L, Cervello M, Soresi M, Notarbartolo M, La Rosa M, Virruso L, et al. Circulating IL-6 and sIL-6R in patients with hepatocellular carcinoma. Ann N Y Acad Sci. 2002;963:46–52.
16. Giannitrapani L, Soresi M, Giacalone A, Campagna ME, Marasa M, Cervello M, et al. IL-6–174G/C polymorphism and IL-6 serum levels in patients with liver cirrhosis and hepatocellular carcinoma. OMICS. 2011;15(3):183–6.
17. Wang XH, Liu BR, Qu B, Xing H, Gao SL, Yin JM, et al. Silencing STAT3 may inhibit cell growth through regulating signaling pathway, telomerase, cell cycle, apoptosis and angiogenesis in hepatocellular carcinoma: potential uses for gene therapy. Neoplasma. 2011;58(2):158–71.
18. Lin L, Amin R, Gallicano GI, Glasgow E, Jogunoori W, Jessup JM, et al. The STAT3 inhibitor NSC 74859 is effective in hepatocellular cancers with disrupted TGF-beta signaling. Oncogene. 2009;28(7):961–72.
19. Arsura M, Cavin LG. Nuclear factor-kappaB and liver carcinogenesis. Cancer Lett. 2005;229(2):157–69.
20. Luedde T, Schwabe RF. NF-kappaB in the liver–linking injury, fibrosis and hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol. 2011;8(2):108–18.
21. Tai DI, Tsai SL, Chen YM, Chuang YL, Peng CY, Sheen IS, et al. Activation of nuclear factor kappaB in hepatitis C virus infection: implications for pathogenesis and hepatocarcinogenesis. Hepatology. 2000;31(3):656–64.
22. Albini A, Tosetti F, Benelli R, Noonan DM. Tumor inflammatory angiogenesis and its chemoprevention. Cancer Res. 2005;65(23):10637–41.
23. Scapini P, Morini M, Tecchio C, Minghelli S, Di Carlo E, Tanghetti E, et al. CXCL1/macrophage inflammatory protein-2-induced angiogenesis in vivo is mediated by neutrophil-derived vascular endothelial growth factor-A. J Immunol. 2004;172(8):5034–40.
24. Li YW, Qiu SJ, Fan J, Zhou J, Gao Q, Xiao YS, et al. Intratumoral neutrophils: a poor prognostic factor for hepatocellular carcinoma following resection. J Hepatol. 2011;54(3):497–505.
25. Gomez D, Farid S, Malik HZ, Young AL, Toogood GJ, Lodge JP, et al. Preoperative neutrophil-to-lymphocyte ratio as a prognostic predictor after curative resection for hepatocellular carcinoma. World J Surg. 2008;32(8):1757–62.
26. Kuang DM, Zhao Q, Wu Y, Peng C, Wang J, Xu Z, et al. Peritumoral neutrophils link inflammatory response to disease progression by fostering angiogenesis in hepatocellular carcinoma. J Hepatol. 2011;54(5):948–55.
27. Condeelis J, Pollard JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell. 2006;124(2):263–6.
28. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer. 2004;4(1):71–8.

29. Movahedi K, Laoui D, Gysemans C, Baeten M, Stange G, Van den Bossche J, et al. Different tumor microenvironments contain functionally distinct subsets of macrophages derived from Ly6C(high) monocytes. Cancer Res. 2010;70(14):5728–39.
30. Zhou J, Ding T, Pan W, Zhu LY, Li L, Zheng L. Increased intratumoral regulatory T cells are related to intratumoral macrophages and poor prognosis in hepatocellular carcinoma patients. Int J Cancer. 2009;125(7):1640–8.
31. Kuang DM, Zhao Q, Peng C, Xu J, Zhang JP, Wu C, et al. Activated monocytes in peritumoral stroma of hepatocellular carcinoma foster immune privilege and disease progression through PD-L1. J Exp Med. 2009;206(6):1327–37.
32. Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nat Immunol. 2010;11(10):889–96.
33. Peng SH, Deng H, Yang JF, Xie PP, Li C, Li H, et al. Significance and relationship between infiltrating inflammatory cell and tumor angiogenesis in hepatocellular carcinoma tissues. World J Gastroenterol. 2005;11(41):6521–4.
34. Yang W, Lu Y, Xu Y, Xu L, Zheng W, Wu Y, et al. Estrogen represses hepatocellular carcinoma (HCC) growth via inhibiting alternative activation of tumor-associated macrophages (TAMs). J Biol Chem. 2012;287(48):40140–9.
35. Yang J, Zhang JX, Wang H, Wang GL, Hu QG, Zheng QC. Hepatocellular carcinoma and macrophage interaction induced tumor immunosuppression via Treg requires TLR4 signaling. World J Gastroenterol. 2012;18(23):2938–47.
36. Ju MJ, Qiu SJ, Fan J, Xiao YS, Gao Q, Zhou J, et al. Peritumoral activated hepatic stellate cells predict poor clinical outcome in hepatocellular carcinoma after curative resection. Am J Clin Pathol. 2009;131(4):498–510.
37. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science. 2003;299(5609):1057–61.
38. Mathai AM, Kapadia MJ, Alexander J, Kernochan LE, Swanson PE, Yeh MM. Role of Foxp3-positive tumor-infiltrating lymphocytes in the histologic features and clinical outcomes of hepatocellular carcinoma. Am J Surg Pathol. 2012;36(7):980–6.
39. Zhou L, Fu JL, Lu YY, Fu BY, Wang CP, An LJ, et al. Regulatory T cells are associated with post-cryoablation prognosis in patients with hepatitis B virus-related hepatocellular carcinoma. J Gastroenterol. 2010;45(9):968–78.
40. Lin GH, Wang J, Li SH, Xu L, Li SP. Relationship and clinical significance of TGF-beta1 expression with Treg cell infiltration in hepatocellular carcinoma. Chin J Cancer. 2010;29(4):403–7.
41. Shen X, Li N, Li H, Zhang T, Wang F, Li Q. Increased prevalence of regulatory T cells in the tumor microenvironment and its correlation with TNM stage of hepatocellular carcinoma. J Cancer Res Clin Oncol. 2010;136(11):1745–54.
42. Zhang HH, Mei MH, Fei R, Liu F, Wang JH, Liao WJ, et al. Regulatory T cells in chronic hepatitis B patients affect the immunopathogenesis of hepatocellular carcinoma by suppressing the anti-tumour immune responses. J Viral Hepat. 2010;17 Suppl 1:34–43.
43. Wu H, Chen P, Liao R, Li YW, Yi Y, Wang JX, et al. Intratumoral regulatory T cells with higher prevalence and more suppressive activity in HCC patients. J Gastroenterol Hepatol. 2013;28(9):1555–64.
44. Yi Y, He HW, Wang JX, Cai XY, Li YW, Zhou J, et al. The functional impairment of HCC-infiltrating gammadelta T cells, partially mediated by regulatory T cells in a TGFbeta- and IL-10-dependent manner. J Hepatol. 2013;58(5):977–83.
45. Kakita N, Kanto T, Itose I, Kuroda S, Inoue M, Matsubara T, et al. Comparative analyses of regulatory T cell subsets in patients with hepatocellular carcinoma: a crucial role of CD25(-) FOXP3(-) T cells. Int J Cancer. 2012;131(11):2573–83.
46. Stone JH, Zen Y, Deshpande V. IgG4-related disease. N Engl J Med. 2012;366(6):539–51.
47. Harada K, Shimoda S, Kimura Y, Sato Y, Ikeda H, Igarashi S, et al. Significance of immunoglobulin G4 (IgG4)-positive cells in extrahepatic cholangiocarcinoma: molecular mechanism of IgG4 reaction in cancer tissue. Hepatology. 2012;56(1):157–64.
48. Kimura Y, Harada K, Nakanuma Y. Pathologic significance of immunoglobulin G4-positive plasma cells in extrahepatic cholangiocarcinoma. Hum Pathol. 2012;43(12):2149–56.
49. Quezada SA, Peggs KS. Exploiting CTLA-4, PD-1 and PD-L1 to reactivate the host immune response against cancer. Br J Cancer. 2013;108(8):1560–5.
50. Ye Y, Zhou L, Xie X, Jiang G, Xie H, Zheng S. Interaction of B7-H1 on intrahepatic cholangiocarcinoma cells with PD-1 on tumor-infiltrating T cells as a mechanism of immune evasion. J Surg Oncol. 2009;100(6):500–4.
51. Hasita H, Komohara Y, Okabe H, Masuda T, Ohnishi K, Lei XF, et al. Significance of alternatively activated macrophages in patients with intrahepatic cholangiocarcinoma. Cancer Sci. 2010;101(8):1913–9.
52. Jarnagin WR. Management of small hepatocellular carcinoma: a review of transplantation, resection, and ablation. Ann Surg Oncol. 2010;17(5):1226–33.
53. Truty MJ, Vauthey JN. Surgical resection of high-risk hepatocellular carcinoma: patient selection, preoperative considerations, and operative technique. Ann Surg Oncol. 2010;17(5):1219–25.
54. Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. N Engl J Med. 1996;334(11):693–9.
55. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008;359(4):378–90.
56. Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced

hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol. 2009;10(1):25–34.
57. El-Serag HB, Marrero JA, Rudolph L, Reddy KR. Diagnosis and treatment of hepatocellular carcinoma. Gastroenterology. 2008;134(6):1752–63.
58. Park J, Kim MH, Kim KP, Park do H, Moon SH, Song TJ, et al. Natural history and prognostic factors of advanced cholangiocarcinoma without surgery, chemotherapy, or radiotherapy: a large-scale observational study. Gut Liver. 2009;3(4):298–305.
59. Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. Structure of the human class I histocompatibility antigen, HLA-A2. Nature. 1987;329(6139):506–12.
60. Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. Nature. 1987;329(6139):512–8.
61. Rosenberg SA. Adoptive immunotherapy of cancer: accomplishments and prospects. Cancer Treat Rep. 1984;68(1):233–55.
62. Unitt E, Marshall A, Gelson W, Rushbrook SM, Davies S, Vowler SL, et al. Tumour lymphocytic infiltrate and recurrence of hepatocellular carcinoma following liver transplantation. J Hepatol. 2006;45(2):246–53.
63. Cai L, Zhang Z, Zhou L, Wang H, Fu J, Zhang S, et al. Functional impairment in circulating and intrahepatic NK cells and relative mechanism in hepatocellular carcinoma patients. Clin Immunol. 2008;129(3):428–37.
64. Kwon S, Kim D, Park BK, Cho S, Kim KD, Kim YE, et al. Prevention and therapy of hepatocellular carcinoma by vaccination with TM4SF5 epitope-CpG-DNA-liposome complex without carriers. PLoS ONE. 2012;7(3):e33121.
65. Yi BR, Hwang KA, Kang NH, Kim SU, Jeung EB, Kim HC, et al. Synergistic effects of genetically engineered stem cells expressing cytosine deaminase and interferon-beta via their tumor tropism to selectively target human hepatocarcinoma cells. Cancer Gene Ther. 2012;19(9):644–51.
66. Gao J, Luo SM, Peng ML, Deng T. Enhanced immunity against hepatoma induced by dendritic cells pulsed with Hsp70-H22 peptide complexes and CD40L. J Cancer Res Clin Oncol. 2012;138(6):917–26.
67. Butterfield LH, Koh A, Meng W, Vollmer CM, Ribas A, Dissette V, et al. Generation of human T-cell responses to an HLA-A2.1-restricted peptide epitope derived from alpha-fetoprotein. Cancer Res. 1999;59(13):3134–42.
68. Liu Y, Daley S, Evdokimova VN, Zdobinski DD, Potter DM, Butterfield LH. Hierarchy of alpha fetoprotein (AFP)-specific T cell responses in subjects with AFP-positive hepatocellular cancer. J Immunol. 2006;177(1):712–21.
69. Jakubovic BD, Jothy S. Glypican-3: from the mutations of Simpson-Golabi-Behmel genetic syndrome to a tumor marker for hepatocellular carcinoma. Exp Mol Pathol. 2007;82(2):184–9.
70. Shirakawa H, Kuronuma T, Nishimura Y, Hasebe T, Nakano M, Gotohda N, et al. Glypican-3 is a useful diagnostic marker for a component of hepatocellular carcinoma in human liver cancer. Int J Oncol. 2009;34(3):649–56.
71. Shirakawa H, Suzuki H, Shimomura M, Kojima M, Gotohda N, Takahashi S, et al. Glypican-3 expression is correlated with poor prognosis in hepatocellular carcinoma. Cancer Sci. 2009;100(8):1403–7.
72. Komori H, Nakatsura T, Senju S, Yoshitake Y, Motomura Y, Ikuta Y, et al. Identification of HLA-A2- or HLA-A24-restricted CTL epitopes possibly useful for glypican-3-specific immunotherapy of hepatocellular carcinoma. Clin Cancer Res. 2006;12(9):2689–97.
73. Sawada Y, Yoshikawa T, Nobuoka D, Shirakawa H, Kuronuma T, Motomura Y, et al. Phase I trial of a glypican-3-derived peptide vaccine for advanced hepatocellular carcinoma: immunologic evidence and potential for improving overall survival. Clin Cancer Res. 2012;18(13):3686–96.
74. Nobuoka D, Yoshikawa T, Sawada Y, Fujiwara T, Nakatsura T. Peptide vaccines for hepatocellular carcinoma. Hum Vaccin Immunother. 2013;9(1):210–2.
75. Yamamoto K, Ueno T, Kawaoka T, Hazama S, Fukui M, Suehiro Y, et al. MUC1 peptide vaccination in patients with advanced pancreas or biliary tract cancer. Anticancer Res. 2005;25(5):3575–9.
76. El Ansary M, Mogawer S, Elhamid SA, Alwakil S, Aboelkasem F, Sabaawy HE, et al. Immunotherapy by autologous dendritic cell vaccine in patients with advanced HCC. J Cancer Res Clin Oncol. 2013;139(1):39–48.
77. Qiu Y, Xu MB, Yun MM, Wang YZ, Zhang RM, Meng XK, et al. Hepatocellular carcinoma-specific immunotherapy with synthesized alpha1,3- galactosyl epitope-pulsed dendritic cells and cytokine-induced killer cells. World J Gastroenterol. 2011;17(48):5260–6.
78. Rosenberg SA, Lotze MT, Muul LM, Chang AE, Avis FP, Leitman S, et al. A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. N Engl J Med. 1987;316(15):889–97.
79. Rosenberg SA, Yannelli JR, Yang JC, Topalian SL, Schwartzentruber DJ, Weber JS, et al. Treatment of patients with metastatic melanoma with autologous tumor-infiltrating lymphocytes and interleukin 2. J Natl Cancer Inst. 1994;86(15):1159–66.
80. Stromnes IM, Fowler C, Casamina CC, Georgopolos CM, McAfee MS, Schmitt TM, et al. Abrogation of SRC homology region 2 domain-containing phosphatase 1 in tumor-specific T cells improves efficacy of adoptive immunotherapy by enhancing the effector function and accumulation of short-lived effector T cells in vivo. J Immunol. 2012;189(4):1812–25.
81. Morisaki T, Onishi H, Koya N, Kiyota A, Tanaka H, Umebayashi M, et al. Combinatorial cytotoxicity of gemcitabine and cytokine-activated killer cells in

hepatocellular carcinoma via the NKG2D-MICA/B system. Anticancer Res. 2011;31(7):2505–10.
82. Takayama T, Sekine T, Makuuchi M, Yamasaki S, Kosuge T, Yamamoto J, et al. Adoptive immunotherapy to lower postsurgical recurrence rates of hepatocellular carcinoma: a randomised trial. Lancet. 2000;356(9232):802–7.
83. Zhong JH, Ma L, Wu LC, Zhao W, Yuan WP, Wu FX, et al. Adoptive immunotherapy for postoperative hepatocellular carcinoma: a systematic review. Int J Clin Pract. 2012;66(1):21–7.
84. Zhou P, Liang P, Dong B, Yu X, Han Z, Xu Y. Phase clinical study of combination therapy with microwave ablation and cellular immunotherapy in hepatocellular carcinoma. Cancer Biol Ther. 2011;11(5):450–6.
85. Zhu AX, Duda DG, Sahani DV, Jain RK. HCC and angiogenesis: possible targets and future directions. Nat Rev Clin Oncol. 2011;8(5):292–301.
86. Garbi N, Arnold B, Gordon S, Hammerling GJ, Ganss R. CpG motifs as proinflammatory factors render autochthonous tumors permissive for infiltration and destruction. J Immunol. 2004;172(10):5861–9.
87. Boige V, Malka D, Bourredjem A, Dromain C, Baey C, Jacques N, et al. Efficacy, safety, and biomarkers of single-agent bevacizumab therapy in patients with advanced hepatocellular carcinoma. Oncologist. 2012;17(8):1063–72.
88. Fang P, Hu JH, Cheng ZG, Liu ZF, Wang JL, Jiao SC. Efficacy and safety of bevacizumab for the treatment of advanced hepatocellular carcinoma: a systematic review of phase II trials. PLoS ONE. 2012;7(12):e49717.
89. McCoy KD, Le Gros G. The role of CTLA-4 in the regulation of T cell immune responses. Immunol Cell Biol. 1999;77(1):1–10.
90. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711–23.
91. Mizukoshi E, Nakamoto Y, Arai K, Yamashita T, Sakai A, Sakai Y, et al. Comparative analysis of various tumor-associated antigen-specific t-cell responses in patients with hepatocellular carcinoma. Hepatology. 2011;53(4):1206–16.
92. Zhang Z, Bian H, Feng Q, Mi L, Mo T, Kuang A, et al. Biodistribution and localization of iodine-131-labeled metuximab in patients with hepatocellular carcinoma. Cancer Biol Ther. 2006;5(3):318–22.
93. Chen ZN, Mi L, Xu J, Song F, Zhang Q, Zhang Z, et al. Targeting radioimmunotherapy of hepatocellular carcinoma with iodine (131I) metuximab injection: clinical phase I/II trials. Int J Radiat Oncol Biol Phys. 2006;65(2):435–44.
94. Xu J, Shen ZY, Chen XG, Zhang Q, Bian HJ, Zhu P, et al. A randomized controlled trial of Licartin for preventing hepatoma recurrence after liver transplantation. Hepatology. 2007;45(2):269–76.
95. Zhu H, Yang B, Yang X, Wang L, Xu J, Liao C, et al. A novel antibody fragment targeting HAb18G/CD147 with cytotoxicity and decreased immunogenicity. Cancer Biol Ther. 2009;8(11):1035–44.
96. The LO. Tapping our immune potential to realise the ultimate goal. Lancet Oncol. 2011;12(13):1175.
97. Finn OJ. Cancer immunology. N Engl J Med. 2008;358(25):2704–15.
98. Rosenberg SA, Restifo NP, Yang JC, Morgan RA, Dudley ME. Adoptive cell transfer: a clinical path to effective cancer immunotherapy. Nat Rev Cancer. 2008;8(4):299–308.
99. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. Nature. 2011;480(7378):480–9.

Immunology and Immunotherapy of Colorectal Cancer

11

Rubén Varela-Calviño and Oscar J. Cordero

Contents

R. Varela-Calviño, PhD
Department of Biochemistry and Molecular Biology, University of Santiago de Compostela, School of Pharmacy, Campus Vida, Santiago de Compostela, A Coruña, Galicia 15782, Spain
e-mail: ruben.varela@usc.es

O.J. Cordero, PhD (✉)
Department of Biochemistry and Molecular Biology, University of Santiago de Compostela, CIBUS Building. Campus Vida, Santiago de Compostela, A Coruña, Galicia 15782, Spain
e-mail: oscarj.cordero@usc.es

11.1 Introduction

When activated, inflammation and immunity induce repair mechanisms to recover tissue function and integrity in addition to the elimination, or at least the control of dissemination or systemic colonization, of pathogens. These responses are essential for the survival of the organism.

Almost 2,000 years ago, the Greek physician Galenus [1] had already described the similarity between cancer and inflammation. More than a century ago, Dvorak [2] observed that inflammation and cancer share some basic developmental mechanisms (angiogenesis) and tissue-infiltrating cell types (lymphocytes, macrophages, and mast cells), and that tumors act like "wounds that do not heal." The close relationship between carcinogenesis and inflammation, which can be secondary to infection, has recently been the subject of many studies at the molecular, cellular, animal, and clinical levels.

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_11,

Some of the most intriguing clinical evidence in humans regarding the association between chronic inflammation and cancer derives from the finding that regular use of aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs) decreases the incidence of cancer. More recently, G. Trinchieri [3] reviewed the systemic role of commensal microbiota in the inflammatory condition of cancer, particularly important in the CRC case.

11.2 Infection and Inflammation

Hanahan and Weinberg [4] in their 2011 update of the "Hallmarks of Cancer" introduced the immune hallmark of cancer focusing on immunosuppression on the one hand and tumor promoting inflammation as an enabling characteristic on the other. Both are two sides of the same coin because the class of inflammation and immunity responsible for tumor initiation and early progression is of the same class that prevents the immune system from eliminating tumors [3]. In fact, when tumors escape immunosurveillance, they are not immunologically silent, as indicated by the roles of inflammation and immunity in editing the tumor cell phenotype [4], or the evidence that, even in progressing tumors, the nature of immunological infiltrates correlates with prognosis [5].

When transformed cells are not killed by the immune response and the tumor starts to grow in an organism, tumor mass is not an extraneous parasitic body, but a complex organized tissue formed by both transformed as well as normal stromal cells in a symbiotic relationship that sustains the growth of the tumor and eventually favors its dissemination to distant tissues. This is reminiscent of an organism's relationship with infectious pathogens that, in the case of chronic infections, establishes a near symbiotic relationship with the reactive surrounding tissues, often reorganized in specific anatomical structures such as the granulomas. As for pathogens, the organism does not ignore the presence of the tumor, but instead triggers tissue and inflammatory responses. Indeed, successful malignant cells coevolve with their environment, and conversely, the microenvironment can restrain cancer progression [6]. Clinical examples are provided by the observation of frequent subclinical prostate and breast tumors identified during autopsy in young individuals deceased from noncancer-related causes [7].

Up to 15 % of tumors can be attributed to an infection [8]. Some pathogens that infect humans directly induce cell transformation such as herpes virus, Epstein Barr virus (EBV), or Human T-Lymphotropic virus-I (HTLV-I). Other viruses (e.g., hepatitis viruses) favor carcinogenesis by inducing chronic inflammation in the infected tissues (in fact, a tumor-promoting effect of inflammation is now suspected also for transforming viruses). Nonetheless, most human tumors originate from tissues with sterile chronic inflammation. Inflammation can start due to mechanical, chemical, irradiation and other types of injury, or to genetic syndromes. For example, bladder carcinoma is associated with chronic indwelling urinary catheter (mechanical) [9], lung cancer and mesothelioma is associated with asbestosis (chemical) [10], and pancreatic carcinoma in patients with pancreatitis is due to a mutation in the tryspinogen gene (genetic) [11].

The inflammation associated with cancer initiation is defined as intrinsic when the mechanisms that are involved in cell transformation, most typically oncogene (in particular Ras, Myc, Src, RET, and microRNAs such as *mi-R155*) [3, 12] overexpression, mutation, DNA damage, or mitochondrial reactive oxygen species (ROS), are also responsible for the activation of a proinflammatory program in the altered cell.

By contrast, extrinsic inflammation is activated by the tissue's response to the malignant cells, and is most prominently mediated by the infiltrating inflammatory cells [3, 12]. Stressed cells produce pro-inflammatory factors (cytokines, chemokines, interferons (IFNs), tissue rearranging enzymes) in response to DNA damage, mitochondrial damage, endoplasmic reticulum (ER) stress, oxidative damage, hypoxia, excessive temperature, excessive nutrients, senescence, or oncogene activation. The tissue-intrinsic inflammatory responses recruit hematopoietic inflammatory cells. These inflammatory cells express specialized innate

receptors, such as Toll-like receptors (TLRs). Other families of cytoplasmic pattern recognition receptors (PRRs) more recently identified - like Nod (Nucleotide binding oligomerization domain)-like receptors (NLRs), C-type lectin receptors, RIG-I (retinoic acid inducible gene-I)-like receptors (RLRs), or DNA sensors - identify pathogen-associated molecular patterns (PAMPs, associated with microbial pathogens or cellular stress and diet-induced inflammation), and also damage-associated molecular patterns (DAMPs, associated with endogenous components released during cell damage) [13–15]. By definition, these receptors are very promiscuous; the same or very similar ligand can bind different receptors in the same cell or in a different population. Many of these receptors have the same signal transduction pathways, adaptor protein MyD88.

Moreover, tumor cells secrete several cytokines and chemokines that attract blood leukocytes. The leukocyte population present on a developing cancer is quite diverse and includes neutrophils, dendritic cells, macrophages, and lymphocytes, all of which are capable of secreting a series of cytokines, ROS, membrane perforating agents or IFNs. These secreted molecules contribute to the inflammatory milieu present in a developing tumor, which may contribute to the growth and dissemination of transformed cells [16]. For example, although tumor-associated macrophages contribute to the elimination of transformed cells once activated by cytokines [17], they also produce potent angiogenic and growth factors such as VEGF-C and –D that potentiate neoplastic progression [18]. The hypothesis that transformed cells arising from areas of infection and inflammation are part of a normal host response is based on several observations [19].

The effect of the commensal flora on local inflammation and on carcinogenesis at interfaces between the organism and the outside environment (not only in the gut, but also in the upper gastrointestinal tract, oral mucosa, bronchioalveolar mucosa, and skin) can be now readily explained by the direct interaction of the bacteria or their products with those innate receptors or sensors in epithelial cells or in the most superficial cell layers. Increasing evidence indicates that these interactions can have additional deep systemic effects through the immune system cells and alteration of the inflammatory environment. How exposure to the developing commensal flora affects the maturation of the immune system after birth has been extensively studied. Other mechanisms affecting metabolic processes or genetic stability remain uncertain, but several animal models of autoimmunity and immunity to pathogens are beginning to unravel the important effects of commensal flora on the systemic inflammation/immunity. For example, syngeneic graft versus host disease (GVHD) and type 1 diabetes (T1D) in mouse models are regulated by the gut microbiota [20, 21]. In addition, there is a bi-directional relationship between the central nervous system and the intestinal microbiota. The central nervous system influences the intestinal microbiota in several pathways of the gastrointestinal physiology such as epithelial functions, permeability, or the production of mucus and antibacterial peptides. Conversely, gut microbiota interacting with local and systemic inflammatory and immune responses activate the production of several metabolites including inflammatory cytokines that affect brain and behavior [22, 23].

Finally, chronic inflammation affects all phases of carcinogenesis. Inflammation favors the initial genetic mutation, functional protein modifications, or epigenetic mechanisms that drive cell transformation and cancer initiation [24, 25]: ROS and reactive nitrogen species (RNS) can induce DNA strand breaks, single base or more complex DNA mutations, as well as epigenetic modifications in proto-oncogenes, tumor-suppressor genes, and other genes encoding proteins that control apoptosis, survival, DNA repair, and cell-cycle checkpoints [24, 26]. Nuclear factor-κB (NF-κB) and signal transducer and activator of transcription-3 (STAT-3) are among the best characterized of the transcription factors induced by the inflammatory mediators [27, 28]. Activation of NF-κB in response to chronic inflammation is of particular relevance to gastrointestinal cancer development, especially in colitis-associated cancer (CAC). Activated NF-κB was detected in lamina propria macrophages and epithelial cells from biopsies or cultured cells of IBD patients

and the number of cells showing NF-κB activation correlated with the degree of mucosal inflammation [29]. Many factors released by the tumor and by the tumor stroma, such as VEGF, interleukin-6 (IL-6), IL-10, IL-11, and IL-23, activate STAT-3 in tumor and stromal cells. Several of these factors are transcriptionally regulated by STAT-3, thus creating a positive-feedback loop [30]. These factors also act as tumor promoters by establishing a tissue microenvironment (e.g., angiogenesis) that allows the tumor to progress and metastasize, as well as prevents the effective immune response against the tumor by establishing immunosuppressive mechanisms. Inflammation also causes systemic metabolic alterations such as cachexia that often represent the primary cause of morbidity and mortality in cancer patients. In all these ways, inflammation also affects the response to therapy.

11.3 Inflammation, Gut Microbiota, and Colorectal Cancer

CRC develops from malignant lesions originated by genetic alterations that affect primarily genes encoding either intestinal homeostatic regulators or DNA-mismatch-repair factors.

It is also known that inflammatory bowel disease (IBD) patients have a several-fold increased susceptibility to cancer. IBDs—both Crohn's disease and ulcerative colitis—are the major risk factors for CRC. Therefore, colorectal cancers in IBD patients are considered typical examples of inflammation-related or CAC. However, the relative increased risk for colorectal cancer in these patients is not higher than threefold compared with healthy controls, and the tumors usually appear after many years of intestinal pathology, with a cumulative lifetime risk of 18 % [31, 32].

Interestingly, IBD patients also have an increased susceptibility to lymphomas/leukemias, hepatocarcinomas, and other tumors, suggesting that the intestinal inflammation due to the pathological immune responsiveness to the commensal microbiota is responsible for both local and systemic tumor-promoting effects. Alternatively, the same genetic alterations that affect inflammatory and immune intestinal homeostasis also predispose an individual to carcinogenesis in other tissues [31, 32]. The immunosuppressive agents used in IBD therapy could also explain this increased susceptibility; however, the types of tumors increased in the IBD patients are different from that observed in patients that had used that therapy in transplants [33].

Thus, most CRCs develop without any obvious preexisting intestinal inflammatory pathology. However, the other known main risk factors including obesity, lack of exercise, fat-rich diets, and use of alcohol and tobacco [19, 34], and particularly those related to nutrition, are environmental agents that affect the commensal microbiota. As the gut inflammatory tone and the development of mucosal innate and adaptive immunity are regulated by the commensal flora, alterations in the percentages of flora composition or changes in the presence of specific bacterial species can modulate that environment, with pro- or anti-inflammatory effects. Also, the gut microbiota may affect gastrointestinal cancer through the catabolism of natural mutagens and carcinogens that require or are regulated by specific enzymatic activity provided by the commensal flora [35].

Very likely those agents are responsible for the geographical variation in the incidence of colorectal cancer since these wide geographical variations are lost in migrating populations that acquire the same risk than the host populations within one generation [36, 37].

Nowadays, many data support that interaction between risk factors and commensal flora cooperates with colorectal carcinogenesis initiated by genetic predisposition or environmental causes. First, several rodent experimental models mimicking human CRC have been used to study the role of inflammation in the development of CAC, clearly establishing that innate and immune cells, cytokines such as TNF, IL-1, IL-6, IL-10, IL-11, IL-17, IL-22, and IL-23, and the STAT-3-NF-κB axis all participate in the induction of inflammatory colitis (predisposing an individual to cancer development) [38].

Later, the role of the gut commensal flora in colon cancer was demonstrated by the lack of

tumor development in germfree animals. For example, germfree or mono-associated with the mildly colitogenic bacterium *Bacteroides vulgatus* azoxymethane (AOM)-treated *Il10*$^{-/-}$ mice, did not develop colitis nor tumors [39]. Lack of tumor formation in germfree mice was also observed in several cancer models in genetically engineered mice (*Il10*$^{-/-}$, *Gpx1*/*Gpx2*$^{-/-}$, and *Tcrb*/*p53*$^{-/-}$) that mimic clinical colorectal carcinoma [40]. Similarly, carcinogenesis induced by AOM and promoted by bile injection was prevented in rats grown germfree [41]. On the contrary, in immunodeficient *Tbet*$^{-/-}$ and *Rag2*$^{-/-}$ ulcerative colitis (TRUC) mice, induced colitis spontaneously progresses to colonic dysplasia and rectal adenocarcinoma [42]. At least two enterobacterial species, *Klebsiella pneumoniae* and *Proteus mirabilis*, were responsible for colitis and cancer in TRUC mice [43], although colonization of germfree mice with those two species alone was insufficient to induce colitis, indicating that either they modify the gut physiology and the normal flora composition or act in synergy with the normal components of the gut flora in activating colon inflammation. Also, a pathogenic human bacterium, the enterotoxigenic *Bacteroides fragilis*, activated the Wnt and NF-κB signaling pathways and promoted colon cancer in adenomatosis polyposis coli (APC) $^{min/+}$ mice (a mouse model of inherited intestinal cancer). Interestingly, activation through the T helper 17 (Th17) cell response was demonstrated [44, 45]. In a similar way, intestinal infection with *Helicobacter hepaticus* enhanced small intestine and colon cancer and also mammary adenocarcinoma in APC$^{min/+}$ mice [46], or chemical or virus transgene-induced hepatocarcinomas [47].

In another model, *Myd88*$^{-/-}$ mice have been used to induce colitis under dextran sulfate sodium (DSS) and irradiation therapy [48]. Unlike what occurs in most other tumor models, MyD88 has a protective role in the development of the colonic tumors that develop following AOM/DSS treatment [49]. Some mice deficient for single TLRs also display increased susceptibility to colitis, though never so dramatic as the susceptibility to AOM/DSS carcinogenesis observed in the *Myd88*$^{-/-}$ mice [36]. Supporting these findings, under AOM/DSS treatment, mice lacking IL-18 and IL-18 receptor (IL-18R) also display an increased susceptibility to CAC with a molecular profile similar to that observed in *Myd88*$^{-/-}$ mice since signaling through the IL-18R also requires MyD88, as most TLRs [13, 49]. However, other studies showed that IL-18 protects from colitis and carcinogenesis [50].

In *Nlrp6*$^{-/-}$ mice, the expansion of bacterial phyla *Bacteroidetes* (*Prevotellaceae*) and *TM7* in the fecal microbiota correlated with susceptibility to colitis [51], thus another innate system receptor of the inflammasome [13–15], NLRP6 (1 of 14 pyrin domain containing members of the NLR family out of PRRs), also regulates colonic microbial ecology.

In this sense, humans infected with *Helicobater pylori* are an example that an infection by a single bacterial species develops in gastric cancer. Even *Escherichia coli* is associated with an increased risk of colorectal carcinoma by downregulating mismatch-repair genes *in vitro* [52].

11.4 Obesity, Metabolic Syndrome, Cancer Cachexia, and Inflammation

Modern lifestyle and diet, although responsible for an overall improvement in health and life expectancy, have also brought a substantial worldwide increase in overweight (body-mass index (BMI) of 25–30 kg/m^2) or obese (BMI > 30 kg/m^2) individuals, affecting approximately half of the population in developed countries. Three recent studies [53–55] show that obesity, in addition to cancer susceptibility, may also affect survival after detection, in particular in endometrial, postmenopausal breast, and CRC cancers.

More than total BMI, visceral adipose tissue has been referred to as the metabolic syndrome [56]. Adipose tissue actively secretes adipokines and cytokines responsible for the induction of a pro-inflammatory, insulin-resistant, and procoagulant environment. These mediators are not only restricted to adipose tissue, but also affect inflammation and immune responsiveness, oxi-

dative metabolism and energy balance ubiquitously [57]. These factors altogether associated with excessive weight are the probable cause of the increased incidence of cancers associated with obesity [19].

This metabolic inflammatory state, also termed *metaflammation*, is a low-grade, chronic inflammation elicited not only by adipocytes, but also by the stromal and inflammatory cells of the tissue in response to excess nutrients and energy [58, 59]. The inflamed adipose tissue has resident cells, adipocytes, and fibroblasts, as well as infiltrating innate and immune cells such as lymphocytes and macrophages. The inflammatory response determines the type and class of the inflammatory infiltrate and does not resolve unless the nutrient levels are dramatically altered.

Excess of nutrients induce an increased production of mitochondrial ROS. ROS induces pro-inflammatory transcriptional factors such as NF-κB and activating protein-1 (AP-1) through the activation of their upstream kinases IκBα and JNK [60, 61]. Adipocytes also express TLR 2 and 4. In addition to the non-bacterial agonists of these receptors such as diet saturated fatty acid or lipopeptides, the commensal flora or their bacterial products such as LPS and lipopeptides may reach cells in adipose tissues and activate TLRs. Studies in humans and mice have confirmed that obesity is associated with several changes in the composition of the commensal flora. Overall, obese individuals exhibit reduced bacterial diversity associated with changes at the phylum level (an increase in Firmicutes and a corresponding decrease in Bacteroidetes) and an altered representation of bacterial genes and metabolic pathways that favor energy harvest [62–64].

The first pro-inflammatory cytokine found to be produced by the adipose tissue was TNF, which was identified in a search for a soluble factor able to suppress insulin signaling and to maintain type 2 diabetes [65]. Other cytokines identified in the adipose tissue include IL-6, IL-1β, IL-18, CCL2 (monocyte chemotactic protein-1, MCP-1), etc. [58]. Markers such as IL-6 and C-reactive protein (CRP) not only are present in the inflamed adipose tissue, but also are elevated in circulation. More restricted to the adipose tissues are adipokines such as leptin (involved in appetite control), adiponectin (an insulin sensitizer and anti-inflammatory mediator), as well as other molecules such as lipocalin 2 and resistin—produced by adipocytes and inflammatory cells—that have both pro-inflammatory and chemotactic functions and promote insulin resistance [66].

Another important link between inflammation and energy metabolism is the wasting syndrome, termed cancer cachexia, observed in most patients with advanced cancers of certain types, up to 80 % of patients with pancreas or other upper gastrointestinal cancers and 60 % of lung cancer patients, and in a proportion of patients with other chronic diseases, for example, kidney diseases, but it is less common in other cancers [67]. Weight loss in adults and growth failure in children are the main manifestations of cachexia, although anorexia, inflammation, insulin resistance, and muscle protein atrophy are also usually associated with this syndrome. It has a profound effect on patients' morbidity and mortality, with a decreased quality of life due to decreased physical activity, ability to interact socially, and perception of body image [3]. The pathology of cancer cachexia is more significantly associated with infection rather than malnutrition due to starvation. Association between systemic inflammation and cancer cachexia both in patients and in experimental animal models was confirmed by these evidences: circulating CRP levels correlate with the grade of cachexia in patients as well as with the risk of death, and cytokines such as TNF, IL-1β, and IL-6 are elevated either systemically or in the tumor microenvironment of cachectic patients [67]. These immune alterations are likely to alter the innate responses to pathogens and to the commensal flora, therefore modifying the inflammatory environment both in the tumor microenvironment and systemically, with the consequent feedback loop, affecting the energy metabolism.

11.5 CRC Prevention by Nonsteroidal Anti-inflammatory Drugs

Cyclooxygenases (COX)-1 and COX-2 enzymes catalyze the production of prostaglandins from fatty acids. The *COX1* gene is constitutively expressed; its product induces basal levels of prostaglandins and contributes to the homeostasis of the gastrointestinal mucosa. The inducible *COX2* gene is predominantly expressed in stromal cells such as fibroblasts and macrophages. When TNF or IL-1 regulates this gene in pro-inflammatory conditions, its product catalyzes the production of much higher concentrations of prostaglandins compared to COX-1 [68].

It was initially described that CRC highly expresses COX-2, whereas COX-1 is not overexpressed in tumoral tissues [69, 70]. Overexpression of COX-2 protein is observed in 80 % of CRCs and in 40 % of adenomas but not in the normal mucosa. In addition, COX-2 may have a prognostic value in human colorectal cancer [71]. Although a causative link between COX-2 expression and colon cancer was experimentally demonstrated by the reduced but not eliminated incidence of polyposis in *COX2*-deficient APC $^{min/+}$ mice [72], COX-2 was found upregulated at early stages of tumorigenesis in most tumor types.

Prostaglandin E2 (PGE2) directly affects innate and immune adaptive cells and also promotes inflammation, in part by dilating blood vessels and allowing immune cells to migrate from blood into tissues. PGE2 also regulates angiogenesis and enhances hematopoietic cell homing, directing progenitor cells to damaged tissue in order to differentiate into the many immune cell types needed for tissue repair. In carcinogenesis, the effects of COX-2 and prostaglandins are complex, affecting both transformed epithelial cells and the inflammatory microenvironment [68].

In summary, PGE2 (1) promotes epithelial cell proliferation through at least Ras-Erk signaling pathways and glycogen synthase kinase-3β [73, 74]; (2) promotes epithelial survival via the induction of bcl-2 and the activation of the PI3K-AKT-PPARδ cascade, proteins implicated in the apoptotic process [61]; (3) promotes angiogenesis by enhancing endothelial cell mobility and upregulating VEGF and fibroblast growth factor 2 (basic) (FGF2) production [75, 76]; (4) regulates tumor and inflammatory cell migration, affecting chemokine production and tissue rearrangement, in part mediated by activation of EGFR signaling [68].

In addition, although prostaglandins stimulate the immune response, PGE2 enhances IL-23 while inhibits IL-12 production by antigen presenting cells (APCs) facilitating tumor progression through the development of tumor-promoting Th17 responses rather than tumor-preventing Th1 responses [77].

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen inhibit both COX-1 and COX-2 and consistent with the research mentioned above, many studies showed that daily use of aspirin (after at least 5 years of aspirin use) and other NSAIDs for extended periods reduced the risk of colorectal cancer or polyp recurrence (both individuals with reoccurring polyps or genetic predisposition but also the risk for sporadic colon cancer), and also partially, 30 % lower, the 20-year risk of cancer death for all solid cancers (e.g., lung or brain) and, 60 % lower, gastrointestinal cancers (e.g., esophageal, pancreatic, stomach) (reviewed by Rothwell et al. [78]). These data came from trials originally planned for prevention of cardiovascular diseases in which patients took aspirin daily. However, these drugs are very toxic and induced a considerable damage to stomach and intestinal lining, or brain hemorrhage [79, 80].

The logical next step was to develop selective COX-2 inhibitors. Since these drugs only inhibit the inducible COX-2 enzyme, which is activated only during inflammation, they do not affect the gastrointestinal homeostasis and were expected to be much safer. In clinical trials, COX-2 inhibitors increased both overall and recurrence-free survival following surgical resection, particularly in the percentage of colorectal cancer patients who overexpressed *COX2* or had mutated forms of the gene [81, 82]. Interestingly, COX-2 inhibitors not only prevented cancer formation, but also decreased the number of already established

polyps in patients with familial adenomatous polyposis, an inherited disorder characterized by the early onset of colon cancer [83]. However, later clinical trials based on these promising results had to be discontinued due to cardiovascular and cerebrovascular toxicity of COX-2 inhibitors [84, 85]. It was discovered that the COX-2 substrate—arachidonic acid—shunted into the 5-lipooxygenase pathway, generating leukotrienes rather than prostaglandins, which suppresses prostacyclin production from the endothelium [79].

Overall, the data from all these clinical trials of cancer prevention using NSAIDs support that inflammation is an underlying cause of cancer even in some tumor types that had not been traditionally considered to originate within chronically inflamed tissues (like lung or prostate). As a consequence, other anti-inflammatory drugs targeting different inflammatory pathways, or in a different way, may play an important role in preventing the initiation and progression of both gastrointestinal and other solid organ cancers.

11.6 Cancer Microenvironment and Immunosuppression

Histopathological studies of solid tumors have demonstrated that the immune system of the host is activated by human colorectal cancer cells, and, both, cells of the innate immune system such as neutrophils [86], macrophages [87], natural killer (NK) cells [88] or DCs [89], and cells of the adaptative immune system such as $CD4^+$ helper and $CD8^+$ cytotoxic T lymphocytes [90, 91] accumulate in sites of tumor development.

The role of this complex microenvironment is less clear. Immune cells can release inflammatory mediators with proangiogenic and prometastasic effects [16]. At the same time, tumor-infiltrating lymphocytes in CRC have been shown to inhibit tumor growth and are associated with improved prognosis [91–93]. However, despite immune surveillance tumors develop in the presence of a completely functional immune system. The concept of cancer immunoediting arised to explain this apparent paradox [94], and try to make progress on new cancer immunotherapies. This concept has been divided into three phases, namely, elimination, equilibrium, and escape [95]. The elimination phase is what has been historically designated cancer immunosurveillance, whereby immune cells detect and eliminate transformed cells with failed intrinsic cell development suppression mechanisms. This elimination could be incomplete, in which case some tumor cells remain either dormant or continue to evolve accumulating further changes that can modulate the expression of tumor-associated antigens (TAAs) or other factors that increase their fitness. During this time, the immune system continues to exert a selective pressure eliminating some transformed clones but if this elimination is again incomplete, the process results in the selection of tumor cell variants that are able to resist, avoid, or suppress the antitumor response, leading to the escape phase [95]. Regarding CRC, it has been clearly demonstrated that these cancer cells are immunogenic [96] and that host immune responses can influence patient survival [97] and these data hold the promise of specifically targeting tumor cells, provided the mechanisms of immune escape and tumor-induced immune suppression are overcome.

It has been shown that CRC induces an immunosuppression state in patients marked by reduced secretion of several cytokines by monocytes/macrophages such as IFN-γ or TNF-α, which is reversible after resection of the affected tissue [98].

During an immune response, $CD4^+$ T lymphocytes can differentiate into two broad phenotypic subtypes: T helper 1 (Th1) or Th2. These two different subtypes secrete different types of cytokines, and consequently, activate different types of immune responses. Th1 lymphocytes secrete IFN-γ and TNF-α, which produce the activation of $CD8^+$ cytotoxic T lymphocytes (CTLs), NK cells, macrophages, and monocytes, all of which contribute to a cellular immune response that is effective against tumor cells. However, Th2 lymphocytes secrete a different set of cytokines such as IL-4, IL-5, IL-10, or IL-13, all of which deviate the response to a humoral immune response, and this kind of immune response is less effective

at eliminating cancer cells [99]. A shift toward a Th2 response has been shown in CRC patients, with reduced levels of Th1 cytokines and normal or elevated levels of Th2 cytokines, an imbalance that becomes more significant the further the disease progresses, with levels of the Th1 cytokines having a prognostic value in terms of patient survival [100–103]. The mechanism through which CRC cells can shift the T-cell immune response could be due in part to the secretion of cytokines that inhibit the development of Th1 responses, such as the transforming growth factor-β (TGF-β) and IL-10, by the CRC cells themselves or other cancer-associated cells such as fibroblasts [104].

TGF-β plays pivotal roles in wound healing, fibrosis, angiogenesis, carcinogenesis, cell differentiation, and immune responses [105, 106]. Patients with high TGF-β protein levels in their primary CRC site are more likely to experience tumor recurrence compared to patients with low levels [107]. TGF-β levels correlates with Duke's stage and plasma levels of active, too, and TGF-β return to normal levels after a curative resection [108]. Many epithelial-derived tumor cells become resistant to the growth-inhibitory effects of TGF-β, including CRC cells, due to mutations in the SMAD proteins or the TGF-β receptors [109]; on the contrary, TGF-β is able to stimulate the same cancer cells to proliferate [110].

In addition, TGF-β contributes to CRC development through immunosuppression, predominantly through effects on T lymphocytes and APCs: TGF-β inhibits the proliferation and differentiation of T lymphocytes preventing naïve T cells from acquiring effector functions [111], and inhibits the ability of tumor-infiltrating lymphocytes (TILs) to kill cancer cells as well as tumor-specific $CD8^+$ cytotoxic responses [112]. TGF-β on APCs, such as macrophages, inhibits secretion of TNF-α, IL-1α and IL-1β [113].

Although the role played by IL-10 in cancer development has been extensively studied, the ultimate role of this cytokine remains somehow controversial. The most controversial topic is the effect that this cytokine has on the immune response against tumor cells. As a result of its ability to inhibit key stages of the adaptative immune response, the mainstream idea is that IL-10 is an immunosuppressive molecule secreted by tumor cells as well as tumor-infiltrating immune cells, allowing transformed cells to escape immunosurveillance [114]. CRC tumor cell lines are able to secrete IL-10 [115] and are able to induce the secretion of this cytokine by other immune cells such as monocytes and lamina propria mononuclear cells [116]. Also, elevated levels of plasma IL-10 have been associated with bad prognosis and their levels return to normal in resected patients [117, 118]. This immunosuppressive effect of the IL-10 is mainly indirect and mediated by DCs and Treg lymphocytes (see below).

Dendritic cells are key APCs that play a central role in the induction of immune responses including antitumor responses [119, 120]. Immature DCs present self-antigens to both $CD4^+$ and $CD8^+$ T cells leading to tolerance of those lymphocytes [121]. On the contrary, activated and matured DCs loaded with antigens induce an antigen-specific response leading to T-cell proliferation and differentiation into helper and effector lymphocytes [122]. It has been shown that CRC patients have DCs infiltrating the tumor or the surrounding tissue forming clusters with T lymphocytes [123] and that this infiltration seems to correlate with a better prognosis [124]. However, the role played by DCs in CRC is controversial due to the fact that some studies have found that tumor cells are able to impair the function of these cells in inducing an effective immune response capable of eliminating transformed cells. In this direction, tissue culture media from CRC explants inhibit DC maturation with reduced levels of CD54, CD86, HLA-DR, and CD83, and induce IL-10 secretion while inhibiting secretion of IL-12p70, factors that inhibit Th1 immune responses and probably protect the tumor from a potent immune response [125]. Moreover, *in vivo* DCs infiltrating the tumor show an immature phenotype [126] and those immature DCs correlate with infiltration by Treg cells and with no detectable tumor-associated antigen systemic response [89]. However, as mentioned before, increased DCs tumor infiltration shows a positive correlation with survival in CRC.

A classical mechanism of transformed cells to avoid the host immune response is the combination of downregulation of key soluble or membrane-associated molecules implicated in the immune response and the upregulation of other molecules that actively inhibit a protective response. In this sense, it has been shown that alterations in the expression of class I human leukocyte antigens (HLA) is frequent in many cancers and related to cancer survival by enabling tumor cells to escape cytotoxic T-cell-mediated responses [127]. Activation of antitumor CTL responses requires the recognition of immunogenic epitopes presented on various types of HLA class I molecules on the tumor [128]. CRC tumors show high levels of HLA class I alterations due to multiple mechanisms such as mutations on the β2-microglobulin chain or defects in the peptide transporters associated with antigen processing [129, 130]. Although expression of HLA class I antigens is associated with poor prognosis in many cancers, there are conflicting results regarding CRC [131], probably indicating that NK cells are important effectors in the antitumor response [132] against CRC.

11.7 Infiltrating T Cells and Tumor-Associated Antigens

As previously mentioned, human CRC tissue is infiltrated by a variety of immune cells, often in the margins of the transformed tissue. This lymphocytic infiltration is antigen-specific and is an independent survival prognostic factor. Several studies have characterized the lymphocyte infiltration of CRC and confirmed the concept of prognostic impact of these TILs [90, 133]. In most cases, the lymphocytes infiltrating the cancer tissue, and most frequently the area along the invasive margin, are either $CD4^+$ and/or $CD8^+$ T cells [134]. Despite their low numbers, $CD8^+$ T lymphocytes infiltrating the neoplastic epithelium have been positively correlated with longer disease-free survival time [135, 136].

All these data suggest that TILs have antitumor activity and are activated by TAAs so the identification of those TAAs is essential for the development of an antigen-specific cancer vaccination directed to class I and class II peptides' epitopes. Many of the TAAs in CRC identified so far was done by the identification of autoantibodies present in the plasma of cancer patients compared to healthy donors, and although the clinical significance of those serologically defined antigens still have to be demonstrated, several are attractive candidates for cancer vaccines. Moreover, antibody responses against some TAAs correlate with $CD8^+$ responses in those patients [137, 138] supporting the idea that the immune response taking place in CRC patients requires coordinated $CD4^+$, $CD8^+$, and B-cell responses.

Some TAAs have been identified as potential targets of cytotoxic $CD8^+$ T lymphocyte responses. By detecting the secretion of IFN-γ by ELISPOT it has been demonstrated a natural response against peptides derived from the epithelial cell adhesion molecule (ep-CAM), her-2/*neu*, and the carcinoembryogenic antigen (CEA) in approximately one half of CRC patients with involvement of lymph nodes or distant metastases [139]. There are also cases where CD8+ T-cell responses against mutated normal antigens have been detected, Ags such as p21-Ras-derived peptide expressing a single amino acid mutation in a colon carcinoma patient [140]. There are many other studies where TAAs have been only identified serologically [141, 142] and the significance of those TAAs regarding their recognition by either T helper $CD4^+$ or cytotoxic T $CD8^+$ cells is still a matter of study.

11.8 Regulatory Cells and CRC

Nowadays, it has been clearly demonstrated that Treg cells characterized by the expression of CD25 and the transcription factor Foxp3 are critical for the prevention of autoimmunity and the regulation of immune responses to foreign and self antigens [143]. Tregs are often found at high frequencies in the peripheral blood and tumors of cancer patients, and for many of those human cancers high densities of such Tregs in the tumor correlates with poor disease outcome [144].

However, conflicting data have accumulated suggesting that high densities of Tregs in the tumor is not always associated with poor prognosis, but on the contrary, can be associated with a favorable survival of CRC patients [144, 145]. These results raise the question whether those tumor-infiltrating $Foxp3^+$ cells are really functional and very few studies have examined this question in human CRC, but when such analysis has been performed on $Foxp3^+$ cells sorted from CRC, it has been shown that those cells have the capacity to suppress T-cell proliferation and IFN-γ secretion [146]; even the $CD8^+Foxp3^+$ cells have this capacity [147]. A hypothesis has been put forward to explain this apparent contradiction indicating that those $Foxp3^+$ Tregs may infiltrate the tumor mass to suppress inflammation and immune responses resulting from the commensal microflora [148].

Alternatively, the presence of other regulatory populations may explain it, since the nature of the regulatory cell types, Tregs, natural killer T (NKT) cells, immature myeloid DCs, plasmacytoid DCs, or Bregs that dominate in any given tumor is not understood at present [149, 150]. Addressing this question for Tregs and also regulatory type I and II NKT cells in mice syngeneic models of colorectal and renal cancer, those mice with both type I and II NKT cells, or those mice with neither type of NKT cell, Treg depletion was sufficient to protect against tumor outgrowth, but in those mice lacking type I NKT cells only, Treg blockade was insufficient for protection, that is, type II NKT cells could suppress tumor immunity even when Tregs were blocked [150].

11.9 Immunotherapy for Colorectal Cancer

Summarizing, the immunosuppressive strategies mediated by tumor cells are as follows: (a) impairment of antigen presentation by APCs (DCs, macrophages, B cells), (b) activation of negative costimulatory signals, (c) elaboration of immunosuppressive network, and (d) recruitment of regulatory cell populations such as Tregs, NKT cells, etc. [149, 151]. Therefore, the countermeasures immunologists have thought to fight cancer are, apart from the possible preventive use of anti-inflammatory drugs (in preclinical stages) mentioned before, different procedures to activate the immune reactivity once damage has occurred in one or more of those branches.

11.9.1 Lymphodepletion and Adoptive Cell Transfer

To block immunosuppressive mechanisms by depletion of suppressive populations, early approaches used lymphodepleting chemotherapy before adaptive cell transfer of stimulated cells [152]. Although adoptive cell therapy (ACT) using tumor-specific T cells is a promising modality for the treatment of cancer [152, 153], generation of autologous tumor-specific T cells able to induce cancer regression in each individual patient is logistically and economically challenging. Redirection of T cells through an antibody-based chimeric antigen receptor (CAR) can potentially create "universal effector T cells" capable of recognizing targets independent of MHC restriction. Nonetheless, recent developments [153] to avoid the limits of this approach, the host-versus-graft (HVG) rejection, on the one hand, and the graft-versus-host (GVH) response, on the other, have not been tested in humans.

New approaches of depletion try to target specific immunosuppressive populations, having accumulated knowledge for the case of Tregs in many cancers [154], including CRC in patients with liver metastasis [155] or with high-level microsatellite-unstable (MSI-H) colorectal carcinomas, which represent a distinct subtype of tumors commonly characterized by dense infiltration with cytotoxic T lymphocyte [156]. In this case, depletion of Treg cells increased the frequency of effector T-cell responses specific for MUC1/CEA-derived peptides [156]. As mentioned, as $FoxP3^+$ T cells were associated with generally good prognosis in colorectal cancer [144, 145], may be this approach must be personalized in humans according to their specific regulatory cells network.

Interestingly, since it is related to the inflammatory origin of CRC, pepducin-mediated chemokine

receptor CXCR2 inhibition reduced tumorigenesis in $APC^{min/+}$ mice. As $Ly6G^+$ neutrophils are the dominant source of CXCR2 in blood, $Ly6G^+$ cell depletion purged CXCR2-dependent tumor-associated leukocytes, suppressing established skin tumor growth and colitis-associated tumorigenesis, and reducing $APC^{min/+}$ adenoma formation [157].

Also, it has been recently shown that bacteria-induced colon cancer is accompanied by differential accumulation of $IL\text{-}17^+IL\text{-}22^+$ colonic innate lymphoid cells (cILCs), which are phenotypically distinct from LTi and NK-22 cells [158]. Their depletion in mice with dysplastic inflammation blocks the development of invasive colon cancer. As $IL\text{-}22^+CD3^+$ and $IL\text{-}22^+CD3^-$ cells were detected in human CRC, this activity of IL-22 in the colon is a nonredundant mediator of the inflammatory cascade required for perpetuation of CRC [158]. This IL-22 axis may be then a novel therapeutic target in colon cancer.

11.9.2 Monoclonal Antibodies

Several tools are available to block negative costimulatory signals or the immunosuppressive network. In addition to COX inhibition already discussed, and indoleamine 2,3-dioxygenase (IDO) inhibition, monoclonal antibodies (mAbs) that block cytotoxic T-lymphocyte antigen-4 (CTLA-4) in T cells, TGF, IL-10, VEGF, galectin-1, and other signaling molecules [149] have been studied. During the past 15 years, FDA has approved some of these mAbs for cancer therapy [159]. mAbs currently approved by EMA [European Medicine Agency] for the treatment of colorectal cancer are cetuximab, panitumumab, and bevacizumab. Bevacizumab is an mAb that blocks the vascular endothelial growth factor, VEGF, as well as interferes with the tumor–stroma interaction, thereby indirectly inhibiting tumor growth. It is also currently employed for the therapy of breast, renal, and lung cancer [160–162].

In addition to activating the immune system against tumor cells (through blocking suppression in the case of bevacizumab), other mAbs have been designed to (1) inhibit cancer-cell-intrinsic signaling pathways, (2) bring toxins in the close proximity of cancer cells, or (3) interfere with the tumor–stroma interaction. Cetuximab and panitumumab are mAbs that directly inhibit tumor-cell autonomous pro-survival cascades through epidermal growth factor receptor, EGFR, blocking [163].

Other mAbs in phase I/II for colorectal cancer [159] are conatumumab and tigatuzumab (also known as AMG 655 and CS-1008, respectively), which target the TNF-related apoptosis-inducing ligand receptor 2 (TRAILR2), a member of the death receptor protein family also known as death receptor 5 (DR5). Both operate as agonists, activating TRAILR2 signaling and inducing the apoptotic demise of TRAILR2-expressing cancer (but not normal) cells. Although the exact mechanism of action is not yet known, conatumumab exhibited promising safety and efficacy profiles in preclinical tests and in initial phase I–II studies. Several other immunoconjugates carry radioactive isotopes: T84.66 and M5A mAbs specifically bind to the tumor-associated CEA, while TF2 is a bi-specific mAb that simultaneously targets CEA and a heterologous hapten peptide (IMP-288).

The immune system can be stimulated either directly (lymphocyte stimulation) or indirectly through the stimulation of APCs or other subsets. Preclinical models approaching the concept of vaccination [159, 164] also use immunostimulatory mAbs, that is, mAbs that facilitate the development of a tumor-specific immune response by targeting the cancer cell/immune system cross talk and the signaling pathways associated to that cross talk, for example, delivering tumor proteins (or protein epitopes) to DCs [159].

11.9.3 Vaccines

APCs, in particular DCs, have been used in the development of antitumor vaccines for a long time [164]. The concept of therapeutic vaccination is to generate an antitumor response in the body by injecting the Ag, or to boost the DCs *in vitro*, before adoptive cell transfer. However, inadequate DCs activation—the blockade of differentiation of mature DCs and accumulation of immature DCs and plasmacytoid DCs—is the

dominant mechanism underlying the development of T-cell tolerance; therefore, traditional Ag-based (such as CEA) vaccination may gain prolonged survival only in some cancer patients, but it fails to eradicate the tumor in most cases, owing partly to the immunosuppressive effects exerted by the tumor microenvironment [165]. Among the approaches to stimulate APCs, inhibition of suppressor mechanisms (silencing RNA or tyrosine kinases inhibitors (ITKs)), blocking CD40, and the use of cytokines, are included [149]. A classical approach to metastatic renal cell carcinoma used *in vitro* monocyte-derived DCs, autologous tumor lysate, and IL-2 plus IFNα2 [166], while a novel one approaching metastatic breast cancer abrogated Tregs with cyclophosphamide, used trastuzumab (an Ab that activates antibody-dependent cell-mediated cytotoxicity (ADCC) and inhibits growth factor VEGF at the same time), and fibroblasts transfected with plasmids expressing recombinant oncoantigen and cytokine (HER-2 and GM-CSF) to activate DCs *in vivo* [167]. This comparison clearly indicates the evolution of these approaches. In 2010, this branch of clinical research culminated with the FDA approval of a DC-based therapeutic vaccine (sipuleucel-T, Provenge®) for use in patients with asymptomatic or minimally symptomatic metastatic hormone-refractory prostate cancer.

Three types of strategies for DC-based anticancer vaccination exists: In the first, DCs are generated by culturing patient-derived hematopoietic progenitor cells or monocytes with specific cytokine combinations, loaded with TAAs *ex vivo* in the presence of an adjuvant or cytokines that promote DC maturation and are eventually reinfused into the patient, usually intradermally in combination with adjuvant. Among the several means to load the antigens tested are the coincubation of DCs with whole tumor cell lysates or with apoptotic tumor cell corpses, with purified TAAs (encompassing both full-length proteins and short peptides), with tumor cell-derived mRNA, with different transfection approaches, or by fusion of tumor and DCs. In the second strategy, TAAs are delivered to DCs *in vivo*, for example, with mAbs (as previously mentioned) [159] or DNA (plasmids or other vectors), which also requires the codelivery of DC maturation signals. The third class of DC-based immunotherapeutic interventions against cancer includes approaches based on DC-derived exosomes. DC-derived exosomes are fully capable of activating adaptive immune responses once loaded with TAAs and inoculated *in vivo* in suitable animal models [reviewed in 168].

Among the wide array of phase I/II clinical trials been launched to test the safety and efficacy of these therapeutic strategies in cancer patients [168], there are only two active phase II trials for CRC (references NCT01348256 and NCT01413295) using DCs pulsed with tumor cell lysates.

When generally talking about vaccines, the knowledge gained during several decades indicates that most antigens are not immunogenics per se and that several considerations should be made for a right formulation, such as the size, the dose, and the route of application or the use of adjuvants. The latter has been the dirty little secret of vaccines. Although the composition of the most used adjuvants is well known, their mechanism of action was unknown for a long time and its use was empiric. For example, the long-known adjuvant system 04 (AS04), which consists of lipid A and alum, is a component in two licensed prophylactic vaccines against cancer, Cervarix™ and Fendrix™, against human papillomavirus (HPV) and hepatitis B virus (HBV), respectively. As04 was approved in humans before it was realized to be a Toll-like receptors (TLR) ligand.

The importance of this discovery was highlighted 2 years ago with the Nobel Prize to Beutler and Hoffmann for the discovery of TLR and their role in the activation of innate immunity; and to Steinman for the discovery of DCs and their role in adaptive immunity. Out of PRRs families, already mentioned, TLRs are transmembrane proteins in the plasma membrane and endosomes mainly expressed by cells of the innate immune system, which serve as receptors for diverse ligands including bacterial, fungal, yeast, or viral components (PAMPs). These ligands activate signaling pathways that launch immune and

inflammatory responses to destroy the invaders. In humans, the TLR family includes 11 proteins (TLR1–TLR11). Most PRRs have the same signal transduction pathways but some of them, such as TLR4, have various signal transduction pathways. In addition, in many cases, such as TLR4, the receptor is a complex with different components participating in the binding event. Triggering of distinct TLRs on DCs and on other leukocyte populations that also express different TLR/PRR combinations, elicits different cytokine profiles and different immune responses [169].

Obviously, this fact led to an explosion of interest in the natural or synthetic agonists for TLRs, resembling PAMPs, and hundreds of clinical trials are currently being carried out for cancer [170]. When these molecules, PAMPs and DAMPs are used alone or as monotherapy, they are called cytokine-like or cytokines, whereas if they are used in combination with antigens, they are called adjuvants [14]. For colorectal cancer, most are used as adjuvants, for example, Poly-ICLC targets TLR-3 as an adjuvant to MUC1 peptide vaccination (reference NCT00773097 in phase II) or IMA910 (targets TLR-7/8) plus GM-CSF with cyclophosphamide (reference NCT00785122 in phase I/II). For TLR-9, out of four studies in phase I, one used a TLR9 agonist as an adjuvant of autologous tumor cells (reference NCT00780988), while the others used them directly or in therapeutic combinations: IMO-2055 versus cetuximab or FOLFIRI (reference NCT00719199), MGN-1703 alone (reference NCT01208194), or ISS1018 plus irinotecan plus cetuximab (reference NCT00403052).

Still in experimental settings, DAMPs are already been tested. High mobility group box 1 (HMGB1) is a DNA-binding nuclear protein actively released, following cytokine stimulation or other ways, as well as passively during cell death. This protein can bind different molecules, like IL-1, a chemokine, or LPS, molecules that have distinct receptors accordingly. On the contrary, different DAMPs can also bind the same receptor [171]. Some studies use plasmids expressing HMGB1 to improve DNA vaccines because they trigger the production of antibodies [172], and the generation of a B-cell response to tumors, knowing the success of many mAbs used in antitumor immunotherapy, can be relevant [169]. Others build recombinant fusion proteins of antigen and DAMP, immunizing with the plasmid plus different adjuvants depending on the type of response required [173].

11.10 Concluding Remarks

Nowadays, it is clear that tumor development and spread not only depends on the capacity of transformed cells to grow uncontrollably but also on the contribution of the immune system to intervene in the development of cancer cells. It is somehow controversial the role that the immune system plays in the development of CRC, since inflammation seems to contribute to the tumorigenesis. In fact, anti-inflammatory drugs prevent CRC. However, CRC is an example of the cross talk between the immune system, inflammation and cancer because of the specificity of the gut microflora in the primary niche of cancer. The increasing knowledge in this field is already used to develop new treatments based not only in monoclonal antibodies but also a diversity of therapeutic vaccines and the depletion of certain immunosuppressive populations, all capable of blocking and/or killing of CRC transformed cells.

References

1. Reedy J. Galen on cancer and related diseases. Clio Med. 1975;10:227–38.
2. Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med. 1986;315:1650–9.
3. Trinchieri G. Cancer and inflammation: an old intuition with rapidly evolving new concepts. Annu Rev Immunol. 2012;30:677–706.
4. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646–74.
5. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science. 2011;331:1565–70.
6. Bindea G, Mlecnik B, Fridman WH, Galon J. The prognostic impact of anti-cancer immune response: a novel classification of cancer patients. Semin Immunopathol. 2011;33:335–40.

7. Bissell MJ, Hines WC. Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression. Nat Med. 2011;17: 320–9.
8. Kuper H, Adami H-O, Trichopoulos D. Infections as a major preventable cause of human cancer. J Intern Med. 2000;248:171–83.
9. Wall BM, Dmochowski RR, Malecha M, et al. Inducible nitric oxide synthase in the bladder of spinal cord injured patients with a chronic indwelling urinary catheter. J Urol. 2001;165:1457–61.
10. Ameille J, Brochard P, Letourneux M, et al. Asbestos-related cancer risk in patients with asbestosis or pleural plaques. Rev Mal Respir. 2011;28:e11–7.
11. Rebours V, Boutron-Ruault MC, Jooste V, et al. Mortality rate and risk factors in patients with hereditary pancreatitis: uni- and multidimensional analyses. Am J Gastroenterol. 2009;104:2312–7.
12. Allavena P, Garlanda C, Borrello MG, et al. Pathways connecting inflammation and cancer. Curr Opin Genet Dev. 2008;18:3–10.
13. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell. 2010;140:805–20.
14. Marichal T, Ohata K, Bedoret D, et al. DNA released from dying host cells mediates aluminum adjuvant activity. Nat Med. 2011;17:996–1002.
15. Barbalat R, Ewald SE, Mouchess ML, Barton GM. Nucleic acid recognition by the innate immune system. Annu Rev Immunol. 2011;29:185–214.
16. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420:860–7.
17. Tsung K, Dolan JP, Tsung YL, Norton JA. Macrophages as effector cells in interleukin 12-induced T cell-dependent tumor rejection. Cancer Res. 2002;62:5069–75.
18. Shoppmann SF, Birner P, Stöckl J, et al. Tumor-associated macrophages express lymphatic endothelial growth factors and are related to peritumoral lymphangiogenesis. Am J Pathol. 2002;161: 947–56.
19. Triantafillidis JK, Nasioulas G, Kosmidis PA. Colorectal cancer and inflammatory bowel disease: epidemiology, risk factors, mechanisms of carcinogenesis and prevention strategies. Anticancer Res. 2009;29:2727–37.
20. Brandon JA, Jennings CD, Kaplan AM, Bryson JS. Murine syngeneic graft-versus-host disease is responsive to broad-spectrum antibiotic therapy. J Immunol. 2011;186:3726–34.
21. Wen L, Ley RE, Volchkov PY, et al. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature. 2008;455:1109–13.
22. Bercik P, Denou E, Collins J, et al. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology. 2011;141:599–609.
23. Collins SM, Bercik P. The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease. Gastroenterology. 2009;136:2003–14.
24. Schetter AJ, Heegaard NH, Harris CC. Inflammation and cancer: interweaving microRNA, free radical, cytokine and p53 pathways. Carcinogenesis. 2010;31:37–49.
25. Colotta F, Allavena P, Sica A, et al. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis. 2009;30:1073–81.
26. Hussain SP, Harris CC. Inflammation and cancer: an ancient link with novel potentials. Int J Cancer. 2007;121:2373–80.
27. Karin M, Greten FR. NF-κB: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol. 2005;5:749–59.
28. Ben-Neriah Y, Karin M. Inflammation meets cancer, with NF-κB as the matchmaker. Nat Immunol. 2011;12:715–23.
29. Rogler G, Brand K, Vogl D, et al. Nuclear factor kB is activated in macrophages and epithelial cells of inflamed intestinal mucosa. Gastroenterology. 1998;115:357–69.
30. Yu H, Kortylewski M, Pardoll D. Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment. Nat Rev Immunol. 2007;7:41–51.
31. Pedersen N, Duricova D, Elkjaer M, et al. Risk of extra-intestinal cancer in inflammatory bowel disease: meta-analysis of population-based cohort studies. Am J Gastroenterol. 2010;105:1480–7.
32. Bernstein CN, Blanchard JF, Rawsthorne P, Yu N. The prevalence of extraintestinal diseases in inflammatory bowel disease: a population-based study. Am J Gastroenterol. 2001;96:1116–22.
33. Adami J, Gabel H, Lindelof B, et al. Cancer risk following organ transplantation: a nationwide cohort study in Sweden. Br J Cancer. 2003;89:1221–7.
34. WCRF/AICR. Expert report: food, nutrition, physical activity and the prevention of cancer: a global perspective. Washington DC: American Institute for Cancer Research; 2007.
35. Saleh M, Trinchieri G. Innate immune mechanisms of colitis and colitis-associated colorectal cancer. Nat Rev Immunol. 2011;11:9–20.
36. Kamangar F, Dores GM, Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. J Clin Oncol. 2006;24:2137–50.
37. Tomatis L, editor. Cancer, causes, occurrence and control. Lyon: IARC Press; 1990.
38. Terzi'c J, Grivennikov S, Karin E, Karin M. Inflammation and colon cancer. Gastroenterology. 2010;138:2101–14.
39. Uronis JM, Muhlbauer M, Herfarth HH, et al. Modulation of the intestinal microbiota alters colitis-associated colorectal cancer susceptibility. PLoS One. 2009;4:e6026.
40. Yang L, Pei Z. Bacteria, inflammation, and colon cancer. World J Gastroenterol. 2006;12:6741–6.
41. Vannucci L, Stepankova R, Kozakova H, et al. Colorectal carcinogenesis in germ-free and

conventionally reared rats: different intestinal environments affect the systemic immunity. Int J Oncol. 2008;32:609–17.
42. Garrett WS, Punit S, Gallini CA, et al. Colitis-associated colorectal cancer driven by T-bet deficiency in dendritic cells. Cancer Cell. 2009;16:208–19.
43. Garrett WS, Gallini CA, Yatsunenko T, et al. Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. Cell Host Microbe. 2010;8:292–300.
44. Wu S, Rhee KJ, Albesiano E, et al. A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. Nat Med. 2009;15:1016–22.
45. Sears CL. Enterotoxigenic bacteroides fragilis: a rogue among symbiotes. Clin Microbiol Rev. 2009;22:349–69.
46. Rao VP, Poutahidis T, Ge Z, et al. Innate immune inflammatory response against enteric bacteria Helicobacter hepaticus induces mammary adenocarcinoma in mice. Cancer Res. 2006;66:7395–400.
47. Fox JG, Feng Y, Theve EJ, et al. Gut microbes define liver cancer risk in mice exposed to chemical and viral transgenic hepatocarcinogens. Gut. 2010;59:88–97.
48. Rakoff-Nahoum S, Medzhitov R. Regulation of spontaneous intestinal tumorigenesis through the adaptor protein MyD88. Science. 2007;317:124–7.
49. Salcedo R, Worschech A, Cardone M, et al. MyD88-mediated signaling prevents development of adenocarcinomas of the colon: role of interleukin 18. J Exp Med. 2010;207:1625–36.
50. Allen IC, TeKippe EM, Woodford RM, et al. The NLRP3 inflammasome functions as a negative regulator of tumorigenesis during colitis-associated cancer. J Exp Med. 2010;207:1045–56.
51. Elinav E, Strowig T, Kau AL, et al. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. Cell. 2011;145:745–57.
52. Maddocks OD, Short AJ, Donnenberg MS, et al. Attaching and effacing Escherichia coli downregulate DNA mismatch repair protein in vitro and are associated with colorectal adenocarcinomas in humans. PLoS One. 2009;4:e5517.
53. Renehan AG, Tyson M, Egger M, et al. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet. 2008;371:569–78.
54. Renehan AG, Soerjomataram I, Tyson M, et al. Incident cancer burden attributable to excess body mass index in 30 European countries. Int J Cancer. 2010;126:692–702.
55. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of US adults. N Engl J Med. 2003;348:1625–38.
56. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. Nature. 2006;444:881–7.
57. Renehan AG, Roberts DL, Dive C. Obesity and cancer: pathophysiological and biological mechanisms. Arch Physiol Biochem. 2008;114:71–83.
58. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. Annu Rev Immunol. 2011;29:415–45.
59. Wellen KE, Thompson CB. Cellular metabolic stress: considering how cells respond to nutrient excess. Mol Cell. 2010;40:323–32.
60. Kamp DW, Shacter E, Weitzman SA. Chronic inflammation and cancer: the role of the mitochondria. Oncology. 2011;25:400–10.
61. Naik E, Dixit VM. Mitochondrial reactive oxygen species drive proinflammatory cytokine production. J Exp Med. 2011;208:417–20.
62. Turnbaugh PJ, Ley RE, Mahowald MA, et al. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444:1027–31.
63. Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. Nature. 2009;457:480–4.
64. Turnbaugh PJ, Ridaura VK, Faith JJ, et al. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Sci Transl Med. 2009;1:6ra14.
65. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. Science. 1993;259:87–91.
66. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol. 2011;11:85–97.
67. Donohoe CL, Ryan AM, Reynolds JV. Cancer cachexia: mechanisms and clinical implications. Gastroenterol Res Pract. 2011:601434.
68. Wang D, Dubois RN. Eicosanoids and cancer. Nat Rev Cancer. 2010;10:181–93.
69. Eberhart CE, Coffey RJ, Radhika A, et al. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology. 1994;107:1183–8.
70. Sinicrope FA, Lemoine M, Xi L, et al. Reduced expression of cyclooxygenase 2 proteins in hereditary nonpolyposis colorectal cancers relative to sporadic cancers. Gastroenterology. 1999;117:350–8.
71. Sheehan KM, Sheahan K, O'Donoghue DP, et al. The relationship between cyclooxygenase-2 expression and colorectal cancer. JAMA. 1999;282:1254–7.
72. Oshima M, Dinchuk JE, Kargman SL, et al. Suppression of intestinal polyposis in ApcΔ716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell. 1996;87:803–9.
73. Castellone MD, Teramoto H, Williams BO, et al. Prostaglandin E2 promotes colon cancer cell growth through a Gs-axin-β-catenin signaling axis. Science. 2005;310:1504–10.
74. Wang D, Buchanan FG, Wang H. Prostaglandin E2 enhances intestinal adenoma growth via activation of the Ras-mitogen-activated protein kinase cascade. Cancer Res. 2005;65:1822–9.
75. Salcedo R, Zhang X, Young HA, et al. Angiogenic effects of prostaglandin E2 are mediated by up-regulation of CXCR4 on human microvascular endothelial cells. Blood. 2003;102:1966–77.

76. Pai R, Szabo IL, Soreghan BA, et al. PGE(2) stimulates VEGF expression in endothelial cells viaERK2/JNK1 signaling pathways. Biochem Biophys Res Commun. 2001;86:923–8.
77. Sheibanie AF, Yen JH, Khayrullina T, et al. The proinflammatory effect of prostaglandin E2 in experimental inflammatory bowel disease is mediated through the IL-23–IL-17 axis. J Immunol. 2007;178:8138–47.
78. Rothwell PM, Fowkes FG, Belch JF, et al. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. Lancet. 2011;377:31–41.
79. McAdam BF, Catella-Lawson F, Mardini IA, et al. Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: the human pharmacology of a selective inhibitor of COX-2. Proc Natl Acad Sci U S A. 1999;96:272–7.
80. Konstantinopoulos PA, Lehmann DF. The cardiovascular toxicity of selective and nonselective cyclooxygenase inhibitors: comparisons, contrasts, and aspirin confounding. J Clin Pharmacol. 2005;45:742–50.
81. Arber N, Eagle CJ, Spicak J, et al. Celecoxib for the prevention of colorectal adenomatous polyps. N Engl J Med. 2006;355:885–95.
82. Bertagnolli MM, Eagle CJ, Zauber AG, et al. Celecoxib for the prevention of sporadic colorectal adenomas. N Engl J Med. 2006;355:873–84.
83. Rostom A, Dube C, Lewin G, et al. Nonsteroidal anti-inflammatory drugs and cyclooxygenase-2 inhibitors for primary prevention of colorectal cancer: a systematic review prepared for the US Preventive Services Task Force. Ann Intern Med. 2007;146:376–89.
84. Menter DG, Schilsky RL, DuBois RN. Cyclooxygenase-2 and cancer treatment: understanding the risk should be worth the reward. Clin Cancer Res. 2010;16:1384–90.
85. Bertagnolli MM, Eagle CJ, Zauber AG, et al. Five-year efficacy and safety analysis of the Adenoma Prevention with Celecoxib Trial. Cancer Prev Res. 2009;2:310–21.
86. Rao H-L, Chen J-W, Li M, et al. Increased intratumoral neutrophil in colorectal carcinomas correlates closely with malignant phenotype and predicts patients' adverse prognosis. PLoS One. 2012;7:e30806.
87. Algars A, Irjala H, Vaittinen S, et al. Typ and location of tumor-infiltrating macrophages and lymphatic vessels predict survival of colorectal cancer patients. Int J Cancer. 2012;131:864–73.
88. Papanikolaou IS, Lazaris AC, Apostolopoulos P, et al. Tissue detection of natural killer cells in colorectal adenocarcinoma. BMC Gastroenterol. 2004;4:20.
89. Nagorsen D, Voigt S, Berg E, et al. Tumor-infiltrating macrophages and dendritic cells in human colorectal cancer: relation to local regulatory T cells, systemic T-cell response against tumor-associated antigens and survival. J Transl Med. 2007;5:62.
90. Koch M, Beckhove P, op den Winkel J, et al. Tumor infiltrating T lymphocytes in colorectal cancer. Tumor-selective activation and cytotoxic activity in situ. Ann Surg. 2006;244:986–93.
91. Naito Y, Saito K, Shiiba K, et al. $CD8^+$ T cells infiltrated within cancer nests as a prognostic factor in human colorectal cancer. Cancer Res. 1998;58:3491–4.
92. Pagès F, Berger A, Camus M, et al. Effector memory T cells, early metastasis, and survival in colorectal cancer. N Engl J Med. 2005;353:2654–66.
93. Diederichsen AC, Hjelmborg JB, Christensen PB, et al. Prognostic value of the CD4+/CD8+ ratio of tumour infiltrating lymphocytes in colorectal cancer and HLA-DR expression on tumour cells. Cancer Immunol Immunother. 2003;52:423–8.
94. Dunn GP, Bruce AT, Ikeda H, et al. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3:991–8.
95. Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. Ann Rev Immunol. 2004;22:329–60.
96. Banerjea A, Bustin SA, Dorudi S. The immunogenicity of colorectal cancers with high- degree microsatellite instability. World J Surg Oncol. 2005;3:26.
97. Ropponen KM, Eskelinen MJ, Lipponen PK, et al. Prognostic value of tumour-infiltrating lymphocytes (TILs) in colorectal cancer. J Pathol. 1997;182:318–24.
98. Heriot AG, Marriott JB, Cookson S, et al. Reduction in cytokine production in colorectal cancer patients: association with stage and reversal by resection. Br J Cancer. 2000;82:1009–12.
99. Kobayashi M, Kobayashi H, Pollard RB, Suzuki F. A pathogenic role of Th2 cells and their cytokine products on the pulmonary metastasis of murine B16 melanoma. J Immunol. 1998;160:5869–73.
100. Tosolini M, Kirilovsky A, Mlecnik B, et al. Clinical impact of different classes of infiltrating T cytotoxic and helper cells (Th1, Th2, Treg, Th17) in patients with colorectal cancer. Cancer Res. 2011;71:1263–71.
101. O'Hara RJ, Greenman J, MacDonald AW, et al. Advanced colorectal cancer is associated with impaired interleukin 12 and enhanced interleukin 10 production. Clin Cancer Res. 1998;4:1943–8.
102. Pellegrini P, Berghella AM, Del Beato T, et al. Disregulation of Th1 and Th2 subsets of CD4+ T cells in peripheral blood of colorectal cancer patients and involvement in cancer establishment and progression. Cancer Immunol Immunother. 1996;42:1–8.
103. Lahm H, Schindel M, Frikart L, et al. Selective suppression of cytokine secretion in whole blood cell cultures of patients with colorectal cancer. Br J Cancer. 1998;78:1018–23.
104. Hawinkels LJAC, Paauwe M, Verspaget HW, et al. Interaction with colon cancer cells hyperactivates TGF-β signaling in cancer-associated fibroblasts. Oncogene. 2014;33(1):97–107.

105. Massagué J. The transforming growth factor-beta family. Annu Rev Cell Biol. 1990;6:597–641.
106. Mo L, Wan YY, Sanjabi S, et al. Transforming growth factor-beta regulation of immune responses. Annu Rev Immunol. 2006;24:99–146.
107. Friedman E, Gold LI, Klimstra D, et al. High levels of transforming growth factor beta 1 correlate with disease progression in human colon cancer. Cancer Epidemiol Biomarkers Prev. 1995;4:549–54.
108. Narai S, Watanabe M, Hasegawa H, et al. Significance of transforming growth factor β1 as a new tumor marker for colorectal cancer. Int J Cancer. 2002;97:508–11.
109. Grady WM, Myeroff LL, Swindler SE, et al. Mutational inactivation of transforming growth factor b receptor type II in microsatellite stable colon cancers. Cancer Res. 1999;59:320–4.
110. Yan Z, Deng X, Friedman E. Oncogenic Ki-ras confers a more aggressive colon cancer phenotype through modification of transforming growth factor-β receptor III. J Biol Chem. 2001;276:1555–63.
111. Gorelik L, Flavell RA. Transforming growth factor-β in T-cell biology. Nat Rev Immunol. 2002;2:46–53.
112. Hsiao YW, Liao KW, Hung SW, Chu RM. Tumor-infiltrating lymphocyte secretion of IL-6 antagonizes tumor-derived TGF-beta 1 and restores the lymphokine-activated killing activity. J Immunol. 2004;172:1508–14.
113. Bogdan C, Paik J, Vodovotz Y, Nathan C. Contrasting mechanisms for suppression of macrophage cytokine release by transforming growth factor-beta and interleukin-10. J Biol Chem. 1992;267:23301–8.
114. Mapara MY, Sykes M. Tolerance and cancer: mechanisms of tumor evasion and strategies for breaking tolerance. J Clin Oncol. 2004;22:1136–51.
115. Gastl GA, Abrams JS, Nanus DM, et al. Interleukin-10 production by human carcinoma cell lines and its relationship to interleukin-6 expression. Int J Cancer. 1993;55:96–101.
116. Kucharzik T, Lügering N, Winde G, et al. Colon carcinoma cell line stimulate monocytes and lamina propria mononuclear cells to produce IL-10. Clin Exp Immunol. 1997;110:296–302.
117. Asadullah K, Sterry W, Volk HD. Interleukin-10 therapy – review of a new approach. Pharmacol Rev. 2003;55:241–69.
118. Galizia G, Orditura M, Romano C, et al. Prognostic significance of circulating IL-10 and IL-6 serum levels in colon cancer patients undergoing surgery. Clin Immunol. 2002;102:169–78.
119. Palucka K, Ueno H, Roberts L. Dendritic cells: are they relevant? Cancer J. 2010;16:318–24.
120. Fong L, Engleman EG. Dendritic cells in cancer immunotherapy. Annu Rev Immunol. 2000;18:245–73.
121. Steinman RM, Hawiger D, Nussenzweing MC. Tolerogenic dendritic cells. Annu Rev Immunol. 2003;21:685–711.
122. Banchereau J, Briere F, Caux C, et al. Immunobiology of dendritic cells. Annu Rev Immunol. 2000;18:767–811.
123. Suzuki A, Masuda A, Nagata H, et al. Mature dendritic cells make clusters with T cells in the invasive margin of colrectal carcinoma. J Pathol. 2002;196:37–43.
124. Dadabayev AR, Sandel MH, Menon AG, et al. Dendritic cells in colorectal cancer correlate with other tumor-infiltrating immune cells. Cancer Immunol Immunther. 2004;53:978–86.
125. Michielsen AJ, Hogan AE, Marry J, et al. Tumor tissue microenvironment can inhibit dendritic cell maturation in colorectal cancer. PLoS One. 2011;6:e27944.
126. Schwaab T, Weiss JE, Schned AR, Barth Jr RJ. Dendritic cell infiltration in colon cancer. J Immunother. 2001;24:130–7.
127. Hicklin DJD, Marincola FM, Ferrone S. HLA class I antigen downregulation in human cancers: T-cell immunotherapy revives an old story. Mol Med Today. 1999;5:178–86.
128. Nagorsen D, Thiel E. HLA typing for peptide-based anti-cancer vaccine. Cancer Immunol Immunother. 2008;57:1903–10.
129. Browning M, Petronzelli F, Bicknell D, et al. Mechanisms of loss of HLA class I expression on colorectal tumor cells. Tissue Antigens. 1996;47:364–71.
130. Kaklamanis L, Townsend A, Doussis-Anagnostopoulou IA, et al. Loss of major histocompatibility complex-encoded transporter associated with antigen presenting (TAP) in colorectal cancer. Am J Pathol. 1994;145:505–9.
131. Menon AG, Morreau H, Tollenaar RAEM, et al. Down-regulation of HLA-A expression correlates with a better prognosis in colorectal cancer patients. Lab Invest. 2002;82:1725–33.
132. Koda K, Saito N, Takiguchi N, et al. Preoperative natural killer cell activity: correlation with distant metastases in curatively research colorectal carcinomas. Int Surg. 1997;82:190–3.
133. Ogino S, Nosho K, Irahara N, et al. Lymphocytic reaction to colorectal cancer is associated with longer survival, independent of lymph node count, microsatellite instability, and CpG island methylator phenotype. Clin Cancer Res. 2009;15:6412–20.
134. Menon AG, Janssen-van Rihijn CM, Morreau H, et al. Immune system and prognosis in colorectal cancer: a detailed immunohistochemical analysis. Lab Invest. 2004;84:493–501.
135. Chiba T, Ohtani H, Mizoi T, et al. Intraepithelial $CD8^+$ T-cell-count becomes a prognostic factor after a longer follow-up period in human colorectal carcinoma: possible association with suppression of micrometastasis. Br J Cancer. 2004;91:1711–7.
136. Deschoolmeester V, Baay M, Van Marck E, et al. Tumor infiltrating lymphocytes: an intriguing player in the survival of colorectal cancer patients. BMC Immunol. 2010;11:19.
137. Jäger E, Nagata Y, Gnjatic S, et al. Monitoring CD8 T cell responses to NY-ESO-1: correlation of

humoral and cellular immune responses. Proc Natl Acad Sci U S A. 2000;97:4760–5.
138. Jäger E, Gnjatic S, Nagata Y, et al. Induction of primary NY-ESO-1 immunity: CD8+ T lymphocyte and antibody responses in peptide-vaccinated patients with NY-ESO-1+ cancers. Proc Natl Acad Sci U S A. 2000;97:12198–203.
139. Nagorsen D, Keilholz U, Rivoltini L, et al. Natural T-cell response against MHC class I epitopes of epithelial cell adhesion molecule, her-2/neu, and carcioembryonic antigen in patients with colorectal cancer. Cancer Res. 2000;60:4850–4.
140. Fossum B, Olsen AC, Thorsby E, Gaudernack G. CD8 T cells from a patient with colon carcinoma, specific for a mutant p21-Ras-derived peptide (GLY^{13}→ASP), are cytotoxic towards a carcinoma cell line harbouring the same mutation. Cancer Immunol Immunother. 1995;40:165–72.
141. Somers VA, Brandwijk RJ, Joosten B, et al. A panel of candidate tumor antigens in colorectal cancer revealed by the serological selection of a phage displayed cDNA expression library. J Immunol. 2002;169:2772–80.
142. Line A, Slucka Z, Stengrevics A, et al. Characterisation of tumor-associated antigens in colon cancer. Cancer Immunol Immunother. 2002;51:574–82.
143. Josefowicz SZ, Lu L-F, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol. 2012;30:531–64.
144. de Leeuw RJ, Kost SE, Kakal JA, Nelson BH. The prognostic value of $FoxP3^+$ tumor-infiltrating lymphocytes in cancer: a critical review of the literature. Clin Cancer Res. 2012;18:3022–9.
145. Ladoire S, Martin F, Ghiringhelli F. Prognostic role of FoxP3+ regulatory T cells infiltrating human carcinomas: the paradox of colorectal cancer. Cancer Immunol Immunother. 2011;60:909–18.
146. Kryczek I, Liu R, Wang G, et al. FOXP3 defines regulatory T cells in human tumor and autoimmune disease. Cancer Res. 2009;69:3995–4000.
147. Chaput N, Louafi S, Bardier A, et al. Identification of $CD8^+CD25^+Foxp3^+$ suppressive T cells in colorectal cancer. Gut. 2009;58:520–9.
148. Erdman SE, Poutahidis T, Tomczak M, et al. CD4+CD25+ regulatory T lymphocytes inhibit microbially induced colon cancer in Rag2-deficient mice. Am J Pathol. 2003;162:691–702.
149. Rabinovich GA, Gabrilovich D, Sotomayor EM. Immunosuppressive strategies that are mediated by tumor cells. Annu Rev Immunol. 2007;25:267–96.
150. Izhak L, Ambrosino E, Kato S, et al. Delicate balance among three types of T cells in concurrent regulation of tumor immunity. Cancer Res. 2013;73:1514–23.
151. Cavallo F, De Giovanni C, Nanni P, et al. The immune hallmarks of cancer. Cancer Immunol Immunother. 2011;60:319–26.
152. Gattinoni L, Powell Jr DJ, Rosenberg SA, Restifo NP. Adoptive immunotherapy for cancer: building on success. Nat Rev Immunol. 2006;6:383–93.
153. Marcus A, Waks T, Eshhar Z. Redirected tumor-specific allogeneic T cells for universal treatment of cancer. Blood. 2011;118:975–83.
154. Hindley JP, Jones E, Smart K, et al. T-cell trafficking facilitated by high endothelial venules is required for tumor control after regulatory T-cell depletion. Cancer Res. 2012;72:5473–82.
155. Brudvik KW, Henjum K, Aandahl EM, et al. Regulatory T-cell-mediated inhibition of antitumor immune responses is associated with clinical outcome in patients with liver metastasis from colorectal cancer. Cancer Immunol Immunother. 2012;61:1045–53.
156. Bauer K, Nelius N, Reuschenbach M, et al. T cell responses against microsatellite instability-induced frameshift peptides and influence of regulatory T cells in colorectal cancer. Cancer Immunol Immunother. 2013;62:27–37.
157. Jamieson T, Clarke M, Steele CW. Inhibition of CXCR2 profoundly suppresses inflammation-driven and spontaneous tumorigenesis. J Clin Invest. 2012;122:3127–44.
158. Kirchberger S, Royston DJ, Boulard O, et al. Innate lymphoid cells sustain colon cancer through production of interleukin-22 in a mouse model. J Exp Med. 2013;210(5):917–31.
159. Galluzzi L, Vacchelli E, Fridman WH, et al. Trial watch: monoclonal antibodies in cancer therapy. Oncoimmunology. 2012;1:28–37.
160. Hurvitz SA, Allen HJ, Moroose RL, et al. A phase II trial of docetaxel with bevacizumab as first-line therapy for HER2- negative metastatic breast cancer (TORI B01). Clin Breast Cancer. 2010;10:307–12.
161. Miller K, Wang M, Gralow J, et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med. 2007;357:2666–76.
162. Sandler A, Gray R, Perry MC, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med. 2006;355:2542–50.
163. Weiner LM, Belldegrun AS, Crawford J, et al. Dose and schedule study of panitumumab monotherapy in patients with advanced solid malignancies. Clin Cancer Res. 2008;14:502–8.
164. Tacken PJ, de Vries IJ, Torensma R, Figdor CG. Dendritic-cell immunotherapy: from ex vivo loading to in vivo targeting. Nat Rev Immunol. 2007;7:790–802.
165. Turriziani M, Fantini M, Benvenuto M, et al. Carcinoembryonic antigen (CEA)-based cancer vaccines: recent patents and antitumor effects from experimental models to clinical trials. Recent Pat Anticancer Drug Discov. 2012;7:265–96.
166. Schwaab T, Schwarzer A, Wolf B, et al. Clinical and immunologic effects of intranodal autologous tumor lysate-dendritic cell vaccine with Aldesleukin (Interleukin 2) and IFN-alpha-2a therapy in metastatic renal cell carcinoma patients. Clin Cancer Res. 2009;15:4986–99.

167. Emens LA. Re-purposing cancer therapeutics for breast cancer immunotherapy. Cancer Immunol Immunother. 2012;61:1299–305.
168. Galluzzi L, Senovilla L, Vacchelli E, et al. Trial watch: dendritic cell-based interventions for cancer therapy. Oncoimmunology. 2012;1:1111–34.
169. Pulendran B, Ahmed R. Translating innate immunity into immunological memory: implications for vaccine development. Cell. 2006;124:849–63.
170. Goutagny N, Estornes Y, Hasan U. Targeting pattern recognition receptors in cancer immunotherapy. Target Oncol. 2012;7:29–54.
171. Sims GP, Rowe DC, Rietdijk ST, et al. 1HMGB1 and RAGE in inflammation and cancer. Annu Rev Immunol. 2010;28:367–88.
172. Fagone P, Shedlock DJ, Bao H, et al. Molecular adjuvant HMGB1 enhances anti-influenza immunity during DNA vaccination. Gene Ther. 2011;18:1070–7.
173. Mansilla C, Berraondo P, Durantez M, et al. Eradication of large tumors expressing human papillomavirus E7 protein by therapeutic vaccination with E7 fused to the extra domain a from fibronectin. Int J Cancer. 2012;131:641–51.

Immunotherapy of Pancreatic Cancer

12

Ryan Stevenson, Martin Goodman, and Muhammad Wasif Saif

Contents

R. Stevenson, MD (✉)
Department of Hematology/Oncology, Tufts University School of Medicine, Boston, MA 02111, USA
e-mail: rstevenson@tuftsmedicalcenter.org

M. Goodman, MD
Department of Hematology/Oncology, Tufts University School of Medicine, Boston, MA 02111, USA

M.W. Saif, MD, MBBS
Department of GI Oncology, Tufts University School of Medicine, Boston, MA 02111, USA
e-mail: wsaif@tuftsmedicalcenter.org

12.1 Introduction

Patients with pancreatic cancer have a very poor prognosis with it being the fourth leading cause of cancer-related death in men and women in the USA [1]. In developing countries, there were an estimated 165,100 new cases and 161,800 estimated deaths in 2011 [2]. In 2010, in the USA, 36,800 deaths were attributed to pancreatic cancer and a 5-year survival <5 % [1].

The management of patients with pancreatic cancer depends on the extent of disease at diagnosis. Surgical resection with negative margins with no lymph node involvement is the only chance for cure. The use of adjuvant chemotherapy improved survival in early-stage pancreatic cancer. The majority of patients present with locally advanced unresectable disease or distant disease, most commonly to the liver or peritoneal surface. Survival for metastatic pancreatic cancer remains poor and less than 20 % survive at the end of 1 year. There are only few chemotherapy agents that have shown an effect in pancreatic cancer including single agent gemcitabine, nab-paclitaxel with gemcitabine, and a new combination of 5-fluorouracil, leucovorin, oxaliplatin, and irinotecan (FOLFIRINOX) [3, 4, 113]. Chemoradiation has shown some benefit in locally advanced unresectable pancreatic cancers; however, it is minimal. Survival of patients with unresectable disease with these modalities is marginal, which warrants further investigation of other therapies. Immunotherapy might be an alternative treatment modality to this deadly disease.

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_12,

12.2 Evidence that Pancreatic Adenocarcinoma Elicits Immune Response

Immune-based therapy for pancreatic cancer has gained attention in every decade and, as such, generates short-lived enthusiasm. Pancreatic cancer is characterized by a highly immunosuppressive environment, with multiple components and pathways that inhibit effective pancreatic cancer-targeted immune responses. Therefore, there is great potential to target these mechanisms of immunosuppression and reverse them to create an environment that supports the infiltration of antitumor immune responses and enables the generation of T cells capable of killing pancreatic tumor cells. Each of these components and pathways represents a potential target for pancreatic cancer immunotherapy based on the supposition that the immune system can discriminate tumor cells from normal cells [5]. The data suggest that cancer patients generate B and T cells that recognize antigens expressed on autologous pancreatic tumor cells [6–12]. In addition, the animal models showed that mice deficient in genes associated with immunity (e.g., *IFN* [13] and *perforin* [14]) are susceptible to cancer development. Moreover, the analysis of immune infiltrates in human tumors has revealed a strong association between prognosis and the presence of a humoral response to pancreatic tumor antigens, such as MUC-1 and mesothelin, and of tumor-infiltrating cytotoxic T lymphocyte and Th1 cells [11, 12, 15, 16]. On the other hand, in a mouse model in which an activating K-Ras mutation is expressed in the pancreas, preinvasive pancreatic lesions are characterized by the infiltration of immune suppressor cells rather than immune effector cells, suggesting that tumor immunity may be blocked from the inception of pancreatic cancer development [17]. All mice with the K-Ras mutation develop pancreatic adenocarcinoma and eventually die of cancer. Another finding that antagonism of negative T-cell regulators, such as cytotoxic T-lymphocyte-associated (CTLA) protein-4 and B- and T-lymphocyte attenuator (BTLA) can augment the antitumor immune response further confirms that patients produce an immune response to the tumor [18, 19].

Despite the presence of the above data that underlines the fact that an antitumor immune response is elicited in cancer patients, unfortunately this response is ineffective and does not result in the killing of the tumor. Given that most tumor antigens are self- or mutated self-antigens and that the pancreatic tumor microenvironment is immunosuppressive, this is not surprising [20]. Interestingly, both the prevalence of Treg in peripheral blood and tumor, and the expression level of programmed cell death ligand 1 (PD-L1) in tumor independently predict a poor survival in pancreatic cancer [21, 22]. Tregs that constitute 5–10 % of $CD4^+$ T cells induce immune tolerance by suppressing host immune responses against self- and nonself-antigens [23–28], hence playing a crucial role in tolerance and the immune response to cancer. These findings strengthen the notion that pancreatic cancers induce antitumor immune responses. Therefore, attempts to improve the clinical efficacy of immunotherapy should involve strategies to neutralize or overcome immune suppression.

12.3 Cellular Targets in Pancreatic Cancer

The expression of an antigen – either mutated or unaltered self – must be restricted to the tumor or only minimally expressed elsewhere in the body to be considered an ideal tumor vaccine candidate. Table 12.1 enumerates a limited list of tumor antigens that fulfill this criterion for pancreatic cancer.

12.4 Immunotherapies in Pancreatic Cancer

Both active and passive immunity have been tested in pancreatic cancer (PC) to elicit immune responses to tumor cells. Targeting active immunity through vaccines attempts to induce long-term cellular (T-cell) immunity against cancer cells, whereas antibody-based immunotherapy targets PC cells, but does not stimulate long-term immunity. Recent active and passive immunotherapies in PC will be discussed in this section.

Table 12.1 Candidate pancreas cancer-associated antigens for immune targeting [29–52]

Antigen	Location	Expression in tumor	Prevalence (%)	Description
CEA	Cell surface (GPI-linked)	Overexpressed	30–100	Glycoprotein, normally expressed only on oncofetal tissues. Functions as cell-adhesion molecule. First tumor antigen to be described
Her2-neu	Transmembrane	Overexpressed	>50	A receptor tyrosine kinase, member of the EGF–receptor family, involved in cell growth and differentiation
K-Ras	Intracellular	Mutated self	90	Mutated form of ras, a GTPase important for cell proliferation, differentiation, and survival
Mesothelin	Cell surface (GPI-linked)	Overexpressed	~100	GPI-linked glycoprotein normally expressed on the surface of mesothelial cells lining the pleura, peritoneum, and pericardium at low levels. Binding partner of CA125/MUC16
MUC-1	Transmembrane	Overexpressed, hypoglycosylation	90	Type 1 transmembrane glycoprotein, expressed on apical surface of ductal and glandular epithelial cells at low levels. Extracellular domain has a polypeptide core with multiple tandem repeats of 20 amino acids
p53	Intracellular	Mutated self	50–70	Tumor suppressor that regulates cell cycle. Normally inhibits survivin at the transcription level and can initiate apoptosis if DNA damage is unrepairable
Survivin	Intracellular	Overexpressed	80	Member of IAP family. Inhibits caspase activation; is found in most human tumors and fetal tissue, but is completely absent in terminally differentiated cells
Telomerase	Intracellular	Overexpressed	95	Ribonucleoprotein that is responsible for RNA-dependent synthesis of telomeric DNA. TERT is its catalytic subunit
VEGFR2	Transmembrane	Overexpressed	64	A tyrosine kinase and member of platelet-derived growth factor family. Receptor for VEGF with functions in blood vessel development

CEA carcinoembryonic antigen, *GPI* glycosylphosphatidylinositol, *IAP* inhibitor of apoptosis protein, *MUC* mucin, *TERT* telomerase reverse transcriptase, *VEGFR* VEGF receptor

12.4.1 Active Immunotherapy

The goal of tumor-specific vaccines is to present tumor-associated antigens (TAA) to immune cells and produce potent and lasting cytotoxic effects against tumor cells. Antigen presenting cells (APC) such as dendritic cells (DCs) and T cells (CD4/CD8) are the targets and effectors of this immune response. Different types of vaccines have been developed and specific examples are reviewed below.

12.4.1.1 Whole Cell Vaccines

Using irradiated tumor cell vaccines can produce potent immune responses to multiple TAAs present on pancreatic tumor cells. Allogeneic or autologous tumor cells can be used to develop vaccines. Advantages of whole cell vaccines include tumor cells can be grown *in vitro*, specific TAAs do not need to be identified, polyclonal tumor specific T cell populations are generated, and cells can be altered to express surface proteins or secretable factors that induce strong immune responses [53].

One such example is granulocyte-macrophage colony stimulating factor (GM-CSF) secreting tumor cells. Dranoff has previously shown that GM-CSF secreting cells induce long-lasting immunity in melanoma models [54]. A phase I study by Jaffee et al. took 14 patients

with PC and inoculated variable doses of GM-CSF secreting allogeneic tumor cells 8 weeks after pancreaticoduodenectomy. Three patients developed delayed-type hypersensitivity responses and remained disease free at 25 months [55].

A phase II, single-institution study treated 60 patients after surgical resection (R0 or R1) with 5×10^8 GM-CSF secreting tumor cells 8–10 weeks after surgery [56]. Patients then received 5 FU chemoradiotherapy and up to four more vaccinations. Median disease-free survival and overall survival were 17.3 and 24.8 months, respectively. The most common side effects were erythema, induration and pain at the vaccination site, and the only grade 3–4 side effect was eosinophilia in two patients (1 %). Disease-free survival was correlated with the induction of mesothelin-specific $CD8^+$ T cells. Mesothelin is a membrane-bound protein that is highly expressed in pancreatic cancer cells, but has low expression in normal tissue, making it a possible target of immune therapy [39] Laheru and colleagues showed that in metastatic cancer, a GM-CSF tumor cell vaccine given with cyclophosphamide also induces a mesothelin-specific $CD8^+$ T-cell response [57].

Cell surface molecule expression can be modified to induce immune responses. The most successful model exploits hyperacute rejection due to alpha-galactosyl (αGal). Nonprimate mammals express αGal, but humans have a nonfunctional gene. Repeated exposure by gut flora to αGal epitopes leads to high expression of anti-αGal antibodies, which constitutes up to 1 % of circulating IgG [58]. The hyperacute antibody-mediated rejection leads to cell-mediated immunity against TAA in murine models of melanoma [59]. A phase II open-label multi-institutional trial evaluated algenpantucel-L with adjuvant gemcitabine and 5-FU-based radiotherapy after surgical resection [60]. Adjuvant therapy was the same as the RTOG-9704 study protocol [61]; 73 patients were enrolled and 69 received 100 million or 300 million cells per injection and a median of 12 vaccinations. The primary endpoint of 1-year disease-free survival was 62 % and secondary endpoint of 1-year overall survival was 86 % with no serious side effects attributed to immunotherapy. Survival with the addition of algenpantucel-L compared favorably with RTOG-9704 study. A phase III multicenter randomized controlled trial to evaluate adjuvant gemcitabine alone or with 5-FU-based radiotherapy with or without algenpantucel-L was completed in January 2014 [62].

12.4.1.2 Peptide Vaccines

Small antigenic protein fragments are used to develop peptide vaccines. These peptides can be produced economically and safely with no risk of infectious material. In addition, no autologous tissue is required. The drawbacks are that they can be poorly immunogenic and adjuvants may be required to induce a meaningful response [63]. Multiple peptide vaccines have been developed to target PC and have shown promising results.

12.4.1.3 KRAS Vaccines

The v-Ki-ras2 Kristin rat sarcoma (KRAS) viral oncogene encodes a GTPase important for signal transduction. Mutations can be found in the majority of pancreatic cancers as well as in lung and colon cancers [36] In 1995, Gjertsen showed that T-cell response could be activated using a Kristin rat sarcoma mutant (codon 12) peptide vaccine [64]. A follow-up phase I/II study exposed autologous APCs to Kristin rat sarcoma peptide vaccine *ex vivo*, then reinfused the activated APCs in five patients. Two out of five produced immune responses to ras [65]. To improve immunogenicity of the vaccine, GM-CSF was used as an adjuvant to the K-Ras vaccine in 48 patients with PC. The vaccine was well tolerated and patients who showed an immune response had superior survival compared to nonresponders (148 days *vs.* 61 days) [66]. Unfortunately, a follow-up study showed the safety of k-Ras/GM-CSF vaccine, but did not produce an immune response [67].

Weden and colleagues were able to induce immune responses using Kristin rat sarcoma vaccines prepared with long synthetic peptides. These peptides require processing and presentation by APC and induce polyclonal T cells that have specificity to mutated Kristin rat sarcoma [68]. In 23 patients who were vaccinated after surgical resection (20 evaluable), 4/20 (20 %)

were alive at 10 years, whereas 0/87 in a cohort of nonvaccinated patients during that same period were alive [69].

12.4.1.4 VEGF Vaccines

Vascular endothelial growth factor (VEGF) is a key signaling protein in angiogenesis. Overexpression of VEGF is seen in pancreatic cancers and is associated with larger tumor size and enhanced local spread [52]. A phase I study used VEGFR2-169 epitope, a peptide vaccine for VEGF receptor 2, in combination with gemcitabine in advanced PC (unresectable or metastatic disease). Of the 18 patients receiving at least 1 vaccination, 61 % developed cytotoxic T lymphocytes specific to VEGFR2 and median survival was 8.7 months [70]. Recently, the results of a phase II/III randomized placebo controlled study of gemcitabine +/−VEGFR2 vaccine showed no survival advantage [71].

12.4.1.5 Telomerase Vaccines

Telomeres are nucleotide sequence repeats at the ends of chromatid that help maintain chromosomes. Telomerase helps to maintain the telomeres, and is reactivated in 85 % of pancreatic cancers [72]. A phase I/II trial looking at GV1001, a peptide vaccine based on the catalytic subunit of telomerase was tested in 48 patients with unresectable pancreatic cancer who were injected with one of three dose levels. The intermediate dose group (300 nmol) showed a median survival of 8.6 months, significantly higher than other groups, and a 1-year survival of 25 % [73]. Follow-up phase III trial did not show a survival advantage of GV1001 versus gemcitabine, but other phase III trials evaluating combination therapy are ongoing [74].

12.4.1.6 Recombinant Vaccines

To increase antigenicity, viral and bacterial antigens can be added to cancer vaccines to induce a more potent immune response. These infectious antigens can activate the innate immune system, thereby recruiting APC to the site [72]. One example is the TRICOM vaccine [75]. This poxvirus-based vaccination uses B7-1, ICAM-1, and LFA-3 to enhance T-cell stimulation. Kaufman combined CEA and MUC1 antigens with TRICOM expressing vaccinia (PANVAC-V) or fowlpox (PANVAC-F) in a phase I trial. Ten patients with advanced PC were primed with PANVAC-V, and then given three boosters of PANVAC-F monthly up to 12 months. Antibodies to vaccinia were seen in all ten patients and five of eight evaluable patients developed antigen-specific T-cell responses. Median overall survival was 6.3 months and significant prolonged survival was seen in patients who developed anti-CEA and anti-MUC1 immune responses (15.1 months versus 3.9 months) [76].

Listeria vaccines have also been combined with TAA in cancer vaccines. A trial of 28 patients looked at patients with hepatic metastases from four primary tumors (pancreatic, mesothelioma, ovarian, and nonsmall-cell lung cancer). Patients were administered live attenuated listeria vaccine expressing mesothelin, which is expressed on the cell surface of the tumors. An overall of 37 % of subjects lived >15 months with minimal adverse events; half of these patients had PC [77].

12.4.1.7 DNA Vaccines

Vaccines using DNA encoding TAAs have also shown some efficacy in murine models of PC. MUC-1 DNA vaccine was injected in mice along with pancreatic cancer cells expressing MUC-1 (panc02-MUC1) or no MUC-1 (panc02). Vaccinated mice developed cytotoxic T-cell responses to MUC-1 and tumor shrinkage and improved survival was seen in mice with panc02-MUC1 cells. Mice injected with panc02 cells did not show any therapeutic benefit [78].

Survivin has also been tested as a DNA vaccine. A member of the inhibitor apoptosis family, survivin expression is found in PC cells, but not normal pancreatic tissue [48]. Zhu and colleagues inoculated mice with a survivin DNA vaccine or control vector followed by panc02 cells. Survivin inoculated mice showed increased lymphocyte infiltration of tumors compared with control mice. Increased survival and decreased tumor size was also seen in the Survivin group [79]. These findings need to be tested further in human subjects.

12.4.1.8 Antigen-Pulsed Dendritic Cells

Antigen presenting cells (APC) acquire and process antigens to present to T-cells on MHC class I and II molecules. Dendritic cells (DC) are the most important APC, often referred to as "professional APC." Multiple DC vaccines have been developed and sipuleucel-T is known as the most successful that improved overall survival in castration-resistant prostate cancer versus placebo in a phase III trial [80]. Multiple DC vaccines have been tested in pancreatic cancer with encouraging results. A phase I/II study took autologous DC pulsed with MUC1 peptide and treated 12 patients with resected pancreatic and biliary tumors. No significant toxicity was seen and four patients were alive at 4 years [81]. MUC1-pulsed DC have also been tested in advanced stage PC in a recent pilot study [82]. The vaccine was well tolerated in all seven enrolled patients with no significant side effects. This phase I study did not show a clinical benefit, but confirmed the safety of the vaccine and needs further study.

Another study looked at DC pulsed with carcinoembryonic antigen (CEA) mRNA. Patients with resected PC following neoadjuvant chemoradiotherapy were given autologous DC vaccine for 6 months. All three patients treated were alive 2.5 years after diagnosis with no evidence of disease [83].

12.4.2 Passive Immunotherapy

12.4.2.1 Antibody-Based Therapies

Monoclonal antibodies (mAbs) can affect tumor cells by a number of different mechanisms. Cytotoxic effects including Antibody-dependent cell-mediated cytotoxicity (ADCC), complement-mediated cytotoxicity (CMC), and antibody-dependent cellular phagocytosis leading to apoptosis as well as blockade of cellular receptors-growth factor/cytokine interaction inhibiting growth and survival can be exploited by antibody therapy [84]. Immunoglobulins can also be conjugated to radioisotopes or cytotoxic agents (chemotherapeutic agents, or toxins) and target-specific cellular targets to limit side effects. Numerous studies have evaluated the role of mAb in pancreatic cancer.

Various proteins expressed by PC cells have been targeted by mAb. One such protein expressed by multiple cancer cells is mesothelin. The vast majority of adenocarcinoma of the pancreas express mesothelin, but it is not seen in normal pancreatic tissue or chronic pancreatitis [38]. A phase I study involving 24 patients with mesothelioma, ovarian and pancreatic cancer testing an mAb to mesothelin (MORAb-009) was reported in 2010. MORAb-009 was well tolerated at 200 mg/m^2 weekly and 11 patients showed stable disease. One pancreatic cancer patient who progressed on gemcitabine had stable disease for 6 months [85]. A recent phase II study looking at MORAb-009 with gemcitabine versus gemcitabine alone has now been completed and results are pending [112].

Epidermal growth factor receptor (EGFR) is a glycoprotein receptor key in signaling cell proliferation and has been a successful target in numerous cancers including lung, head, and neck cancers. Multiple trials have tested addition of EGFR inhibition to standard chemotherapy in pancreatic cancer. Erlotinib, a small molecule tyrosine kinase inhibitor of EGFR has shown improved survival, but immunotherapy trials have not been as successful [86]. Cetuximab, a chimeric mAb was tested in a large phase III trial run by SWOG comparing gemcitabine with cetuximab versus gemcitabine alone in advanced pancreatic cancer patients. No improvement in progression-free or overall survival was seen with the addition of cetuximab [87]. A fully humanized antibody to EGFR, matuzumab, has also been tested in pancreatic cancer. A phase I study combining matuzumab with gemcitabine was well tolerated and 67 % (8/12 patients) showed partial response or stable disease [88].

Trastuzumab is an mAb that binds epidermal growth factor receptor 2 (HER2), a tyrosine kinase receptor that is part of the EGFR family. It has been effective in both breast and gastric cancers, and is expressed in a significant percent of pancreatic tumors [53]. Treatment with trastuzumab in mouse models has shown efficacy.

Nude mice injected with human pancreatic tumor cells showed prolonged survival and decreased metastasis when treated with trastuzumab [89]. Combination therapy has also shown benefit. Larbouret showed that trastuzumab combined with cetuximab resulted in superior survival in mice with human pancreatic cancer xenografts compared to gemcitabine [90]. A phase I/II trial of trastuzumab and cetuximab as second-line therapy in metastatic PC has been completed, but results are not published [91].

Angiogenesis has also been a target of passive immunotherapy, specifically antibodies to vascular endothelial growth factor (VEGF). As noted previously, VEGF and its receptors are often overexpressed in PC [52], and a phase II study showed partial response (21 %) and stable disease (46 %) in metastatic PC patients treated with bevacizumab (anti-VEGF Ab) and gemcitabine [92]. Benefit was not seen in the phase III follow-up study by the Cancer and Leukemia group B (CALGB80303). Overall, 602 patients with advanced PC (85 % metastatic disease) were randomly assigned to gemcitabine+bevacizumab or gemcitabine+placebo. No statistical difference in progression-free or overall survival was seen with the addition of bevacizumab [93]. Bevacizumab has also been tested in combination with gemcitabine and erlotinib in the AVITA study. The addition of bevacizumab increased progression-free survival, but not overall survival [94].

12.5 Radioimmunotherapy

Delivery of radioactive substances by tumor-specific antibodies has produced promising results. Anti-Muc-1 antibody, PAM4, is expressed in 85 % of pancreatic cancers, but not in normal pancreatic tissue [95]. Humanized PAM4 (clivatuzumab tetraxetan) conjugated with yttrium-90, a beta-emitting nucleotide with a radiation path length of 5 mm (^{90}Y-hPAM4), was studied in a phase I trial of 38 untreated patients with advanced PC (86 % stage IV disease). Weekly gemcitabine was used as a radiosensitizer and ^{90}Y-hPAM4 was given weekly starting week 2 on 4 week cycles. Six patients had partial responses (16 %) and 16 (42 % showed stable disease). Median survival was 7.7 months. Grade 3–4 thrombocytopenia or neutropenia developed in 20/38 treated patients after cycle 1. The authors concluded that ^{90}Y-hPAM4 has promising therapeutic activity with manageable side effects [96].

12.6 Immunoconjugates

Antibodies conjugated to cytotoxic agents can concentrate chemotherapy or other toxins at tumor sites and spare normal tissue. CEA has been the target of early immunoconjugate studies in PC. CEA is frequently overexpressed in gastrointestinal tumors including PC. hMN-14 or labetuzumab is an anti-CEA antibody that induces ADCC *in vitro* in murine colon cancer models [97]. In a pilot study, Labetuzumab was conjugated to SN-38 and infused to mice with human colon and pancreatic cancer xenografts. SN-38 is the active metabolite (two to three times potency) of irinotecan. Improved survival and decreased tumor size was observed in both colon and pancreatic cancer xenografts compared to controls [98].

12.7 Pancreatic Neuroendocrine Immunotherapy

Pancreatic neuroendocrine (pNET) or islet cell tumors comprise 2–3 % of primary pancreatic tumors with increasing incidence over the past 30 years [99, 100]. Early-stage disease is treated with surgical resection, but until recently few options were available for metastatic or unresectable disease. Cytotoxic agents such as streptozocin, dacarbazine, and temozolomide have shown activity, but their application has been limited due to side effects.

Immunotherapy has also been previously studied with interferon. Antitumor effects with interferon alpha include T-cell stimulation as well as cell cycle arrest [101]. Retrospective studies have reported improvement in symptoms and tumor stabilization, but two prospective trials comparing somatostatin analogs with or without

interferon therapy did not show any significant difference in tumor response rates or progression-free survival [102, 103]. A large multicenter trial evaluating octreotide with either interferon or bevacizumab is currently enrolling [104].

New targeted therapies including everolimus (mTOR inhibitor) and sunitinib, along with octreotide have shown improvement in progression-free survival [105–107]. Sunitinib is a multikinase inhibitor that binds to various tyrosine kinase receptors including VEGF receptor. VEGF expression has been associated with risk of metastasis in low-grade neuroendocrine tumors [108]. Anti-VEGF antibody bevacizumab has shown activity in combination with gemcitabine and S1 in PC mouse models [109]. A phase II study by Chan and colleagues looked at 34 patients with gastroenteropancreatic neuroendocrine tumors (44 % pNET, 56 % carcinoid) treated with bevacizumab plus temozolomide. Overall response rate was 15 %, but 33 % of pNET patients (5/15) had a response compared to 0/19 with carcinoid tumors. Both median progression-free (14.3 months *vs.* 7.3 months) and overall survival (41.7 months vs. 18.8 months) were higher in pNET versus carcinoid [110].

Little research has been pursued in vaccine development for pancreatic neuroendocrine tumors. A case report using autologous DCs pulsed with tumor cell lysate was delivered subcutaneously to a patient with metastatic pNET. A DTH reaction developed and specific T-cell response was noted. The patient had stable disease at 20 months after starting therapy [111].

12.8 Concluding Remarks

Pancreatic cancer continues to be a highly lethal disease in which new therapeutics are desperately needed. Immunotherapy in pancreatic cancer is in its infancy, but gives new targets and new ways to deliver therapeutics to the tumor. Vaccines, mAbs and drug- or radioimmunotherapy has shown promising results in multiple preclinical models and numerous therapies are currently in clinical trials. Although no current immunotherapy is standard of care in pancreatic cancers, they may prove to be key components of treatment in the future.

References

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin. 2010;60:277–300.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69–90.
3. Oettle H, Post S, Neuhaus P, et al. Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. JAMA. 2007;297:267–77.
4. Conroy T, Desseigne F, Ychou M, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med. 2011;364:1817–25.
5. Goldman B, DeFrancesco L. The cancer vaccine roller coaster. Nat Biotechnol. 2009;27(2):129–39.
6. Yokokawa J, Palena C, Arlen P, et al. Identification of novel human CTL epitopes and their agonist epitopes of mesothelin. Clin Cancer Res. 2005;11(17):6342–51.
7. Andersen MH, Pedersen LO, Becker JC, Straten PT. Identification of a cytotoxic T lymphocyte response to the apoptosis inhibitor protein survivin in cancer patients. Cancer Res. 2001;61(3):869–72.
8. Johnston FM, Tan MC, Tan Jr BR, et al. Circulating mesothelin protein and cellular antimesothelin immunity in patients with pancreatic cancer. Clin Cancer Res. 2009;15(21):6511–18.
9. Kotera Y, Fontenot JD, Pecher G, Metzgar RS, Finn OJ. Humoral immunity against a tandem repeat epitope of human mucin MUC-1 in sera from breast, pancreatic, and colon cancer patients. Cancer Res. 1994;54(11):2856–60.
10. Kubuschok B, Neumann F, Breit R, et al. Naturally occurring T-cell response against mutated p21 ras oncoprotein in pancreatic cancer. Clin Cancer Res. 2006;12(4):1365–72.
11. Wenandy L, Sorensen RB, Sengelov L, Svane IM, Thor SP, Andersen MH. The immunogenicity of the hTERT540–548 peptide in cancer. Clin Cancer Res. 2008;14(1):4–7.
12. Yanagimoto H, Mine T, Yamamoto K, et al. Immunological evaluation of personalized peptide vaccination with gemcitabine for pancreatic cancer. Cancer Sci. 2007;98(4):605–11.
13. Dunn GP, Koebel CM, Schreiber RD. Interferons, immunity and cancer immunoediting. Nat Rev Immunol. 2006;6(11):836–48.
14. Swann JB, Smyth MJ. Immune surveillance of tumors. J Clin Invest. 2007;117(5):1137–46.
15. Hamanaka Y, Suehiro Y, Fukui M, Shikichi K, Imai K, Hinoda Y. Circulating anti-MUC1 IgG antibodies as a favorable prognostic factor for pancreatic cancer. Int J Cancer. 2003;103(1):97–100.
16. Pages F, Galon J, Dieu-Nosjean MC, Tartour E, Sautes-Fridman C, Fridman WH. Immune infiltration in human tumors: a prognostic factor that should not be ignored. Oncogene. 2010;29(8):1093–102.

17. Clark CE, Beatty GL, Vonderheide RH. Immunosurveillance of pancreatic adenocarcinoma: insights from genetically engineered mouse models of cancer. Cancer Lett. 2009;279(1):1–7.
18. Fong L, Small EJ. Anti-cytotoxic T-lymphocyte antigen-4 antibody: the first in an emerging class of immunomodulatory antibodies for cancer treatment. J Clin Oncol. 2008;26(32):5275–83.
19. Paulos CM, June CH. Putting the brakes on BTLA in T cell-mediated cancer immunotherapy. J Clin Invest. 2010;120(1):76–80.
20. Pardoll D. Does the immune system see tumors as foreign or self? Annu Rev Immunol. 2003;21:807–39.
21. Ikemoto T, Yamaguchi T, Morine Y, et al. Clinical roles of increased populations of $Foxp3^{+}CD4^{+}$ T cells in peripheral blood from advanced pancreatic cancer patients. Pancreas. 2006;33(4):386–90.
22. Hiraoka N, Onozato K, Kosuge T, Hirohashi S. Prevalence of $FOXP3^{+}$ regulatory T cells increases during the progression of pancreatic ductal adenocarcinoma and its premalignant lesions. Clin Cancer Res. 2006;12(18):5423–34.
23. Nomi T, Sho M, Akahori T, et al. Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. Clin Cancer Res. 2007;13(7):2151–57.
24. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science. 2003;299(5609):1057–61.
25. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol. 1995;155(3):1151–64.
26. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of $CD4^{+}CD25^{+}$ regulatory T cells. Nat Immunol. 2003;4(4):330–36.
27. Khattri R, Cox T, Yasayko SA, Ramsdell F. An essential role for Scurfin in $CD4^{+}CD25^{+}$ T regulatory cells. Nat Immunol. 2003;4(4):337–42.
28. Linehan DC, Goedegebuure PS. $CD25^{+}$ $CD4^{+}$ regulatory T-cells in cancer. Immunol Res. 2005;32(1–3):155–16.
29. Huang EH, Kaufman HL. CEA-based vaccines. Expert Rev Vaccines. 2002;1(1):49–63.
30. Beatty JD, Romero C, Brown PW, Lawrence Jr W, Terz JJ. Clinical value of carcinoembryonic antigen: diagnosis, prognosis, and follow-up of patients with cancer. Arch Surg. 1979;114(5):563–67.
31. Ona FV, Zamcheck N, Dhar P, Moore T, Kupchik HZ. Carcinoembryonic antigen (CEA) in the diagnosis of pancreatic cancer. Cancer. 1973;31(2):324–27.
32. Ladjemi MZ, Jacot W, Chardes T, Pelegrin A, Navarro-Teulon I. Anti-HER2 vaccines: new prospects for breast cancer therapy. Cancer Immunol Immunother. 2010;59(9):1295–12.
33. Lei S, Appert HE, Nakata B, Domenico DR, Kim K, Howard JM. Overexpression of HER2/neu oncogene in pancreatic cancer correlates with shortened survival. Int J Pancreatol. 1995;17(1):15–21.
34. Yamanaka Y, Friess H, Kobrin MS, et al. Overexpression of *HER2/neu* oncogene in human pancreatic carcinoma. Hum Pathol. 1993;24(10):1127–34.
35. Downward J. Targeting RAS and PI3K in lung cancer. Nat Med. 2008;14(12):1315–16.
36. Almoguera C, Shibata D, Forrester K, Martin J, Arnheim N, Perucho M. Most human carcinomas of the exocrine pancreas contain mutant *c-K-ras* genes. Cell. 1988;53(4):549–54.
37. Li M, Bharadwaj U, Zhang R, et al. Mesothelin is a malignant factor and therapeutic vaccine target for pancreatic cancer. Mol Cancer Ther. 2008;7(2):286–96.
38. Hassan R, Laszik ZG, Lerner M, Raffeld M, Postier R, Brackett D. Mesothelin is overexpressed in pancreaticobiliary adenocarcinomas but not in normal pancreas and chronic pancreatitis. Am J Clin Pathol. 2005;124(6):838–45.
39. Argani P, Iacobuzio-Donahue C, Ryu B, et al. Mesothelin is overexpressed in the vast majority of ductal adenocarcinomas of the pancreas: identification of a new pancreatic cancer marker by serial analysis of gene expression (SAGE). Clin Cancer Res. 2001;7(12):3862–68.
40. Kaneko O, Gong L, Zhang J, et al. A binding domain on mesothelin for CA125/MUC16. J Biol Chem. 2009;284(6):3739–49.
41. Tang CK, Katsara M, Apostolopoulos V. Strategies used for MUC1 immunotherapy: human clinical studies. Expert Rev Vaccines. 2008;7(7):963–75.
42. Qu CF, Li Y, Song YJ, et al. MUC1 expression in primary and metastatic pancreatic cancer cells for *in vitro* treatment by (213)Bi-C595 radioimmunoconjugate. Br J Cancer. 2004;91(12):2086–93.
43. Chen F, Wang W, El-Deiry WS. Current strategies to target p53 in cancer. Biochem Pharmacol. 2010;80(5):724–30.
44. Scarpa A, Capelli P, Mukai K, et al. Pancreatic adenocarcinomas frequently show *p53* gene mutations. Am J Pathol. 1993;142(5):1534–43.
45. Ryan BM, O'Donovan N, Duffy MJ. Survivin: a new target for anti-cancer therapy. Cancer Treat Rev. 2009;35(7):553–62.
46. Kanwar RK, Cheung CH, Chang JY, Kanwar JR. Recent advances in anti-survivin treatments for cancer. Curr Med Chem. 2010;17(15):1509–15.
47. Qiao JG, Zhang YQ, Yin YC, Tan Z. Expression of Survivin in pancreatic cancer and its correlation to expression of Bcl-2. World J Gastroenterol. 2004;10(18):2759–61.
48. Satoh K, Kaneko K, Hirota M, Masamune A, Satoh A, Shimosegawa T. Expression of survivin is correlated with cancer cell apoptosis and is involved in the development of human pancreatic duct cell tumors. Cancer. 2001;92(2):271–8.
49. Liu JP, Chen W, Schwarer AP, Li H. Telomerase in cancer immunotherapy. Biochim Biophys Acta. 2010;1805(1):35–42.

50. Hiyama E, Kodama T, Shinbara K, et al. Telomerase activity is detected in pancreatic cancer but not in benign tumors. Cancer Res. 1997;57(2):326–31.
51. Shibuya M. Vascular endothelial growth factor (VEGF)-receptor2: its biological functions, major signaling pathway, and specific ligand VEGF-E. Endothelium. 2006;13(2):63–9.
52. Itakura J, Ishiwata T, Friess H, et al. Enhanced expression of vascular endothelial growth factor in human pancreatic cancer correlates with local disease progression. Clin Cancer Res. 1997;3(8):1309–16.
53. Niccolai E, Prisco D, D'Elios MM, Amedei A. What is recent in pancreatic cancer immunotherapy? BioMed Res Int. 2013;2013:492372.
54. Dranoff G, Jaffee EM, Lazenby A, et al. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte/macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. Proc Natl Acad Sci U S A. 1993;90:3539–43.
55. Jaffee EM, et al. Novel allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: a phase I trial of safety and immune activation. J Clin Oncol. 2001;19(1):145–56.
56. Lutz E, et al. A lethally irradiated allogeneic granulocyte-macrophage colony stimulating factor-secreting tumor vaccine for pancreatic adenocarcinoma: a phase II trial of safety, efficacy, and immune activation. Ann Surg. 2011;253(2):328–35.
57. Laheru D, et al. Allogeneic granulocyte macrophage colony-stimulating factor-secreting tumor immunotherapy alone or in sequence with cyclophosphamide for metastatic pancreatic cancer: a pilot study of safety, feasibility, and immune activation. Clin Cancer Res. 2008;14(5):1455–63.
58. Galili U, et al. Evolutionary relationship between the natural anti-Gal antibody and the Gal alpha 1—3Gal epitope in primates. Proc Natl Acad Sci U S A. 1987;84(5):1369–73.
59. Rossi GR, et al. Allogeneic melanoma vaccine expressing alphaGal epitopes induces antitumor immunity to autologous antigens in mice without signs of toxicity. J Immunother. 2008;31(6):545–54.
60. Hardacre J, et al. Addition of algenpantucel-L immunotherapy to standard adjuvant therapy for pancreatic cancer: a phase 2 study. J Gastrointest Surg. 2013;17(1):94–100.
61. Regine W, et al. Fluorouracil vs gemcitabine chemotherapy before and after fluorouracil-based chemoradiation following resection of pancreatic adenocarcinoma: a randomized controlled trial. JAMA. 2008;299(9):1019–26.
62. Clinicaltrials.gov identifier: NCT01072981. Principal director: Nicholas N Vahanian, M.D. A Phase III study of chemotherapy and chemoradiotherapy with or without hyperacute®-pancreas (algenpantucel-L) immunotherapy in subjects with surgically resected pancreatic cancer. Opened 2/18/2010.
63. Purcell A, et al. More than one reason to rethink the use of peptides in vaccine design. Nat Rev Drug Discov. 2007;6(5):404–14.
64. Gjertsen M. Vaccination with mutant ras peptides and induction of T-cell responsiveness in pancreatic carcinoma patients carrying the corresponding RAS mutation. Lancet. 1995;346(8987):1399–400.
65. Gjertsen M, et al. Ex vivo ras peptide vaccination in patients with advanced pancreatic cancer: results of a phase I/II study. Int J Cancer. 1996;65(4):450–3.
66. Gjertsen M, et al. Intradermal ras peptide vaccination with granulocyte-macrophage colony-stimulating factor as adjuvant: clinical and immunological responses in patients with pancreatic adenocarcinoma. Int J Cancer. 2001;92(3):441–50.
67. Abou-Alfa G, et al. Targeting mutated K-ras in pancreatic adenocarcinoma using an adjuvant vaccine. Am J Clin Oncol. 2011;34(3):321–5.
68. Bijker M, et al. Superior induction of anti-tumor CTL immunity by extended peptide vaccines involves prolonged, DC-focused antigen presentation. Eur J Immunol. 2008;38(4):1033–42.
69. Weden S, et al. Long-term follow-up of patients with resected pancreatic cancer following vaccination against mutant K-ras. Int J Cancer. 2011;128(5):1120–8.
70. Miyazawa M, et al. Phase I clinical trial using peptide vaccine for human vascular endothelial growth factor receptor 2 in combination with gemcitabine for patients with advanced pancreatic cancer. Cancer Sci. 2010;101(2):433–9.
71. Yamaue H, et al. Phase II/III clinical trial with VEGFR2-epitope peptide and gemcitabine for patients with locally advanced, metastatic, or unresectable pancreatic cancer: Pegasus-PC study. J Clin Oncol. 2012;30(Suppl 34):abstr 223.
72. Soares K, et al. Vaccines for pancreatic cancer. Cancer J. 2012;18(6):642–52.
73. Bernhardt S, et al. Telomerase peptide vaccination of patients with non-resectable pancreatic cancer: a dose escalating phase I/II study. Br J Cancer. 2006;95(11):1474–82.
74. Gunturu K, et al. Immunotherapy updates in pancreatic cancer: are we there yet? Ther AdvMed Oncol. 2013;5(1):81–9.
75. Garnett C, et al. TRICOM vector based cancer vaccines. Curr Pharm Des. 2006;12(3):351–61.
76. Kaufman H, et al. Poxvirus-based vaccine therapy for patients with advanced pancreatic cancer. J Transl Med. 2007;5:60.
77. Le D, et al. A live-attenuated Listeria vaccine (ANZ-100) and a live-attenuated Listeria vaccine expressing mesothelin (CRS-207) for advanced cancers: phase I studies of safety and immune induction. Clin Cancer Res. 2012;18(3):858–68.
78. Rong Y, et al. Induction of protective and therapeutic anti-pancreatic cancer immunity using a reconstructed MUC1 DNA vaccine. BMC Cancer. 2009;9:191.
79. Zhu K, et al. Survivin DNA vaccine generated specific antitumor effects in pancreatic carcinoma and

lymphoma mouse models. Vaccine. 2007;25(46): 7955–61.
80. Kantoff P, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010;363(5):411–22.
81. Lepisto A, et al. A phase I/II study of a MUC1 peptide pulsed autologous dendritic cell vaccine as adjuvant therapy in patients with resected pancreatic and biliary tumors. Cancer Ther. 2008;6(B):955–64.
82. Rong Y, et al. A phase I pilot trial of MUC1-peptide-pulsed dendritic cells in the treatment of advanced pancreatic cancer. Clin Exp Med. 2012;12(3):173–80.
83. Morse M, et al. The feasibility and safety of immunotherapy with dendritic cells loaded with CEA mRNA following neoadjuvant chemoradiotherapy and resection of pancreatic cancer. Int J Gastrointest Cancer. 2002;32(1):1–6.
84. Jurcic J, et al. Monoclonal antibody therapy of cancer. In: Kaufman H, Wolchok JD, editors. General principles of tumor immunology: basic and clinical applications of tumor immunology. Dordrecht: Springer; 2008. p. 321–42.
85. Hassan R, et al. Phase I clinical trial of the chimeric anti-mesothelin monoclonal antibody MORAb-009 in patients with mesothelin-expressing cancers. Clin Cancer Res. 2010;16(24):6132–8.
86. Moore M, et al. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol. 2007;25(15):1960–6.
87. Philip P, et al. Phase III study comparing gemcitabine plus cetuximab versus gemcitabine in patients with advanced pancreatic adenocarcinoma: Southwest Oncology Group-directed intergroup trial S0205. J Clin Oncol. 2010;28(22):3605–10.
88. Graeven U, et al. Phase I study of the humanised anti-EGFR monoclonal antibodymatuzumab (EMD 72000) combined with gemcitabine in advanced pancreatic cancer. Br J Cancer. 2006;94(9):1293–9.
89. Pratesi G, Petrangolini G, Tortoreto M, et al. Antitumor efficacy of trastuzumab in nude mice orthotopically xenografted with human pancreatic tumor cells expressing low levels of HER-2/neu. J Immunother. 2008;31(6):537–44.
90. Larbouret C, Robert B, Bascoul-Mollevi C, et al. Combined cetuximab and trastuzumab are superior to gemcitabine in the treatment of human pancreatic carcinoma xenografts. Ann Oncol. 2010;21(1):98–103.
91. ClinicalTrials.gov Identifier:NCT00923299. Cetuximab and trastuzumab in treating patients with metastatic pancreatic cancer that progressed after previous treatment with gemcitabine primary investigator: Marc Ychou MD, PhD. Opened 6/17/2009.
92. Kindler HL, Friberg G, Singh DA, et al. Phase II trial of bevacizumab plus gemcitabine in patients with advanced pancreatic cancer. J Clin Oncol. 2005;23(31):8033–40.
93. Kindler HL, Niedzwiecki D, Hollis D, et al. Gemcitabine plus bevacizumab compared with gemcitabine plus placebo in patients with advanced pancreatic cancer: phase III trial of the Cancer and Leukemia Group B (CALGB 80303). J Clin Oncol. 2010;28(22):3617–22.
94. Van Cutsem E, Vervenne WL, Bennouna J, et al. Phase III trial of bevacizumab in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. J Clin Oncol. 2009;27(13):2231–7.
95. Gold DV, Karanjawala Z, Modrak DE, et al. PAM4-reactive MUC1 is a biomarker for early pancreatic adenocarcinoma. Clin Cancer Res. 2007;13(24):7380–7.
96. Ocean AJ, Pennington KL, Guarino MJ, et al. Fractionated radioimmunotherapy with (90) Y-clivatuzumab tetraxetan and low-dose gemcitabine is active in advanced pancreatic cancer: a phase 1 trial. Cancer. 2012;118(22):5497–506.
97. Blumenthal RD, Osorio L, Hayes MK, et al. Carcinoembryonic antigen antibody inhibits lung metastasis and augments chemotherapy in a human colonic carcinoma xenograft. Cancer Immunol Immunother. 2005;54(4):315–27.
98. Govindan SV, Cardillo TM, Moon SJ, et al. CEACAM5-targeted therapy of human colonic and pancreatic cancer xenografts with potent labetuzumab-SN-38 immunoconjugates. Clin Cancer Res. 2009;15(19):6052–61.
99. Fesinmeyer MD, Austin MA, Li CI, et al. Differences in survival by histologic type of pancreatic cancer. Cancer Epidemiol Biomarkers Prev. 2005;14(7):1766–73.
100. Yao JC, Hassan M, Phan A, et al. One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. J Clin Oncol. 2008;26(18):3063–72.
101. Detjen KM, Welzel M, Farwig K, et al. Molecular mechanism of interferon alfa-mediated growth inhibition in human neuroendocrine tumor cells. Gastroenterology. 2000;118(4):735–48.
102. Faiss S, Pape U-F, Böhmig M, et al. Prospective, randomized, multicenter trial on the antiproliferative effect of lanreotide, interferon alfa, and their combination for therapy of metastatic neuroendocrine gastroenteropancreatic tumors – The International Lanreotide and Interferon Alfa Study Group. J Clin Oncol. 2003;21(14):2689–696.
103. Arnold R, Rinke A, Klose KJ, et al. Octreotide versus octreotide plus interferon-alpha in endocrine gastroenteropancreatic tumors: a randomized trial. Clin Gastroenterol Hepatol. 2005;3(8):761–71.
104. ClinicalTrials.gov Identifier:NCT00569127. Octreotide acetate and recombinant interferon alfa-2b or bevacizumab in treating patients with metastatic or locally advanced, high-risk neuroendocrine tumor.
105. Yao JC, Shah MH, Ito T, et al. RAD001 in advanced neuroendocrine tumors, third trial (RADIANT-3) study group. Everolimus for advanced pancreatic

neuroendocrine tumors. N Engl J Med. 2011;364(6):514–23.
106. Raymond E, Dahan L, Raoul JL, et al. Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. N Engl J Med. 2011;364(6):501–13.
107. Rinke A, Müller HH, Schade-Brittinger C, Klose KJ, PROMID Study Group, et al. Placebo-controlled, double-blind, prospective, randomized study on the effect of octreotide LAR in the control of tumor growth in patients with metastatic neuroendocrine midgut tumors: a report from the PROMID Study Group. J Clin Oncol. 2009;27(28):4656–63.
108. Zhang J, Jia Z, Li Q, Wang L, et al. Elevated expression of vascular endothelial growth factor correlates with increased angiogenesis and decreased progression-free survival among patients with low-grade neuroendocrine tumors. Cancer. 2007;109(8):1478–86.
109. Kasuya K, Nagakawa Y, Suzuki M, et al. Combination therapy of gemcitabine or oral S-1 with the anti-VEGF monoclonal antibody bevacizumab for pancreatic neuroendocrinecarcinoma. Exp Ther Med. 2012;3(4):599–602.
110. Chan JA, Stuart K, Earle CC, et al. Prospective study of bevacizumab plus temozolomide in patients with advanced neuroendocrine tumors. J Clin Oncol. 2012;30(24):2963–8.
111. Schott M, Feldkamp J, Lettmann M, et al. Dendritic cell immunotherapy in a neuroendocrine pancreas carcinoma. Clin Endocrinol (Oxf). 2007;55(2):271–7.
112. ClinicalTrials.gov Identifier:NCT00570713. A phase 2 randomized, placebo-controlled, double-blind study of the efficacy of MORAb-009 in combination with gemcitabine in patients with advanced pancreatic cancer. Opened 7 Dec 2007.
113. Von Hoff D, Ervin T, Arena FP, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N Engl J Med. 2013;369(18):1691–703.

Immune Modulation by Agents Used in the Prevention and Treatment of Colon and Pancreatic Cancers

13

Naveena B. Janakiram, Altaf Mohammed, Mark L. Lang, and Chinthalapally V. Rao

Contents

N.B. Janakiram, PhD (✉) • A. Mohammed, PhD
C.V. Rao, PhD
Hematology Oncology Section, Department of Medicine, Center for Cancer Prevention and Drug Development, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA

Department of Medicine, PCS Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA
e-mail: njanakir@ouhsc.edu

M.L. Lang, PhD
Department of Microbiology and Immunology, PCS Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA

13.1 Introduction

Inflammation, lifestyle, and other high-risk factors influence development of cancers in the Western world. Cancers are often associated with deregulated immune balances and responses, which may lead to immune tolerance helping the tumor growth. Recently, a lot of interest is shown towards developing safe and effective drugs/agents which can modulate immune disorders, break down tolerance mechanisms developed by the growing tumor, and effectively inhibit tumor growth. Immune modulation for prevention and treatment of cancers opens novel avenues and holds promise to control and optimally reverse important pathologic processes which play key roles in driving cancer growth and development. Although our increasing knowledge of biology of immune responses has led to development of new targets for cancers, still there is a need to understand how best these responses can be altered by different classes of safe agents to modify or enhance the immune responses against the

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_13,

tumors. Few studies showed immune modulation in mouse models of colon and pancreatic cancers and are tried in human clinical trials for treatment.

In this chapter, we will examine strategies of immune modulation and efficacies shown by agents in preclinical studies of colon and pancreatic cancers and also in human clinical trials and future prospects of these agents in inhibiting colon and pancreatic cancers in prevention and treatments by immune modulation.

13.2 Colorectal and Pancreatic Cancers Remain as Unsolved Health Problems

Colorectal cancer (CRC) cancer is the fourth most common cancer among men and women in the USA; it accounted for 9 % of all cancer deaths in 2012 [1]. Surgical techniques for CRC and survival after surgery have improved over the past 15 years. Surgery can cure approximately 90 % of CRCs when they are diagnosed without metastatic disease. The number of new cases and deaths from CRC in 2013 are estimated to be 142,820 and 50,830, respectively [2]. The 10-year survival rate for CRC limited to the mucosa is 90 %; with extension through the bowel wall, it is 70–80 %; with positive lymph nodes, it is 30–50 %; and with metastatic disease, it is <20 %. Most patients are diagnosed at late stages. If trends in reducing risk factors, increasing screening, and improving prevention and treatment strategies continue, CRC mortality could decline by a further 36 % by 2020. The predicted decline in CRC-associated mortality is contingent upon full delineation of the genetic and immunological changes associated with tumor initiation and progression. A complete genetic and immunological profile of CRC cells is expected to identify biological markers and molecular targets that guide disease prevention and treatment.

Despite recent advances in the use of genomic and proteomic analysis and in biomarker identification, these methods are yet to produce reliable candidate markers or targets for CRC. The current treatment modalities for CRC that has spread to lymph nodes include surgery and chemotherapy (predominantly with 5-fluorouracil, bevacizumab, and cetuximab). Despite their efficacy in increasing overall survival, the toxicity of chemotherapeutic drugs limits their use in combination with other agents (capecitabine, irinotecan, and oxaliplatin) and often makes surgery the only option. Additionally, the cancer cells may have intrinsic or acquired resistance towards these drugs. Therefore, there is an urgent need for development of improved prevention and treatment options for CRC.

Pancreatic cancer (PC) is the fourth leading cause of cancer deaths in both men and women in the USA. It is expected that in 2013, more than 45,000 Americans over 65 years old will be diagnosed with pancreatic cancer [3]. The Pancreatic Action Network estimates that by 2020, PC will become the second leading cause of cancer-related deaths in the USA. Pancreatic cancer is often difficult to diagnose and usually is identified at later stages, when it cannot be surgically resected. Even if the tumor is resected, recurrence of PC occurs in the majority of patients, and the median survival of these patients is only 18 months. Overall, the 1-year survival rate of people with PC is 26 %, and the 5-year survival rate is only 6 %. The lifetime risk of developing PC is 1.47 %. The high mortality rate is because of the usual presence of metastatic disease at the time of diagnosis. The cancer drugs approved for the treatment of PC are fluorouracil, erlotinib, gemcitabine, and mitomycin. Usually these drugs help in delaying cancer recurrence up to 6 months. The benefit of combining other drugs (cisplatin, irinotecan, paclitaxel, docetaxel, capecitabine, or oxaliplatin) with these standard drugs is very minimal [4–7]. Despite continued research, limited progress has been made in the prevention and treatment of PC.

In this chapter, the immunomodulatory effects of commonly used anticancer agents that have shown efficacy in preclinical models of CRC and PC and also in clinical studies will be discussed. In addition, antitumor and pro-tumorigenic immune cell functions will be briefly discussed, and the current status of agents that modify those

functions and the future prospects for the use of these agents in immunomodulation in standard prevention and treatment will be outlined. Moreover, the efficacy and toxicity patterns of various agents that belong to different classes including nonsteroidal anti-inflammatory drugs (NSAIDs), statins, selective estrogen receptor modulators (SERMs), rexinoids, antidiabetic, and natural anticancer agents will be discussed. Finally, strategies to select and develop agents to reduce immune cell toxicities and to improve natural killer (NK) cell cytotoxicity towards tumor cells and reduce regulatory T cell (Treg) functions for preventive efficacy in CRC and PC will be discussed.

13.3 Immunotherapy: Unsuccessful in Regressing Colorectal Tumors and Pancreatic Tumors

Many clinical trials have been carried out using therapeutic vaccine strategies (with tumor cells, specific tumor proteins, monoclonal antibodies (mAbs), multi-peptides, viral vectors, anti-idiotypes, naked DNA, and dendritic cells (DC)) alone or in combination with standard treatments for CRC; but unfortunately, none of the patients with metastatic disease has shown tumor regression. The reasons for failure to achieve retardation of tumor growth include tumor tolerance and immune suppression by tumor cells [8–10]. However, it is possible that immunotherapy may be more successful in patients with early stages of the disease when there is less tumor-induced immunosuppression. An in-depth understanding of the interactions between immune cells and tumor growth will help in better design and development of agents that augment antitumor immune responses or block or overcome resistance that is due to immune destruction, for prevention and treatment of CRC. Attempts at immunotherapy for PC treatment have been made over the past 30 years. However, trials to date have targeted tumors that are well established and already induce suppression of immune responses against PC [11, 12]. Unfortunately, these methods have been unsuccessful in regressing PC, with very few successes in other cancers. It is evident that most clinically apparent tumors have escaped adaptive immunity and developed resistance to immune pressure. To overcome these immunosuppressive factors, development of novel immune modulatory agents that can break immune tolerance against tumors alone or in synergy with standard preventive therapies is necessary.

13.4 Immune Surveillance and Tolerance During Initiation and Progression of Tumors

13.4.1 Tumor Microenvironment

The tumor microenvironment consists of normal epithelial cells, fibroblasts, infiltrating immune cells, extracellular matrix (protein fibers that hold cells together), cytokines, chemokines, oxygen, and nitrogen species. It is this tumor microenvironment that influences the growth, invasion, and metastasis of the tumor cells. Immune surveillance is a process that helps in destruction/elimination of the tumor cells before they can establish a tumor, progress, and metastasize. In a process called cancer immune editing, the tumors induce immune tolerance and suppression, which results in an increase in pathogenic behavior of tumor cells in the presence of immune effectors and leads to loss of immunogenicity. The immune editing involves the phases of elimination (initiation of antitumor responses), equilibrium (tumor cells that survive elimination will be stable, at peace with the immune system, and under control), and eventually an escape phase, in which the tumor cells overcome immune defenses, progress into invasive cancer, and metastasize. The immune surveillance theory, suggesting that a natural function of the immune system is to destroy dysfunctional or aberrant cells, was supported by observations of the infiltration of lymphocytic cells such as NK and natural killer T (NKT) cells into tumors; these infiltrates were taken to be a sign of good prognosis [13]. On the

other hand, lymphatic cell inactivation, immune escape, and the presence of high numbers and activity of Tregs are usually associated with poor prognosis and an indication of metastases [14]. Interactions between cytokines and chemokines produced by these immune cells, stromal cells, and tumor cells also play important roles and result in a balance between tumor promotion and tumor controlling effects. However, in a committed tumor cell, one cannot expect to have antitumor responses due to the presence of altered genomic status and tumor microenvironment that favor tumor growth.

13.4.2 Antitumor Innate and Adaptive Immunity

Naturally, the immune system detects and eliminates tumor cells, preventing them from establishing tumors. Various cells such as T cells and NK cells play vital roles in controlling the development of lesions *in vivo*. Cell-mediated immunity is provided by $CD4^+$ T cells, which identify peptides presented and activated by major histocompatibility complex (MHC) II molecules, and by $CD8^+$ T cells, which recognize peptides presented and activated by MHC I molecules and play an important role in adaptive anticancer immunity [15]. $CD4^+$ T cells, through T helper (Th) type 1 responses, including production of the cytokines interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and interleukin (IL)-2, can boost the functions of cells such as macrophages and NK cells and also support the clonal expansion of other cells like Cytotoxic T lymphocytes (CTLs). NK cells respond to the cytokines IFNs, IL-2, IL-12, and IL-15 with increased cytolytic, secretory, proliferative, and antitumor functions.

Dendritic cells play a central role in initiating and coordinating immune responses to tumor-associated antigens (Ag). Immature DCs actively internalize exogenous Ag by endocytosis, pinocytosis, and phagocytosis, resulting in the intracellular proteolytic processing of tumor-derived Ag into peptides that can be loaded onto MHC molecules. The majority of exogenous Ag-derived peptide associates with MHC II and is transported to the DC surface wherein it is engaged by MHC II/peptide-specific T-cell Ag receptors on the surface of $CD4^+$ class II-restricted T cells. DCs prime $CD4^+$ T cells, which subsequently develop into helper T cells skewed towards production of Th1 cytokines (IFNg, IL-12, IL-18), Th2 cytokines (IL-4, IL-10, IL-13), Th17 cells (IL-17, IL-23), and to regulatory T cells (Treg) [16]. Different $CD4^+$ T-cell subsets can therefore exert distinct effector functions towards tumors, promoting either tolerance or immune responses that destroy the tumor. Usually, Th1 responses provide antitumor functions, and any significant increase in Th2 responses leads to inhibition of tumor rejection. The tumorigenic cells can escape or develop tolerance against the immune surveillance through various mechanisms. Activated NK cells influence development of adaptive immune responses by regulating activation and maturation of DCs during tumorigenesis [17]. Also, DC cells are reported to trigger NK cell antitumor responses [18]. Despite this immune surveillance by various components of immune system, the preneoplastic lesions establish and develop into tumors that are clinically detectable.

13.4.3 Immune Responses During CRC

In CRC, chronic inflammation plays a vital role during transformation from a preneoplastic stage to adenomas and then into carcinomas, wherein cell-mediated immunity involving NK and CTLs is diminished and an increase in immune suppressing cells like Tregs and Th2 cytokines is observed as the disease progresses [13] (Fig. 13.1). The importance of activated tumor-specific $CD8^+$ cytotoxic T lymphocytes in tumor inhibition is supported by several reports [19–22]. Colorectal cancer patients with no signs of metastatic spread (vascular emboli, lymphatic invasion, or perineural invasion) had increased infiltration by immune cells (CTLs) with increased content of cytotoxins [23]. An improved survival was observed with

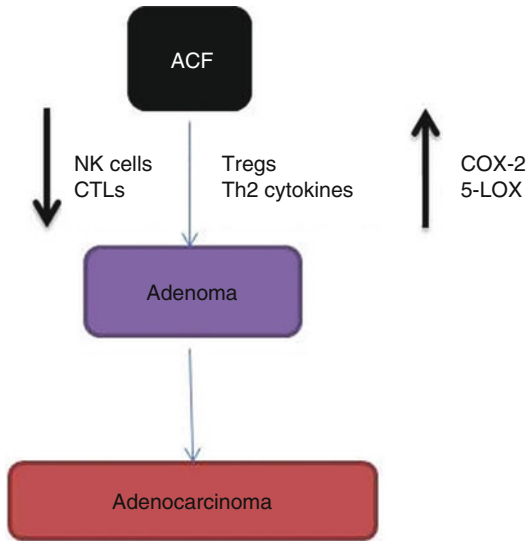


Fig. 13.1 A stepwise development of CRC includes ACF, adenoma, and adenocarcinoma formation which eventually metastasize. ACFs preneoplastic lesions are develop when an insult occurs in the colonic mucosa upon carcinogen treatment in rodents, and few of these develop into adenomas and adenocarcinomas. During this process, the NK cells activity towards tumor cell is inhibited by increased number and activity of Tregs with increased activity of COX-2 and 5-LOX

gastrointestinal cancer patients having granulocytes, lymphocytes, and macrophages mobilized at the invasive cancer border [24, 25]. There is a growing consensus based on vaccine trials that cooperation between $CD4^+$ Th1 cells and activated $CD8^+$ cytotoxic T lymphocytes is necessary for adequate antitumor immune responses. A very recent report suggested that NK cells can recognize and kill colorectal carcinoma-derived cancer-initiating cells through the natural cytotoxicity receptors Nkp30 and Nkp44 [26]. It is interesting to note that human tumors frequently lose expression of HLA molecules, and reduction or total loss has been confirmed in colorectal carcinoma [27–29]. Immune cells fail to recognize and exert function on the tumor cells because of deficient MHC signaling, whereas NK cells may recognize MHC class I-negative tumor cells. Thus, immunomodulatory agents that decrease number and activity of Tregs and restore NK cells may favor inhibition of CRC.

13.4.4 Immune Responses During PDAC

In pancreatic ductal adenocarcinoma (PDAC), a decreased number of NK and NKT cells are observed in patients with more aggressive tumor growth, and the numbers of these cells are inversely correlated with disease progression. Clinical samples of PDAC showed no NK cell infiltration, but significantly high numbers of macrophages were observed in patients with metastatic disease spreading to lymph nodes (Fig. 13.2). The presence of inflammatory cells contributes to the angiogenic phenotype in PDAC. Preclinical *in vivo* and *in vitro* studies have shown that IFN-α has a direct toxic effect on PC cells. A phase III trial combining chemoradiotherapy with IFN-α showed immediate activation of antigen-presenting cells (APCs) and NK cells followed by antigen-specific activation [30]. It is evident from these reports that restoration of NK cells and their functions by administration of immunomodulatory agents leads to enhanced cellular immune response against PC in clinical and preclinical models. PC cells can produce cytokines (including transforming growth factor-β (TGF-β), IL-10, and IL-6) and express surface antigens, such as programmed death-1 (PD1), indoleamine 2,3-dioxygenase (IDO), vascular endothelial growth factor (VEGF), and Fas-L that suppress immune responses against PC. Immune cell infiltrates consisting of tolerogenic DCs, tumor-associated macrophages (TAMs), and Tregs facilitate tumor growth and metastasis [31, 32] (Fig. 13.2).

$CD8^+$ T cells are the predominant immune cells found in pancreatic cancer. However, downregulation of activation markers on $CD8^+$ T cells is reported to reduce their cytotoxic effects towards tumor cells, suggesting that these cells become ineffective against tumors. Tregs, IL-6, and transforming growth factor (TGF)-β1 levels are high in locally advanced metastatic pancreatic cancer patients, and an increased number of Tregs was observed in peripheral blood of patients who had progressive pancreatic tumor growth. Hence, in PDAC, a high prevalence of Tregs seems to be a marker of poor prognosis

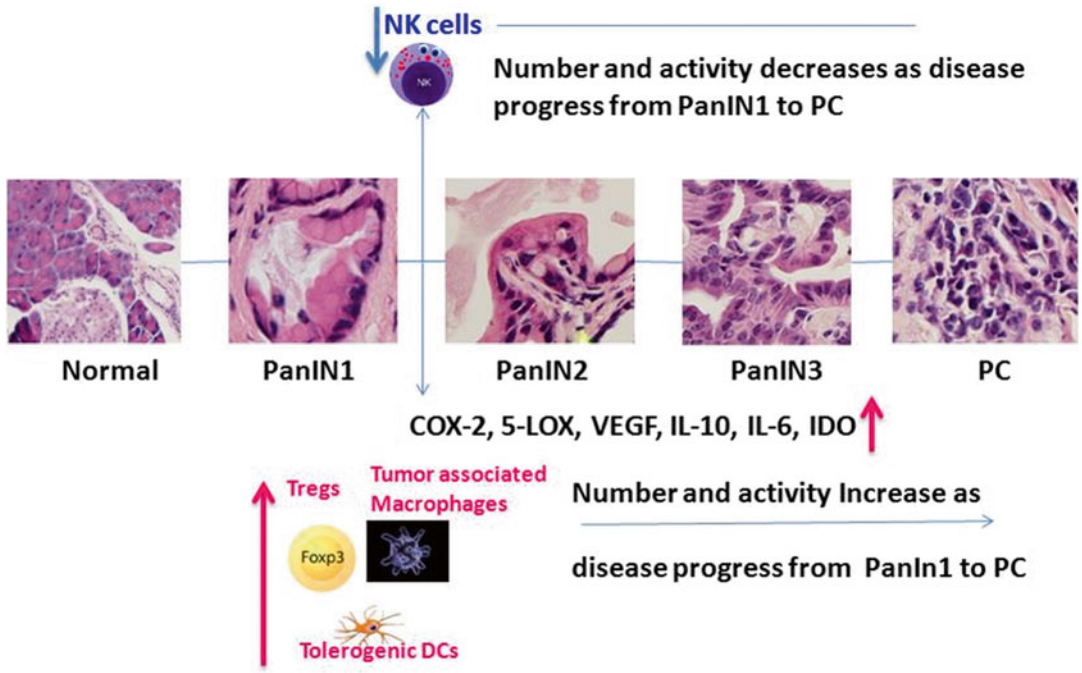


Fig. 13.2 Immune cell modulation during PanINs progression to PC. During stepwise PanIN 1 progression to PanIN 2, Treg number and activity increase, along with other immune cells such as tumor-associated macrophages, which makes Dcs tolerogenic towards PanIN 3 into PC progression. During this tumor initiation process, NK cell activity and number decrease which eventually helps the lesions to progress to PC. Also arachidonic acid metabolites, angiogenic factors, and cytokines (COX-2, VEGF, IL-10, IL-6 and IDO) are highly expressed and cooperate during PanINs progression to PC

[33]. These cells also were observed among tumor-infiltrating cells during multistage development of PDAC. In general, naturally occurring (n)Tregs help in developing self-antigen tolerance by suppressing NK cells, NKT cell activation, and maturation of DCs. DCs help to balance tolerance to self with elicitation of immunity towards tumors. DCs are reported to activate pancreatic stellate cells by increasing their chemokine production [34, 35], migration, and expression of toll-like receptors (TLRs). Infiltrating DCs in chronic pancreatitis exacerbate inflammation, which can lead to PC. Induced Tregs (iTregs) are responsible for regulating immunity against foreign (microbial) and tissue antigens. Tumor cells induce cytokines (IL-2, IL-10, TGF-β) and other enzymes (cyclooxygenase (COX)-2, 5-lipoxygenase (LOX), IDO) that can enhance production of Tregs and, thus, help the tumor escape from immune surveillance (Fig. 13.2). The observation of depleted Tregs helps to provide a mechanism for preexisting immune suppression by them against the tumor despite the presence of tumor antigens. The evidence from both preclinical and clinical studies supporting a critical role of Tregs in immune suppression and evasion by tumors suggests the importance of targeting Tregs or modulating their functions with preventive and therapeutic agents.

13.4.5 Immunomodulatory Effects of NSAIDs

Multiple endogenous mediators regulate differentiation, maturation, and activation of various immune cells. These include various cytokines and chemokines, bioamines, purines, and COX-/LOX-mediated production of lipid mediators derived from arachidonic acid (AA) such as pros-

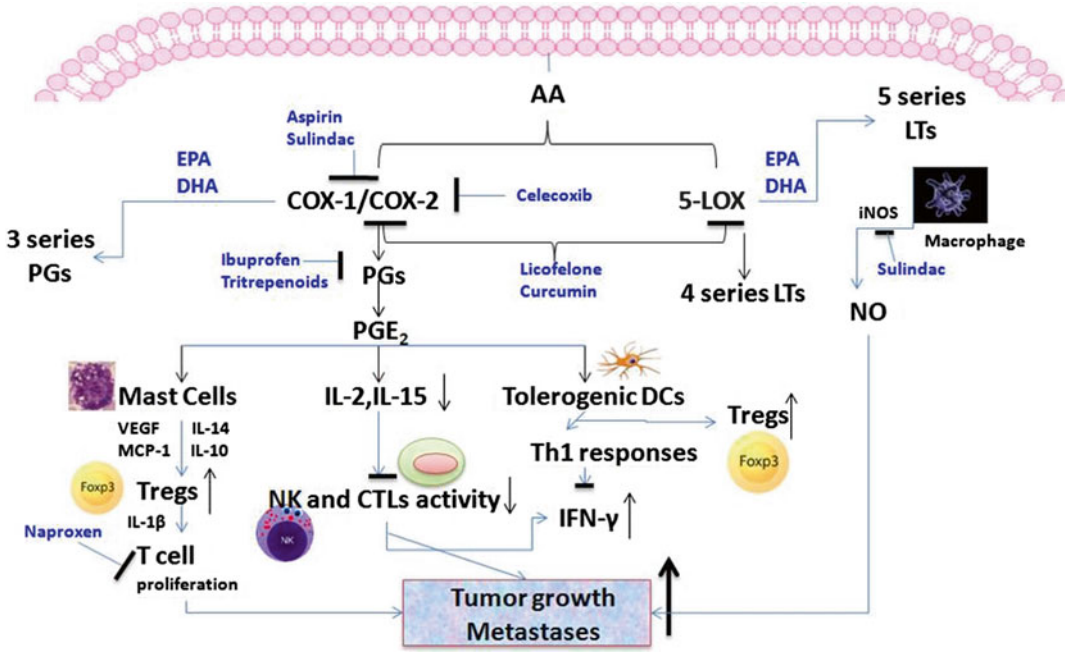


Fig. 13.3 Arachidonic acid (*AA*) metabolites are very well known for their tumor-promoting effects in colon and pancreatic cancer. Notably COX downstream molecule PGE-2 is reported to enhance tumor growth by helping in the development of tolerogenic DCs and Tregs and decrease NK cell activity. 5-LOX metabolites involve 4 series leukotrienes (*LTs*) which play vital role during tumor growth and progression. Formation of NO favors inflammatory conditions and helps in tumor growth and development. NSAIDs, COX-LOX inhibitors, and natural agents (triterpenoids, curcumin) are reported to inhibit COX-LOX activities and PGE-2 levels and inhibit tumor formation. Also, omega-3 fatty acids, EPA and DHA, modulate the COX-LOX activities by forming less potent metabolites such as three series PGEs and five series LTs

taglandins (PGs) and leukotrienes (LTs) that are recognized to have immune regulatory effects. NSAIDs, including salicylates (aspirin), propionic acid derivatives (ibuprofen, naproxen), acetic acid derivatives (sulindac), enolic acid derivatives (piroxicam), and selective COX-2 inhibitors (celecoxib), inhibit COX-1 and/or COX-2 and LOX pathways. Thus, they inhibit the production of PGs that contribute to pain and inflammation and are widely used for treating arthritis. They function as anti-inflammatory agents, but they may exert immune modulatory effects differentially on macrophages and lymphocytes.

COX-1 and COX-2 act on unsaturated 20 carbon essential fatty acids such as AA and generate prostaglandin H2 (PGH_2). Cell-specific PG synthases catalyze the conversion of PGH_2 to five primary bioactive prostanoids PGD_2, PGE_2, $PGF_2\alpha$, PGI_2, and thromboxane A2 (TXA_2). Each of these prostanoids is generated in macrophages and DCs. The prostanoids that are downstream of the COX-2 pathway influence many functions of cells involved in the immune system such as macrophages, DCs, NKs, and T and B cells and play an important role in the physiology and pathology of immunologic responses during inflammation, tumor growth, and development [36, 37]. The role of PGE_2 in these responses is prominent; it reduces activity of NK cells and CTLs by decreasing IL-2, IL-15, and, most likely, IL-2 production and expression of the IL-2 receptor on effector cells [36, 38, 39] (Fig. 13.3). It also reduces expression of IFN-γ by NK cells, thereby abrogating the NK cell helper function in mediating DC-induced Th1 responses [40]. Reduced NK cell function in response to PGE_2 increases establishment of metastases in preclinical animals [41]. PGE_2 also promotes the production of pro-angiogenic and immunosuppressive

VEGF and monocyte chemotactic protein 1 (MCP-1) by mast cells [37, 42] and leads to an increase in Tregs (Fig. 13.3).

Roles of COX and LOX are well established in CRC, wherein generated PGs and other mediators may initiate and promote cancer by suppressing immune responses, triggering cell proliferation, inhibiting apoptosis, and stimulating angiogenesis. These enzymes are also responsible for increased production of the Th2 cytokines IL-14 and IL-10, which help to increase Treg number and activity (Fig. 13.3). In tumor-bearing mice, Tregs can help switch Th functions and contribute to the increased inflammatory conditions promoting cancer in polyposis conditions. 5-LOX metabolites such as LTB_4 increase the activity of immune suppressor cells and increase growth of tumors.

Aspirin has received a lot of appreciation in preventive and therapeutic uses for its traditional anti-inflammatory properties and for having life-saving activity against cardiovascular events such as heart attacks. In a retrospective cohort study, aspirin use was associated with reduced cancer risk in a subgroup of patients whose colon tumors were expressing COX-2. However, aspirin inhibits both COX-1 and COX-2 (Fig. 13.3) and can lead to gastrointestinal (GI) ulceration, bleeding, and hemorrhagic stroke. The GI risk lies between 0.1 % and 1 %. Modified forms of aspirin, including nitric oxide derivatives such as NO aspirin, are gaining much attention due to their gastroprotective effects. Inhibition of COX pathways may cause an increase in LOX catalytic pathways, and LOX metabolites may cause deleterious effects and may stimulate cell proliferation in tumors. It has been established that low doses of aspirin trigger synthesis of factors that promote resolution of inflammation, called aspirin-triggered lipoxins (APLs), such as 15R-epilipoxin A4. Several large epidemiological studies have shown that long-term, frequent aspirin intake is associated with a 50–60 % reduction in the risk of colorectal adenomas and carcinomas [43–45]. The authors' preclinical results are consistent with inhibition of COX-2-mediated PGE_2 synthesis by aspirin; NO aspirin resulted in inhibition of CRC in both mice and rats [46].

Aspirin may also have some additional immunopharmacological properties, although available information is limited. An *in vitro* study demonstrated no effect of aspirin on spontaneous or INF-β-stimulated NK cell activity [47]. Therapeutic doses of aspirin have been shown to increase selectively nTreg number and function in mice [48, 49]. Various preclinical evidence has shown that aspirin may have profound effects on maturation and differentiation of different DC phenotypes, causing DC tolerance and impairing antigen presentation by these cells. Aspirin-induced tolerogenic DCs may help in de novo generation of Tregs or in induction of regulatory functions in naïve T cells. These reports suggest that aspirin may cause immune suppression by directly enhancing Treg activity or indirectly by generating tolerogenic DCs. Hence, aspirin has potential to suppress immune responses in certain diseases such as autoimmunity. The authors and others have demonstrated the existence of Tregs in CRC during initial stages of the disease progression. Thus, although, meta-analyses and systematic reviews suggest that aspirin use is associated with a decreased incidence of colonic adenomas, colorectal cancer, metastatic colorectal cancer, and death due to colorectal cancer [50, 51], one needs to be cautious with the use of aspirin for cancer treatment in cases where immunosuppression by Tregs is observed. The potential benefit of aspirin in cancer is dependent on dose, timing, and number of years of use [44]. At least 7–10 years of aspirin use at 325 mg daily has been shown to decrease cancer. Similar estimates were made in a later meta-analysis of four placebo-controlled trials of aspirin; the beneficial effects of aspirin in reducing cancer risks were very consistent in various clinical trials [52]. Several intervention trials have evaluated the benefits of aspirin in prevention trials, and aspirin showed modest benefit on reducing recurrence of colorectal adenomas. These studies were conducted in patients already at the adenoma or cancer stages and not simply in patients having a risk of developing adenomas. Two meta-analyses have been published which suggest that the risk of recurrent adenomas is reduced by about 13–18 % [51, 52]. Trials focusing on prevention

of colon cancer by preventing adenoma recurrence did not show consistent results with the use of aspirin [43, 53–59]. A recent report on regular use of aspirin in a cancer screening trial showed a reduced risk of hyperplastic and adenomatous polyps, with a slightly greater risk reduction in an age group of 70–74 compared with the 55–69 age group [60]. Overall, these trials suggest that prolonged use of aspirin reduces the risk of colon cancer. A recent study in 904 pancreatic cancer patients and 1,223 similarly matched healthy controls suggested that regular use of aspirin at least once a month resulted in 29 % reduced risk of developing PC. Those who took low-dose aspirin regularly had a greater 35 % risk reduction for PC. In a preclinical study, LsL-KrasG12D;LsL-Trp53R172H;Pdx1-Cre transgenic mice were randomly assigned to receive mock treatment, gemcitabine, or a combination of gemcitabine and aspirin; results showed suppression of Tregs in the mice receiving the combination treatment [61], which, in part, may have affected the increase in survival of diseased animals.

Ibuprofen shares with other NSAIDs such as aspirin the ability to inhibit the activity of COX-1 and COX-2, thus reducing the generation of pro-inflammatory stimuli. Lower ibuprofen drug concentrations act as activators of splenocyte proliferation, possibly through inhibition of thromboxane (TX)/hydroperoxyeicosatetraenoic acid (HETE) synthesis. Higher drug concentrations also activate splenocyte proliferation through inhibition of PGE2 and PGI2 [62]. Thus, both high doses and low doses of ibuprofen are able to regulate PG synthesis in cells (Fig. 13.3). Dietary administration of ibuprofen at 400 ppm resulted in a 41 % reduction of azoxymethane (AOM)-induced colon tumor incidence in F344 rats [63]. Ibuprofen caused a significant growth inhibition of HT29 xenografts overexpressing Rac1b (as occurs in serrated tumors having BRAF mutations), but the growth inhibition was independent of COX inhibition [64]. These findings indicate that the beneficial effect of NSAIDs in CRC may not rely solely on an anti-inflammatory response. Cameron et al. [65] reported that ibuprofen transformed noncytotoxic macrophages into cytotoxic ones and also enhanced the cytotoxic activity of macrophages in colon cancer patients [65]. A case-controlled study showed that, as with low-dose aspirin, intake of one or more pills per week of regular ibuprofen (200 mg) was associated with a significant (~68 %) reduction in the risk of colon cancer [66, 67]. A study in a large general risk population supports previous work showing that recent use of ibuprofen is associated with a decreased risk of colorectal adenomas [60]. Ibuprofen also shares with aspirin similar GI toxicity, which restricts the use of this drug to patients suffering from ulcerative colitis. Hence, a derivative of ibuprofen, phospho-ibuprofen, was designed and tested by the authors and others in AOM-induced colon cancer in F344 rats and was observed to have better efficacy in inhibiting development of aberrant crypt foci (ACF) and colon cancer compared with ibuprofen. The authors also tested phospho-ibuprofen in a transgenic mouse model of PC and found that it significantly inhibited progression from pancreatic intraepithelial neoplasias (PanINs) to PDAC.

NSAIDs have been shown to reduce spleen lymphocyte proliferation and to inhibit T-cell proliferation [68–70]. The S enantiomer of naproxen was shown to cause apoptosis of polymorphonuclear neutrophils (PMNs), which is a crucial mechanism for PMN, removal while resolving inflammation [71]. Immunotoxicology studies to assess the effects of naproxen on cell-mediated immunity showed no effect on NK cell activity [72]. Although high (supra-pharmacologic) concentrations of these drugs have been shown to induce some *in vitro* immunomodulatory effects on the innate immune system, they failed to show any effects *in vivo*. More detailed studies of naproxen effects *in vivo* are therefore needed to understand how it influences immunity in cancer patients.

Anti-inflammatory NSAIDs releasing NO (NO-NSAIDs) are a new class of anti-inflammatory drugs to which an NO-releasing moiety is added. NO-naproxen was developed with the goal of reducing the gastrointestinal toxicity associated with regular use of NSAIDs. These compounds have been shown to retain the

anti-inflammatory, analgesic, and antipyretic activity of the parent compound but to be devoid of gastrointestinal (GI) toxicity [73]. Naproxen was reported to reduce IL-1β, but NO-naproxen reduced both IL-1β and TNF-α plasma levels [74]. NO-naproxen and naproxen both reduced the proliferation of T cells, whereas naproxen needed double the dose of NO-naproxen to show a similar function (Fig. 13.3). NO-naproxen has a greater effect on T-cell responses due to the presence of the NO moiety [74]. Both agents showed potent anti-inflammatory and antitumor properties in preclinical animal models of CRC.

$Apc^{Min/+}$ mice genetically predisposed to spontaneous development of colon tumors who were fed low-dose naproxen for 45 days had 70.3 % fewer small tumors than the control animals, and mice fed low-dose NO-naproxen had 64.0 % fewer tumors than control animals. An 89.3 % reduction in microadenomas was observed when mice were fed high-dose naproxen. Relevant human serum concentrations of naproxen (1 μg/mL) were found to have chemopreventive effects in human colon polyp cells and to reduce the expression of signaling pathway genes (Ras, Fos, Myc, Jun, Vav1, ELK1, ELK4, multiple map kinases, and TGFB receptor (R) 1) implicated in cancer and growth [75]. Sulindac and naproxen, individually and in combination with atorvastatin, caused significant reduction in AOM-induced colon tumors in F344 rats and inhibited key inflammatory markers such as inducible nitric oxide synthase (iNOS), COX-2, and phospho-p65 as well as the inflammatory cytokines TNF-α, IL-1β, and IL-4. These studies also provided evidence for a strategy of NSAID combination with statins for reduced GI toxicity.

Sulindac has long been used to help prevent the development of intestinal polyps that can lead to colon cancer and is well established as a chemopreventive agent for CRC. Min mice receiving sulindac had fewer intestinal tumors [76]. In a randomized, placebo-controlled, double-blind study, administration of sulindac at dose of 300 mg/day for 6–12 months resulted in complete eradication of polyps in patients with familial adenomatous polyposis (FAP) [77]. Sulindac and several other NSAIDs were able to control growth of desmoid tumors in patients affected by FAP or Gardner's syndrome [45, 78–80]. A significant reduction in the size of adenomas was reported in FAP patients after long-term therapy with sulindac [77, 79–81]. As in animal studies, sulindac was more effective when given at early stages of tumor development. Preclinical *in vivo* studies showed that sulindac inhibits dimethylhydrazine (DMH)-induced colon tumor incidence and multiplicity in mice when administered in the diet throughout the period of carcinogen administration, but not when given 17 weeks after DMH administration [82, 83]. In another study, sulindac (10 mg/kg body weight administered twice daily by gavage) inhibited DMH-induced primary colon tumor development and growth in rats. Ahnen et al. [84] showed that dietary administration of sulindac and its metabolite sulindac sulfone significantly inhibited AOM-induced colon carcinogenesis in F344 rats [84]. Sulindac inhibits COX activities as well as polyamine synthesis, for which it has been combined with the ornithine decarboxylase inhibitor DFMO in a large clinical trial for chemoprevention of sporadic colorectal adenocarcinoma [85, 86]. A striking chemopreventive effect was observed in the combination arm, with a 95 % decrease in advanced adenomas, which are most likely to progress to carcinoma [87]. Sulindac inhibition of COX resulted in inhibition of β-catenin signaling by enhancing its degradation in colon cancer [88]. Several studies have demonstrated the effectiveness of sulindac in reducing the size and number of adenomas in familial polyposis [77, 89]. Recently Stein et al. [90] reported that sulindac was a potent inhibitor of metastatic tumors in colon cancer [90]. There is a possibility that sulindac may exert an inhibitory effect on IFNγ-inducible chemokine expression and thus reduce the host immune response against tumorigenesis. Sulindac was shown to inhibit selectively IFNγ-induced expression of the chemokine CXCL9 in mouse macrophage RAW264.7 cells without affecting IFNγ-induced signal transducer and activator of transcription 1 (STAT1) activation [91]. The mechanism for the selective inhibition of CXCL9 is not yet known.

Many studies have shown that several PG synthesis inhibitors, such as indomethacin and piroxicam, suppress colon carcinogenesis in laboratory animal models [92–98]. Piroxicam was found to be an effective chemopreventive agent when administered in the diet during the initiation, post-initiation, and progression stages of colon carcinogenesis in laboratory rodents [92, 93]. Piroxicam at 200 and 400 ppm caused a dose-dependent decrease (45–64 %) in colon tumor incidence in F344 rats [93]. At a dose of 20 mg/day, it reduced mean rectal prostaglandin concentration by 50 % in individuals with a history of adenomas [99]. Bayer et al. [69] and Muller et al. [100] reported decreased expression of Treg-associated molecules such as forkhead box P3 (FOXp3) and IL-10 in indomethacin-treated tumors (Fig. 13.3). Based on evaluation of various immune cell markers [(HLA-DM, HLA-DO (peptide loading), HLA-DP, HLA-DQ, HLA-DR (antigen presentation), granzyme B, H, perforin and FCGR3A (CD16) (cytotoxicity), $CD8^+$ cytotoxic T lymphocyte and $CD4^+$ T helper cells]. Lonroth et al. [101] suggested that specific and nonspecific NSAIDs (indomethacin and celecoxib) alter colorectal cancer progression by affecting immune surveillance. They showed that indomethacin treatment increased in the infiltration of B cells, macrophages, $CD4^+$ T helper cells, and $CD8^+$ cytotoxic T lymphocyte and reduced Foxp3 expression in tumor tissue [101]. These NSAIDs have immunomodulatory effects in addition to their effects on AA metabolism that contribute to tumor inhibition.

Specific COX-2 inhibitors were designed to achieve greater efficacies and reduce GI toxicity. These drugs showed consistent strong chemopreventive effects in preclinical animal models [102, 103]. However, even the nonspecific COX inhibitor aspirin showed risk reductions similar in magnitude to specific COX-2 inhibitors. Studies by Talmadgea et al. [104] reported a significant expansion of immature myeloid suppressor cells (IMSCs) (phenotype $Gr1^+CD11b^+$ cells) and a reduction in $CD4^+$ lymphocytes in the spleen during growth of 1,2-DMH-induced intestinal tumors in mice. The tumor-bearing mice showed increased expression of inflammatory molecules such as COX-2, iNOS, and arginine. Therapy with clinically relevant doses of celecoxib resulted in a reduced number of colon tumors and delayed tumor development by reducing immunosuppressive IMSCs. This study supports the role of COX-2 in inducing immunosuppressive activity during tumor development by causing apoptosis of $CD4^+$ T cells. A population-based retrospective cohort study of individuals aged 65 years and older suggested that long-term use of non-aspirin NSAIDs was associated with reduced risk of CRC [105]. A randomized clinical trial with celecoxib showed significant reduction in the incidence of colonic adenomas [106, 107].

Chemopreventive effects of celecoxib and rofecoxib with daily standard dosages (200 and 25 mg, respectively) were demonstrated as a 69 % decrease in CRC. These drugs were approved in 1999; however, rofecoxib was withdrawn from the market in 2004 due to increased risk of cardiovascular events [108–110]. During this very short window of usage, both of these drugs showed significant reductions in CRC, but their associated risk of cardiotoxicity resulted in new searches for better COX inhibitors that retain potent preventive properties without the toxic effects. Significant inhibition of PanIN progression to PC in transgenic KrasG12D/+ mice upon dietary administration of the dual COX-LOX inhibitor licofelone has been reported by the authors [111] (Fig. 13.3). This study suggests the use/development of agents that can show well-balanced inhibition of COX and 5-LOX activities, with high efficacy in tumor inhibition and less or no toxicity. A complete blockade of progression of PanINs to PC was observed when licofelone was combined with gefitinib in transgenic KrasG12D/+ mice [112]. Hence, targeting multiple pathways with low nontoxic doses of potent agents is encouraging in prevention of PC.

Immunological effects of NSAIDs or non-NSAIDS in PC have not been studied much. The available literature on risk reduction in CRC and PC with NSAID usage suggests that chronic unresolved inflammation involving immune cells plays a critical role in tumor initiation, growth, and development. A thorough understanding of

the functions of NKs, T cells, macrophages, and their related cytokines during different stages of tumor development is needed to help find effective approaches and to establish appropriate doses and administration schedules to suppress the tumors.

13.5 Immunomodulatory Effects of Statins

Since their discovery in 1976, statins have been in use for their beneficial effects in reducing serum cholesterol, low-density lipoprotein (LDL) and triglyceride levels and for increasing high-density lipoprotein (HDL) cholesterol [113]. Statins exhibit these effects by inhibiting HMG-CoA reductase in the cholesterol synthesis pathway (Fig. 13.4). Downstream metabolites of this pathway regulate posttranslational prenylation of Rho and Ras. Statins inhibit isoprenylation of Rho and Ras, which inhibits function of these proteins in cell mobility, shape, proliferation, differentiation, and survival. Since HDLs enhance anti-inflammatory responses by reducing cytokines (TNF-α, IL-1), statins also exhibit anti-inflammatory effects and are effective against inflammation and CRC in preclinical animal models. Although the beneficial effects of statins are predominantly via their lipid-lowering properties, they recently have been noted to have additional immunomodulatory actions that might exert beneficial effects. There is ample evidence that they exert anti-inflammatory and anti-oxidative actions and can induce tumor suppressors in colorectal cancer. A trial of statin therapy was conducted in Northern Israel between 1998 and 2004 in a population-based, case-controlled

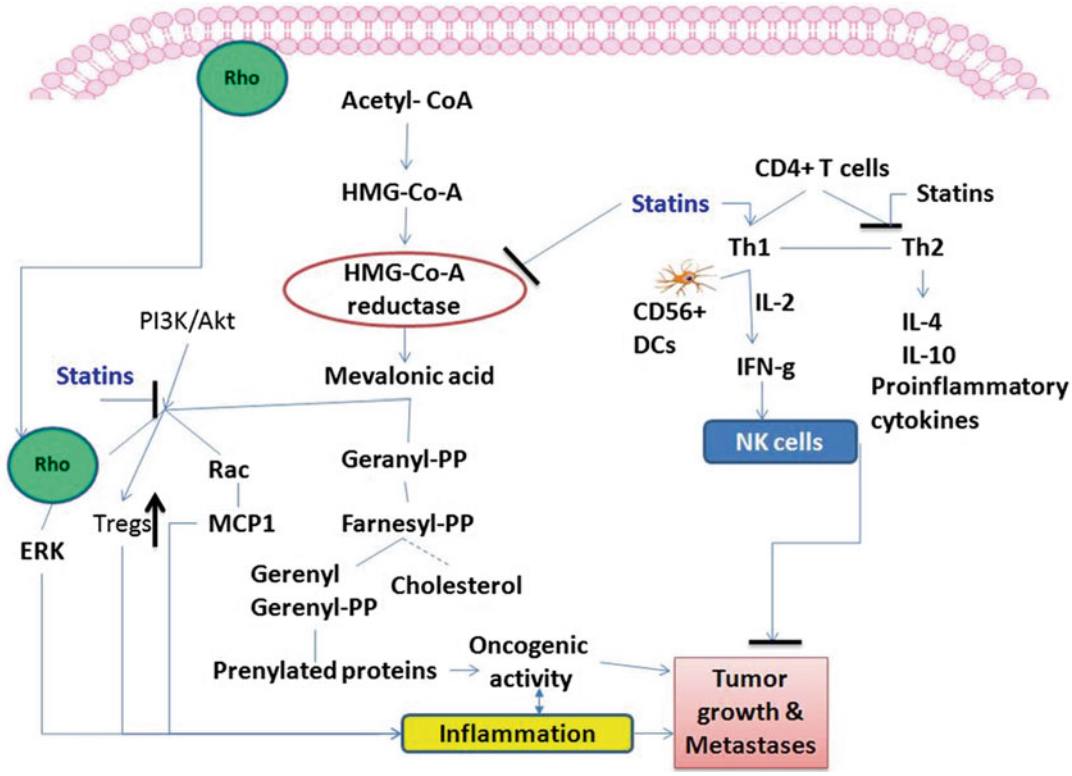


Fig. 13.4 Statins inhibit the cholesterol metabolite pathway and reduce the activation of Rho and Rac family proteins which are involved during tumor formation. Also, statins help in modulation of $CD4^+$ T cells towards Th1 differentiation in the presence of $CD56^+$ DCs that will activate NK cells which help in eliminating tumor cells. Further statins inhibit formation of pro-inflammatory cytokines

study of patients with a diagnosis of CRC. A modest reduction in CRC was shown in the general population without inflammatory bowel disease (IBD), but a substantial 94 % risk reduction was observed in a small number of IBD patients in a subset analysis [114]. A recent meta-analysis of 16 studies involving 1,692,863 participants and 7,807 PC cases showed no association between statin use and PC [115]. However, in a transgenic KrasG12D/+ mouse model, the authors and others have shown delayed progression of pancreatic lesions PanIN 1, PanIN 2, and PanIN 3 to PDAC in atorvastatin-treated mice compared with untreated controls via modulation of phosphatidylinositol 3-kinase (PI3K)/AKT signaling molecules [116]. Since the Akt pathway regulates Foxp3 expression and Treg development in the thymus, atorvastatin may have delayed PC development by decreasing Treg development [117, 118] (Fig. 13.4). Another study reported that fluvastatin and lovastatin reduced liver tumor formation and growth of established liver metastases in PC [119].

Although there are studies suggesting immunosuppressive function of statins, it was shown that statins act cooperatively with IL-2 to induce IFN-γ production in CD56dim NK cells [120–122] (Fig. 13.4). Previously, it was reported that statin effects on NK cells were inhibitory, but all of those studies focused on NK cell-target cell interactions with purified NK cells in co-culture with target cells [123–126]. In contrast, Gruenbacher et al. [121] reported that statins enhanced tumor death by increasing NK cell activity against tumor cells and increasing INF-γ production, effects mediated by $CD56^{+}HLA\text{-}DR^{+}CD14^{+}$ DC-like accessory cells (Fig. 13.4). Atorvastatin and pravastatin showed anti-inflammatory properties by attenuating T-cell activation and proliferation [127, 128]. Mausner-Fainberg et al. [129] reported that treatment of human peripheral blood mononuclear cells (PBMCs) with atorvastatin, but not with mevastatin or pravastatin, increased the number of $CD4^{+}CD25high$ and $CD4^{+}CD25^{+}Foxp3^{+}$ cells [129]. No difference was seen in Tregs in C57BL/6 mice with these agents. This report suggests that each statin is different in its mode of function. In trials of statin treatment for CRC, we showed decreased expression of COX-2 by rosuvastatin in AOM-induced colon tumors in rats, suggesting anti-inflammatory effects. Simvastatin was reported to reduce expression of the chemokines MCP1, MIP1α, and MIP1β as well as of the chemokine receptors CCR1, CCR2, CCR4, and CCR5 in human macrophages [130]. A clinical trial showed blocked expression of T-cell activation markers by atorvastatin [131]. A recent study showed that atorvastatin significantly decreased the expression of six cytokines (IL-6, IL-8, IL-1, plasminogen activator inhibitor type (PAI-1), TGF-β1, TGF-β) and five chemokines (CCL2, CCL7, CCL13, CCL18, CXCL1) and affected the expression of many inflammatory genes, as analyzed via DNA microarray analysis of human peripheral blood lymphocytes from normal subjects [132].

Fluvastatin demonstrated inhibitory activity against colitis and carcinogenesis in a mouse model, and it reduced oxidative DNA damage and activity of the DNA-synthesizing enzyme thymidine kinase in colorectal tissues [133]. Simvastatin was reported to cause significant reduction of tumor development by induction of apoptosis and suppression of angiogenesis in another colitis-associated CRC model. It also suppressed tumors by inhibiting VEGF and inducing apoptosis of tumor cells in a colon cancer xenograft model. Pitavastatin was effective in inhibiting AOM- and dextran sulfate sodium (DSS)-induced colitis-related colon carcinogenesis and significantly inhibited multiplicity of colon adenocarcinoma through modulation of mucosal inflammation, oxidative and nitrosative stress, and cell proliferation [134]. A 3-month treatment with 80 mg per day of atorvastatin resulted in reduced plasma chemokine CXCL10 levels and inflammation in Crohn's disease patients [135]. Atorvastatin therapy was shown to reduce inflammation by reducing monocyte migration [136, 137]. The authors recently presented evidence for chemopreventive effects of rosuvastatin in AOM-induced colon tumors, with significant inhibition of the transformation from adenoma to adenocarcinoma [138]. Although this statin has the greatest lipid-lowering effect, con-

cerns about its safety have been raised. It is evident from all these reports that considerable progress has been made in our understanding of some of the mechanisms underlying the beneficial effects of statins and that these include some actions besides inhibition of HMG-CoA reductase. The available preclinical animal data are supportive of chemopreventive properties of statins [133, 134, 138], but very little is known about the effects of these agents in high-risk subgroups, such as patients with resected colon cancer, and the effectiveness of statins as chemopreventive agents remains to be established in prospective randomized trials. Design and development of new statins and improved understanding of how statins manipulate immune cell functions is warranted to inhibit pro-inflammatory conditions in CRC and PC.

13.6 Immunomodulatory Effects of Selective Estrogen Receptor Modulators

17β-Estradiol is the most potent endogenous ligand for the estrogen receptor (ER)-β, but it binds equally well with ER-α and ER-β. The distribution and also the functions of these receptors are different in different tissues, and these features were behind the drive to identify or design selective ligands or selective estrogen receptor modulators (SERMS). Evidence gathered in recent years demonstrates roles for sex hormones in immunity. A 17β-estradiol- dependent increase in FOXp3 and PD1 expression with expansion of $CD4^{+}CD25+$ T cells in mice has been suggested [139, 140]. And an enhanced functional activity of Tregs in suppressing immunity was observed upon 17β-estradiol treatment in mice [140]. It is evident that high Treg numbers and associated PD1 expression in CRC and PDAC are associated with poor prognosis. In ER-α knockout (KO) (Esr1–/–, ERKO) and ER-β KO (Esr2–/– (BERKO)) mice, Treg functional capacity and PD1 expression were significantly reduced compared with wild-type mice [140] (Fig. 13.5). Pretreatment with 17β-estradiol partially restored the functional Treg suppression of PD-1 KO mice [140].

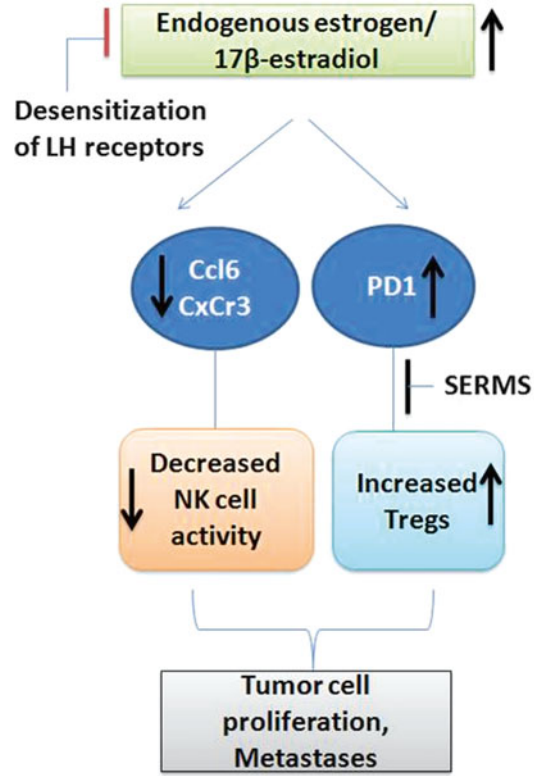


Fig. 13.5 Increased endogenous estrogen levels are linked to formation of more colonic tumors. They are reported to decrease the NK cell activating cytokines and chemokines and increase Tregs by increasing PD1 expression. Selective estrogen receptor modulators are reported to decrease formation of Tregs and CRC

17β-Estradiol is reported to enhance metastasis of both syngeneic and allogenic tumors in nude mice [141]. A link between female hormones and PC has been controversial. ERs were observed in both PC and normal pancreas [142], but a higher level of estrogen binding was reported in patient PC tissues compared with controls [142]. High ER-β RNA levels were reported in PC [143]. Specifically, ER-β2 mRNA was observed to play a more important role than ER-α in PC. However, a weak or no association of PC risk with the use of hormone replacement therapy (HRT) or contraceptives was reported in five cohort studies [144]. A similar result was reported in five case-controlled studies [144]. There is not much convincing data for a hormone dependence of PC, arguing against a role of

estrogens or their antagonists in preclinical or clinical prevention or treatment of PC.

In nude mice with colon cancer xenografts, prolonged systemic administration of 17-estradiol suppressed NK activity resulting in frequent and large metastases to the liver and lungs and the mesenteric, omental, and mediastinal lymph nodes, supporting the role of NKs in controlling metastatic potential of primary tumors [145]. We have observed increased Ccl6 and Cxcr3, indicating increased NK cell activity, with suppressed colon tumor formation by decreasing endogenous estrogen production through desensitization of luteinizing hormone (LH) receptors in an $Apc^{Min/+}$ mouse intestinal tumorigenesis model [146] (Fig. 13.5). Hence, the use of SERMs may provide protection in CRC by enhancing NK cell activity against tumor growth and suppressing Treg functions.

Anti-inflammatory properties of estrogens have been studied extensively [147]. Estrogen therapy lowers cytokine levels in postmenopausal women [148]. Estrogens modulate inflammatory genes through ER-α and ER-β. Since the discovery of ER-β, scientists have made enormous progress in understanding its biology. Its role is well established in the ovary, cardiovascular system, and brain, as well as in several animal models of inflammatory diseases, but less is known of its role in CRC and PC. Estrogens and their receptors are important regulators of colon physiology, but the roles in colonic epithelial cells are not well understood, and there is controversy about the role of ERs in inflammation and CRC [149]. One study reported that loss of ER-β did not cause any colonic tumor formation, suggesting that it is not associated with colonic neoplasia, nor were any significant differences found in colonic tumor formation in ApcMin/+ and ER-β–/– compound mice [150]. Some studies have indicated that ERs can suppress pro-inflammatory genes such as *IL-6* and TNF-*α* [151–153], whereas both ER-α and ER-β have been implicated in repression of inflammatory genes by 17β-estradiol, with ER-β ligands exhibiting more potency in repressing genes induced by TNF-α [154].

Menopausal HRT use has been associated with a decreased colorectal cancer risk, suggesting that estrogen signaling is involved in colon physiology and cancer etiology. However, a French E3N prospective cohort study suggested that while menopausal HRT was not associated with significant colorectal adenoma or adenocarcinoma risk, any use of estrogens alone was associated with the risk of colorectal adenoma and cancer in opposite directions [155]. A very recent report suggests that estrogens promote tumor development in the context of inflammatory damage in a DSS-AOM mouse model. In this study, estrogen-treated animals had formation of invasive adenocarcinomas as compared with untreated animals. The pro-tumorigenic effect of estrogen was related to both ER-α and ER-β. A preclinical AOM-induced colitis model in an ER-β knockout mice revealed that ER-β-deficient animals had greater numbers and sizes of colon polyps with increased expression of IL-6, IL-17, and TNF-α. These results suggest that, in the presence of carcinogen insult, ER-β may provide protection against inflammatory conditions by healing mucosal damage. However, there is evidence suggesting a positive role of ER in enhancing colon tumor formation. The authors have reported an effect of the phytoestrogen genistein in enhancing AOM-induced colon carcinogenesis [156]. This result is consistent with evidence of the association of ER-β expression with elevated cell proliferation markers in tumors [157]. Data represented by the authors suggest that raloxifene, an ER-β antagonist, significantly inhibits AOM-induced formation total ACF (31–40 %) and multicrypt ACF (23–50 %) and suppresses colon adenocarcinoma multiplicity (to 3.28 ± 0.31 with 1.5 ppm raloxifene; to 2.96 ± 0.30 with 3 ppm raloxifene) in F344 rats [158, 159]. It is also observed >70 % fewer polyps with sizes of >1 mm upon raloxifene treatment in $Apc^{Min/+}$ mice [16], and a significant decrease in AOM-induced colonic adenocarcinomas associated with inhibition of the Th2 cytokine IL-4 is found with raloxifene treatment [159] (Fig. 13.5).

Our findings and others suggest that modulation of immune cells by suppressing endogenous estrogen levels in CRC and targeting ERs may be useful as a potential preventive approach to modulate inflammatory responses in CRC [160]. Although the precise clinical indications still are being

defined, the reported mechanistic and animal studies suggest that ER-modulating agents might represent a new class of drugs to prevent and treat inflammatory disorders. Ultimately, clinical trials with synthetic as well as natural plant-derived ER-β-selective compounds are needed to assess the potential translation of drugs that target ER-β for the prevention and treatment of human CRC associated with inflammatory disorders. No preclinical data exist on the use of SERMs in immunomodulation or prevention of PC.

13.7 Immunomodulatory Effects of Rexinoids

Retinoid X receptors (RXRs) and their rexinoid ligands regulate a family of genes having RXR receptor elements. They control differentiation, growth, and, in certain situations, induce apoptosis in tumor cells [161]. Rexinoids can cause cell differentiation and proliferation in T lymphocytes and inhibit activation and prevent apoptosis in B cells [162]. These effects are predominantly through the IL-2R in T and B cells; rexinoids may induce expression of IL-2Rα [163]. The RXR agonist AGN194204 enhanced Th2 development upon antigenic stimulation with splenic APCs [164] (Fig. 13.6). RXRα1/− mice were more susceptible to inflammation than are wild-type mice, and the RXR agonist LG101305 reduced IL-1β expression, providing genetic evidence that RXRs are protective and have anti-inflammatory functions [165]. A chemopreventive property of the rexinoid bexarotene in an $Apc^{Min/+}$ colon cancer model is previously reported by the authors. It decreased the inflammatory cytokines granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-2 and IL-12, increased RXRα levels, and suppressed total intestinal polyps and colon tumors in male and female mice, by 38.2–9.9 % ($P<0.015$ to $P<0.0001$) or 8.5–36.9 %, respectively ($P<0.007$) [166] (Fig. 13.6). A significant inhibition of colonic adenocarcinomas was observed with bexarotene alone and in combination with raloxifene treatment administered in the diet to F344 rats 8 weeks after AOM treatment [159]. Bexarotene-treated colonic tumors

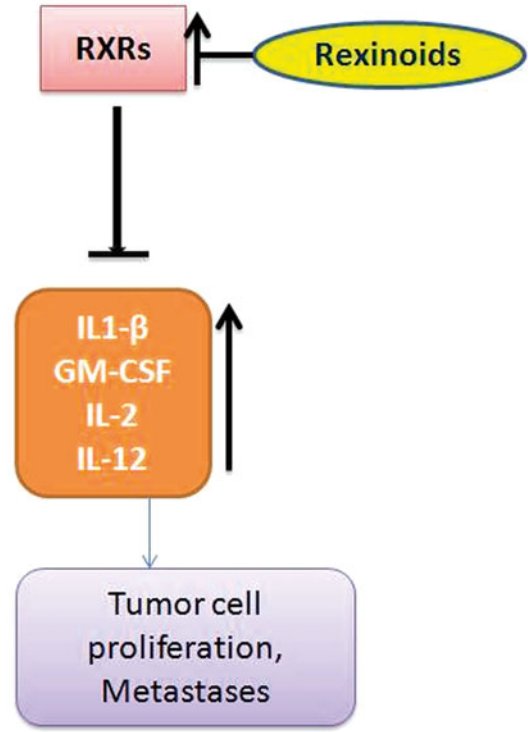


Fig. 13.6 Decreased expressions of RXRs are observed in CRC. Rexinoids increase RXR expression and decrease pro-inflammatory cytokines and increase the Th2 cytokine productions which will help in immunity development against tumor formation

exhibited decreased IL-6 expression [159]. A recent study by Liby et al. [167] reported that the rexinoid LG100268 (LG268), alone or in combination with triterpenoids, increased survival in a *LSL-Kras*$^{G12D/+}$;LSL-Trp53$^{R172H/+}$;Pdx-1^{-Cre} (KPC) triple mutant mouse model of PC. Collectively, these observations support a role for RXRs in immune cells, but, to our knowledge, there are no reports on the specific functions of RXRs in immune cells such as NKs, DCs, and Tregs in CRC and PC.

13.8 Immunomodulatory Effects of Antidiabetic Agents

Many genes involved in diabetes play a role in cancer progression. There is mounting data suggesting that diabetics are more prone to certain

cancers [168]. The antidiabetic drug metformin activates AMP kinase (pAMPK) in liver cells and inhibits production of glucose. Thus, it affects insulin signaling, whole body metabolism, and energy balance. Metformin was reported to increase immune memory cells and $CD8^+$ T cells and to enhance tumor regression [169]. The use of metformin has been linked to a reduction in overall cancer incidence and specifically to reduction of breast cancer and PC. A report by Bodmer et al. [170] suggested that metformin was associated with a slightly increased risk of colon cancer (observed risk 1.43; 1.81 in men *vs.* 1.00 in women), and our results with metformin in CRC and PC are similar. In contrast, Hosono et al. [171] tested metformin for the inhibition of intestinal ACFs in BALB/c mice, where ACFs were formed after 6 weeks of AOM treatment and polyps after 32 weeks, and found a significant inhibitory effect on ACFs and a modest effect on polyps, suggesting that metformin may possess chemopreventive properties. The same group showed an inhibitory effect of metformin (250 mg/day) on preneoplastic ACF lesions in patients [172]. Another study in the $Apc^{Min/+}$ mouse model observed that administration of metformin at 250 mg/kg in the diet significantly reduced the number of large polyps [173]. Metformin efficiently inhibited colon tumor growth in nude mice [174], and a significant decrease in PanINs and PDAC was observed in metformin-treated transgenic LSL-Kras mice [175, 176]. However, we observed a nonsignificant increase in AOM-induced CRC in metformin-treated F344 rats. Metformin-treated PC tissues had an increase in $CD8^+$ T cells and a decrease in Tregs compared with control PC tissues. No effect of metformin on NK cell numbers or INFγ production in PC tissues was observed by the authors. Currently, a phase III clinical trial is recruiting patients for a study in which metformin plus modified FOLFOX 6 will be used in patients with metastatic PC. The primary outcome of this study will be to determine whether this treatment will have any effect on median survival (ClinicalTrials.gov ID-NCT01666730). The authors' preclinical data in CRC suggest the need to evaluate metformin doses thoroughly and establish the mechanisms for its pro-tumorigenic effects in CRC and the conditions under which it may have any inhibitory effect in CRC. Studies of metformin in PC suggest that its tumor inhibitory action is based on pAMPK activation, reduced activity of mTOR, and immune-modulating effects. The potential inhibitory effects of metformin in PC support its further evaluation.

13.9 Immunomodulatory Effects of Natural Agents

The source of most biologically active compounds is plants. Not only are 25 % of all medicines derived from plants, but the starting material for many semisynthetic drugs is obtained from plant components. The compounds derived from plants are not only considered safer but are also cost-effective. Immunomodulatory functions of natural agents such as curcumin on T cells, DCs, and NKs have been investigated via cellular, immunological, biochemical, and molecular technologies and with the use of mouse models.

Curcumin has been shown to exhibit antitumor efficacy; however, clinical studies did not support adequate bioavailability with oral doses due to its poor absorption [177]. Effective delivery methods are under investigation to improve the efficacy of curcumin against CRC. High doses of curcumin (10 or 12 g/day) did result in a significant increase in serum levels in a clinical toxicology study [178], and some studies reported that 4 g/day of curcumin showed similar results [179]. In a double-blind study for ulcerative colitis, continuous consumption of 2 g/day curcumin with a standard diet provided significant protection against inflammation [180]. Fewer relapses were seen in the group taking curcumin. Similar effects were observed in ulcerative colitis and Crohn's disease in another study [181]. These studies suggest that curcumin was able to reach the colonic tissue to exert its effects. Administration of 3.6 g/day of curcumin orally resulted in effective concentrations of 7.7 +/− 1.8 nmol/g in normal colorectal tissue and 12.7 +/− 5.7 umol/L in malignant tissue [182].

Curcumin was effective in modulating TNF-α, COX-2, and 5-LOX activities [183, 184]. Oral administration of curcumin (2–4 g per day for 30 days) in an open-label clinical trial in 44 eligible smokers with eight or more ACF on screening colonoscopy was found to reduce the number of ACFs significantly (40 %); however, a lower dose was ineffective [185]. This study suggests that, although systemic bioavailability is a problem, a significant inhibitory effect of curcumin at high concentrations was observed on ACF. Curcumin did not show any effect on proliferation markers or on AA metabolism. Immune modulatory effects of curcumin were not analyzed in this study.

A few reports on the immune-modulating effects of curcumin are available, but the mechanisms of action are not yet fully understood [186]. Available reports suggest that curcumin restores progenitor and effector circulating T cells. In an $Apc^{Min/+}$ mouse model, curcumin injections led to an increase in intestinal $CD4^+$ T cells and retarded growth of adenomas [187]. Varalakshmi et al. [188] reported that curcumin injections enhanced the mitogen- and antigen-induced proliferation potential of T cells, but did not impair or increase the cytotoxic potential of NK cells or Th1 regulatory cytokine production. However, it still is not clear whether curcumin functions similarly to provide stronger immune responses in the pathological conditions of CRC and PC. It has been previously reported that dietary administration of curcumin at different stages of colon tumor development had significant inhibitory effects on CRC. In CF1 mice, dietary administration of curcumin showed significant suppression of chemically induced forestomach, duodenal, and colon tumors when given during initiation and/or post-initiation periods of tumor development [189]. Curcumin also reduced early preneoplastic lesions, dysplasia, and ACF in the colons of rodents [189, 190]. In another study, administration of 0.8 and 1.6 % curcumin continuously during the initiation and post-initiation phases of tumor formation significantly reduced AOM-induced adenomas in rats [191]. A study by the authors showed similar results; continuous administration of 0.25 % curcumin during the initiation and post-initiation stages of tumor formation significantly inhibited both incidence and multiplicity of AOM-induced colon adenocarcinomas and the tumor burden in F344 rats [192]. Although preclinical efficacy data with curcumin have shown significant inhibitory effects in CRC, achieving clinically efficacious doses is still a challenge. Hence, combinations of curcumin with other natural agents including piperine or omega 3 fatty acids, or with agents that can enhance the uptake of curcumin to increase its bioavailability, may provide greater benefit against CRC.

Triterpenoids, another unique group of phytochemicals among the terpenoids [193], are present in common edible foods such as apples and olives. These triterpenoids have been reported to possess anticancer activities in preclinical studies. Chemopreventive effects of oleanolic acid and its synthetic analog 18α-olean-12-ene-3β-23,28-triol (OT) on AOM-induced ACFs in F344 rats have been reported by the authors. The synthetic analog OT (250 ppm) exhibited similar efficacy against ACFs at half the dose of olelonic acid (500 ppm) [194]. The chemopreventive effect was accompanied by decreased expression of COX-2 in colon cancer cells. Synthetic triterpenoids also decreased expression of pro-inflammatory COX-2 and iNOS [195] (Fig. 13.3). Other investigators reported that dietary administration of crude oleanolic acid extract (200 ppm) had inhibitory effects on formation of ACF in F344 rats [196]. A synthetic triterpenoid, C-28 methyl ester of 2-cyano-3,12-dioxooleana-1,9,-dien-28-oic acid (CDDO-Me; bardoxolone methyl), suppressed myeloid-derived suppressor cell (MDSC) activity and inhibited tumor growth in an MC38 colon carcinoma cell line xenograft model [197, 198]. MDSCs have been recently shown to induce $CD8^+$ T-cell tolerance, causing a nonspecific immune suppression, which may enhance tumor promotion, vascularization, and invasion. CDDO-Me and gemcitabine improved T-cell immune responses in patients with stage II–III or IV PC [198]. Since MDSCs play a role during initial stages of tumor formation and growth, triterpenoids may

have more benefits if given at the early stages of cancer development. The data available so far strongly support the potential chemopreventive and immunomodulatory properties of triterpenoids and make them promising agents for CRC and PC. Considerable work remains to be done to identify the target proteins, immune cells, and pathways through which these triterpenoids may function in CRC and PC. Long-term preclinical and clinical efficacy studies are warranted as well as better characterization of immune-modulating function.

Fish oil is a rich source of omega-3 (n-3) polyunsaturated fatty acids (PUFAs), of which there are two important types: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). N-3 PUFAs compete for the same binding site on the COX enzymes as do PUFAs like AA, but they generate biologically less potent prostanoids of the three series [198]. Metabolism of omega-3 PUFAs by the LOX pathway leads to generation of five series LTs, which are less potent than type 4 LTs [199, 200] (Fig. 13.3). The compounds formed from n-3 PUFAS have been shown to possess anti-inflammatory properties. N-3 PUFAs have anti-inflammatory effects in both animals and humans [201], and they modulate both NK cells and T cells [202]. Dietary supplementation with fish oil alters cytokine functions, T-cell proliferation, and T-cell-mediated cytotoxicity [203]. However, Treg functions were reported to be suppressed by DHA [204].

Quite a few studies have reported on the use of omega-3 fatty acids and colon cancer risk. Reddy [205] reviewed various epidemiological, preclinical, and clinical studies describing effects of nutrients and dietary constituents and the potential of n-3 FAs in prevention of CRC. Woodworth et al. [206] studied a smad3−/− mouse model of colitis in which mice infected with *Helicobacter hepaticus* had high FoxP3$^+$ CD25$^+$ CD4$^+$ Treg cell frequency, FoxP3 expression, and altered L-selectin expression. Administration of fish oil enriched with DHA to the mice induced severe colitis and adenocarcinoma formation. Although results using n-3 PUFAs are controversial, most evidence from epidemiological, preclinical, and clinical studies suggests that PUFAs, especially those of the n-3 type, are beneficial in gastrointestinal inflammation [203, 207–209]. EPA and DHA seem to exert protective immunomodulatory effects under chronic inflammatory conditions. The dosage and timing for the use of these PUFAs need to be evaluated for better protective effects.

A single-arm, phase II trial in Leicester, UK, showed that treatment of patients with advanced pancreatic adenocarcinoma with a weekly ω-3FA-rich intravenous infusion plus gemcitabine was safe. Further investigations are needed to determine whether ω-3FA contributed to low manose binding activity along with gemcitabine. The authors have recently reported decreased PanIN 3 lesion formation and incidence of pancreatic ductal adenocarcinoma in Fat-1.p48$^{Cre/+}$.LSL-Kras$^{G12D/+}$ transgenic mice [210]. This study suggests that increased omega-3 fatty acids in tissues may help in preventing PC progression. Further studies are warranted to identify the potent metabolites and targets of these natural dietary agents for designing better prevention modalities.

13.10 Concluding Remarks

It is evident from the gathered literature that most diseases with an inflammatory component, including CRC and PC, are initiated by chronic unresolved inflammation driven by certain immune cells. A lot of information is accumulating on how the immune cells battle the tumor cells and on how the tumor cells overcome the immune responses to grow and metastasize. However, we still need to understand better the interactions and mutual regulation between the tumor cells, the tumor microenvironment, and the various cells in immune response networks in order to develop better strategies for modulating the immune response without incurring toxicities. The tumor-promoting role of Tregs during initial stages of tumor growth remains elusive in both CRC and PC, and the regulatory interactions between NK cells and Tregs in these cancers remain to be elucidated. Available data suggest

that Tregs suppress NK cell activity during tumor formation and lead to tumor growth and metastases. However, experimental evidence is needed to support this notion. It is clear from various vaccine trails that chemotherapy is often associated with side effects on immune cells and immune suppression. Drugs that do not exhibit such toxicities on immune cells or that selectively inhibit or activate certain immune cells need to be developed and tested for CRC and PC prevention and treatment. Selection of safe drug doses should enable initiation or extension of the antitumor immune responses rather than termination of the response in a tumor-bearing host. AA metabolites play important roles in creating protumorigenic conditions, and COX and LOX activities have critical functions, as evident from efficacy of NSAIDs and coxibs in various inflammatory conditions. Although human studies support the use of these NSAIDS in reducing the risk of CRC and PC, many of these agents, other than aspirin, have not been tested for their effects on modulation of immune responses nor have dosages or duration of administration been optimized for better outcome in tumor inhibition. The role of statins in lowering blood lipids is well known, and its role in inflammation is emerging. A few studies suggest modulatory effects of statins on various cells of the immune system. The preclinical chemopreventive effects of statins are very consistent, but clinical studies are needed to confirm the results obtained in preclinical studies. Large-scale trials and preclinical evaluation of statins for immune modulatory effects and for their use as preventive agents alone or in combination with vaccines are needed. SERMs and rexinoids have been shown to possess anti-inflammatory and cancer-preventive effects in CRC and PC, and they also have been shown to affect cytokines and inflammatory markers in tumor tissues. However, very little to no data exist on how these agents may modulate immune cells involved in the pathology of CRC and PC. Many natural agents known from ancient times have been found recently to induce immune modulation of NK cells in animal models. How these agents function under conditions of tumor initiation and growth in CRC and PC needs to be studied in depth in clinical trials to establish proper dosage and administration schedules and to elucidate the mechanisms for their inhibitory effects on tumor formation. In some cases, low doses are immunostimulatory, and high doses are immunosuppressive. Anticancer agents need to be used judiciously to retain beneficial immune responses and to avoid loss of the efficacy against CRC and PC. Very little work has been done in humans to verify the immune-modulating effects of the agents reviewed. Although immunotherapy has not been that effective, especially when administered in the setting of advanced cancers, immune responses in patients suggest that tumor-associated antigens exist and this information can be used for the development of immunomodulatory agents in combination with vaccines for the prevention of CRC and PC.

Acknowledgements We want to acknowledge the grant support NCI-R01-94962 and NCI-CN-53300 for the work quoted in this chapter and Dr. Julie Sando for her scientific and language editing of this chapter. Dr. Janakiram acknowledges the grant support from college of medicine alumni grant and 1P20GM103639-01 from the COBRE Program of the National Institutes of Health for some of the work mentioned in this chapter.

References

1. Tenesa A, Dunlop MG. New insights into the aetiology of colorectal cancer from genome-wide association studies. Nat Rev Genet. 2009;10:353–8.
2. American Cancer Society. Colorectal cancer facts & figures 2011–2013. Atlanta: American Cancer Society; 2011.
3. American Cancer Society. Cancer facts and figures (2013). Atlanta: American Cancer Society (ACS); 2013.
4. Heinemann V. Gemcitabine: progress in the treatment of pancreatic cancer. Oncology. 2001;60(1):8–18.
5. Mamon HJ, Niedzwiecki D, Hollis D, Tan BR, Mayer RJ, Tepper JE, et al. A phase 2 trial of gemcitabine, 5-fluorouracil, and radiation therapy in locally advanced nonmetastatic pancreatic adenocarcinoma: cancer and Leukemia Group B (CALGB) 80003. Cancer. 2011;117(12):2620–8.
6. Kim MP, Gallick GE. Gemcitabine resistance in pancreatic cancer: picking the key players. Clin Cancer Res. 2008;14(5):1284–5.
7. Hidalgo M. Pancreatic cancer. N Engl J Med. 2010;362:1605–17.

8. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. Nat Med. 2004;10:909–15.
9. Zou W. Immunosuppressive networks in the tumour environment and their therapeutic relevance. Nat Rev Cancer. 2005;5:263–74.
10. Mocellin S, Mandruzzato S, Bronte V, Lise M, Nitti D. Part I. Vaccines for solid tumours. Lancet Oncol. 2004;5:681–9.
11. Schwartz RH. T cell anergy. Annu Rev Immunol. 2003;21:305–34.
12. Greenwald RJ, Boussiotis VA, Lorsbach RB, Abbas AK, Sharpe AH. CTLA-4 regulates induction of anergy in vivo. Immunity. 2001;14:145–55.
13. Halama N, Braun M, Kahlert C, Spille A, Quack C, Rahbari N, et al. Natural killer cells are scarce in colorectal carcinoma tissue despite high levels of chemokines and cytokines. Clin Cancer Res. 2011;17(4):678–89.
14. Liyanage UK, Moore TT, Joo HG, Tanaka Y, Herrmann V, Doherty G, et al. Prevalence of regulatory T cells is increased in peripheral blood tumor microenvironment of patients with pancreas or breast adenocarcinoma. J Immunol. 2002;169:2756–61.
15. Male D, Brostoff J, Roth D, Roitt I. Modes of immune response. In: Immunology. 8th ed. Edinburgh: Mosby Elsevier; 2012. p. 107–99.
16. Berger A. Th1 and Th2 responses: what are they? BMJ. 2000;321:424.1.
17. Zamai L, Ponti C, Mirandola P, Gobbi G, Papa S, Galeotti L, Cocco L, Vitale M. NK cells and cancer. J Immunol. 2007;178:4011–401.
18. Fernandez NC, Lozier A, Flament C, Ricciardi-Castagnoli P, Bellet D, et al. Dendritic cells directly trigger NK cell functions: cross-talk relevant in innate anti-tumor immune responses in vivo. Nat Med. 1999;5:405–11.
19. Ohtani H. Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in human colorectal cancer. Cancer Immun. 2007;7:4.
20. Funada Y, Noguchi T, Kikuchi R, Takeno S, Uchida Y, Gabbert HE. Prognostic significance of CD8+ T cell and macrophage peritumoral infiltration in colorectal cancer. Oncol Rep. 2003;10:309–13.
21. Hiraoka K, Miyamoto M, Cho Y, Suzuoki M, Oshikiri T, Nakakubo Y, Itoh T, Ohbuchi T, Kondo S, Katoh H. Concurrent infiltration by CD8+ T cells and CD4+ T cells is a favourable prognostic factor in non-small-cell lung carcinoma. Br J Cancer. 2006;94:275–80.
22. Bui JD, Uppaluri R, Hsieh CS, Schreiber RD. Comparative analysis of regulatory and effector T cells in progressively growing versus rejecting tumors of similar origins. Cancer Res. 2006;66:7301–9.
23. Pages F, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molidor R, et al. Effector memory T cells, early metastasis, and survival in colorectal cancer. N Engl J Med. 2005;353:2654–66.
24. Klintrup K, Makinen JM, Kauppila S, Vare PO, Melkko J, Tuominen H, Tuppurainen K, Makela J, Karttunen TJ, Makinen MJ. Inflammation and prognosis in colorectal cancer. Eur J Cancer. 2005;41:2645–54.
25. Cuschieri A, Talbot IC, Weeden S. Influence of pathological tumour variables on long-term survival in resectable gastric cancer. Br J Cancer. 2002;86:674–9.
26. Tallerico R, Todaro M, Di Franco S, Maccalli C, Garofalo C, Sottile R, et al. Human NK cells selective targeting of colon cancer-initiating cells: a role for natural cytotoxicity receptors and MHC class I molecules. J Immunol. 2013;190(5):2381–90.
27. Dalerba P, Maccalli C, Casati C, Castelli C, Parmiani G. Immunology and immunotherapy of colorectal cancer. Crit Rev Oncol Hematol. 2003;46:33–57.
28. Hillman GG, Kallinteris NL, Lu X, Wang Y, Wright JL, Li Y, et al. Turning tumor cells in situ into T-helper cell-stimulating, MHC class II tumor epitope-presenters: immuno-curing and immuno-consolidation. Cancer Treat Rev. 2004;30:281–90.
29. Watson NF, Ramage JM, Madjd Z, Spendlove I, Ellis IO, Scholefield JH, Durrant LG. Immunosurveillance is active in colorectal cancer as downregulation but not complete loss of MHC class I expression correlates with a poor prognosis. Int J Cancer. 2006;118:6–10.
30. Schmidt J, Jäger D, Hoffmann K, Büchler MW, Märten A. Impact of interferon-alpha in combined chemoradioimmunotherapy for pancreatic adenocarcinoma (CapRI): first data from the immunomonitoring. J Immunother. 2007;30:108–15.
31. Clark CE, Beatty GL, Vonderheide RH. Immunosurveillance of pancreatic adenocarcinoma: insights from genetically engineered mouse models of cancer. Cancer Lett. 2009;279:1–7.
32. Clark CE, Hingorani SR, Mick R, Combs C, Tuveson DA, Vonderheide RH. Dynamics of the immune reaction to pancreatic cancer from inception to invasion. Cancer Res. 2007;67:9518–27.
33. Yamamoto T, Yanagimoto H, Satoi S, Toyokawa H, Hirooka S, Yamaki S, Yui R, Yamao J, Kim S, Kwon AH. Circulating CD4+CD25+ regulatory T cells in patients with pancreatic cancer. Pancreas. 2012;41(3):409–15.
34. Apte M, Pirola RC, Wilson JS. Pancreatic stellate cells: a starring role in normal and diseased pancreas. Front Physiol. 2012;3:344.
35. Mace TA, Ameen Z, Collins A, Wojcik S, Mair M, Young GS, et al. Pancreatic cancer-associated stellate cells promote differentiation of myeloid-derived suppressor cells in a STAT3-dependent manner. Cancer Res. 2013;73(10):3007–18.
36. Walker W, Rotondo D. Prostaglandin E2 is a potent regulator of interleukin-12- and interleukin-18-induced natural killer cell interferon-gamma synthesis. Immunology. 2004;111(3):298–305.
37. Nakayama T, Mutsuga N, Yao L, Tosato G. Prostaglandin E2 promotes degranulation-independent release of MCP-1 from mast cells. J Leukoc Biol. 2006;79(1):95–104.

38. Bankhurst ADJ. The modulation of human natural killer cell activity by prostaglandins. Clin Lab Immunol. 1982;7(2):85–91.
39. Goto T, Herberman RB, Maluish A, Strong DM. Cyclic AMP as a mediator of prostaglandin E-induced suppression of human natural killer cell activity. J Immunol. 1983;130(3):1350–5.
40. Mailliard RB, Alber SM, Shen H, Watkins SC, Kirkwood JM, Herberman RB, Kalinski P. IL-18-induced CD83+CCR7+ NK helper cells. J Exp Med. 2005;202(7):941–53.
41. Yakar I, Melamed R, Shakhar G, Shakhar K, Rosenne E, Abudarham N, Page GG, Ben-Eliyahu S. Prostaglandin e(2) suppresses NK activity in vivo and promotes postoperative tumor metastasis in rats. Ann Surg Oncol. 2003;10(4):469–79.
42. Abdel-Majid RM, Marshall JS. Prostaglandin E2 induces degranulation-independent production of vascular endothelial growth factor by human mast cells. J Immunol. 2004;172:1227–36.
43. Giovannucci E, Egan KM, Hunter DJ, Stampher MJ, Colditz GA, Willett WC, Speizer FE, et al. Aspirin and the risk of colorectal cancer in women. N Engl J Med. 1995;333:609.
44. Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A, Willett WC. Aspirin use and the risk for colorectal cancer and adenoma in male health professionals. Ann Intern Med. 1994;121:241–6.
45. Thun MH, Namboodiri MM, Heath CW. Aspirin use and reduced risk of fatal colon cancer. N Engl J Med. 1991;325:1593–6.
46. Rao CV, Reddy BS, Steele VE, Wang CX, Liu X, Ouyang N. Nitric oxide-releasing aspirin and indomethacin are potent inhibitors against colon cancer in azoxymethane-treated rats: effects on molecular targets. Mol Cancer Ther. 2006;5(6):1530–8.
47. Porzsolt F, Wolf W. Modulation of natural killer activity by aspirin: I. In vitro effect of aspirin. J Interferon Res. 1983;3:11–7.
48. Javeed A, Zhang B, Qu Y, Zhang A, Sun C, Zhang L, et al. The significantly enhanced frequency of functional CD4+ CD25+ Foxp3+ T regulatory cells in therapeutic dose aspirin-treated mice. Transpl Immunol. 2009;20:253–60.
49. Maldonado RA, von Andrian UH. How tolerogenic dendritic cells induce regulatory T cells. Adv Immunol. 2010;108:111–65.
50. Dubé C, Rostom A, Lewin G, Tsertsvadze A, Barrowman N, Code C, et al. The use of aspirin for primary prevention of colorectal cancer: a systematic review prepared for the U.S. Preventive Services Task Force. Ann Intern Med. 2007;146(5):365–75.
51. Rostom A, Dubé C, Lewin G, Tsertsvadze A, barrowman N, Code C, et al. Nonsteroidal anti-inflammatory drugs and cyclooxygenase-2 inhibitors for primary prevention of colorectal cancer: a systematic review prepared for the U.S. Preventive Services Task Force. Ann Intern Med. 2007;146:376–89.
52. Flossmann E, Rothwell PM, British Doctors Aspirin Trial and the UK-TIA Aspirin Trial. Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. Lancet. 2007;369:1603.
53. Rosenberg L, Palmer JR, Zauber AG, et al. A hypothesis: nonsteroidal anti-inflammatory drugs reduce the incidence of large-bowel cancer. J Natl Cancer Inst. 1991;83:355.
54. Gann PH, Manson JE, Glynn RJ, et al. Low-dose aspirin and incidence of colorectal tumors in a randomized trial. J Natl Cancer Inst. 1993;85:1220.
55. Stürmer T, Buring JE, Lee IM, Kurth T, Gaziano JM, Glynn RJ. Colorectal cancer after start of nonsteroidal anti-inflammatory drug use. Am J Med. 2006;119(6):494–502.
56. Chan AT, Giovannucci EL, Meyerhardt JA, et al. Long-term use of aspirin and nonsteroidal anti-inflammatory drugs and risk of colorectal cancer. JAMA. 2005;294:914.
57. Collet JP, Sharpe C, Belzile E, et al. Colorectal cancer prevention by non-steroidal anti-inflammatory drugs: effects of dosage and timing. Br J Cancer. 1999;81:62.
58. Thun MJ, Namboodiri MM, Calle EE, et al. Aspirin use and risk of fatal cancer. Cancer Res. 1993;53:1322.
59. Friis S, Sørensen HT, McLaughlin JK, Johnsen SP, Blot WJ, Olsen JH. A population-based cohort study of the risk of colorectal and other cancers among users of low-dose aspirin. Br J Cancer. 2003;88(5):684–8.
60. Johnson CC, Hayes RB, Schoen RE, Gunter MJ, Huang WY, PLCO Trial Team. Non-steroidal anti-inflammatory drug use and colorectal polyps in the prostate, lung, colorectal, and ovarian cancer screening trial. Am J Gastroenterol. 2010;105(12):2646–55.
61. Plaßmeier L, Knoop R, Waldmann J, Kesselring R, Fichtner-Feigl MBS, Bartsch DK. Aspirin prolongs survival and reduced the number of Foxp3+ regulatory T cells in a genetically engineered mouse model of pancreatic cancer. Ger Med Sci. 2013;398(7):989–96.
62. Sergeeva MG, Gonchar MV, Grishina ZV, Mevkh AT, Varfolomeyev SD. Low concentrations of nonsteroidal anti-inflammatory drugs affect cell functions. Life Sci Phramacol Lett. 1995;56(16):313–9.
63. Reddy BS, Tokumo K, Kulkarni N, Aligia C, Kelloff G. Inhibition of colon carcinogenesis by prostaglandin synthesis inhibitors and related compounds. Cartinogenesis. 1992;13(6):1019–23.
64. Matos P, Kotelevets L, Gonçalves V, Henriques A, Zerbib§ P, Moyer MP, et al. Ibuprofen inhibits colitis-induced overexpression of tumor related Rac1b1. Neoplasia. 2013;15:102–11.
65. Cameron DJ, O'Brien P, Majeski JA. Effects of in vivo treatment with ibuprofen on macrophage function in breast and colon cancer patients. J Clin Lab Immunol. 1986;20(1):23–8.
66. Harris RE, Beebe-Donk J, Doss H, Burr-Doss D. Aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs in cancer prevention: a critical

review of non-selective COX-2 blockade (review). Oncol Rep. 2005;13(4):559–83.
67. Harris RE, Beebe-Donk J, Alshafie GA. Similar reductions in the risk of human colon cancer by selective and nonselective cyclooxygenase-2 (COX-2) inhibitors. BMC Cancer. 2008;8:237.
68. Waltz DT, Dimartino MJ, Misher A. Adjuvant-induced arthritis in rats. II drug effects on physiological, biochemical and immunologic parameters. J Pharmacol Exp Ther. 1971;178:223–31.
69. Bayer BM, Beaven MB. Evidence that indomethacin reversibly inhibits cell growth in the G1 phase of the cell cycle. Biochem Pharmacol. 1979;28:441–3.
70. Seng GF, Benensohn J, Bayer BM. Changes in T and B lymphocyte proliferative responses in adjuvant-arthritic rats: antagonism by indomethacin. Eur J Pharmacol. 1990;178:267–73.
71. Zielińska-Przyjemska M, Główka FK, Klaczyńska J. Modulatory effect of chiral nonsteroidal anti-inflammatory drugs on apoptosis of human neutrophils. Chirality. 2008;20(2):159–65.
72. Furst SM, Khan KN, Komocsar WJ, Mennear J. Screening new drugs for immunotoxic potential: III. Assessment of the effects of selective and non-selective COX-2 inhibitors on T-cell-dependent antibody and natural killer cell responses in the rat. J Immunotoxicol. 2005;2(3):161–70.
73. Fiorucci S, Antonelli E, Burgaud JL, Morelli A. Nitric oxide-releasing NSAIDs: a review of their current status. Drug Saf. 2001;24(11):801–11.
74. Cicala C, Ianaro A, Stefano F, Calignano A, Mariarosaria B, Roberto G, et al. NO-naproxen modulates in¯ammation, nociception and downregulates T cell response in rat Freund's adjuvant arthritis. Br J Pharmacol. 2000;130:1399–405.
75. Elmore E, Steele VE, Redpath JL. Evaluation of the effects of naproxen in human colon polyp cells using gene expression and pathway analysis. Cancer Prev Res. 2010;3(12):A99.
76. Boolbol SK, Dannenberg AJ, Chadburn A, et al. Cyclooxygenase-2 overexpression and tumor formation are blocked by sulindac in a murine model of familial adenomatous polyposis. Cancer Res. 1996;56:2556–60.
77. Labayle D, Fischer D, Vielh P, et al. Sulindac causes regression of rectal polyps in familial adenomatous polyposis. Gastroenterology. 1991;3(101):635–9.
78. Klein WA, Miller HH, Anderson M, Decosse JJ. The use of indomethacin, sulindac and tamoxifen for the treatment of desmoid tumors associated with familial polyposis. Cancer. 1987;60:2863–8.
79. Belliveau P, Graham AM. Mesenteric desmoid tumor in Gardener's syndrome treated by sulindac. Dis Colon Rectum. 1984;27:53–4.
80. Waddel RW, Ganser GF, Cerise EJ, Loughry RW. Sulindac for polyposis of the colon. Am J Surg. 1989;157:175–9.
81. Cruz-Correa M, Hylind LM, Romans KE, Booker SV, Giardiello FM. Long-term treatment with sulindac in familial adenomatous polyposis: a prospective cohort study. Gastroenterology. 2002;122(3):641–5.
82. Moorghen M, Ince P, Finney KJ, Sunter JP, Appleton DR, Watson AJA. A protective effect of sulindac against chemically-induced primary colonic tumors in mice. J Pathol. 1988;156:341–7.
83. Skinner SA, Penney AG, O'Brien PE. Sulindac inhibits the rate of growth and appearance of colon tumor in the rat. Arch Surg. 1991;126:1094–6.
84. Ahnen D, Hixson L, AlbCrtS D, Bogert C, Einspahr K, Brendel P, et al. Sulindac and its sulfone metabolite (FGN-1) both inhibit rat colon carcinogenesis but neither inhibit colonic proliferation. Proc Am Assoc Cancer Res. 1994;35:631.
85. Meyskens FL, McLaren CE, Pelot D, Fujikawa-Brooks S, Carpenter PM, Hawk E, et al. Difluoromethylornithine plus sulindac for the prevention of sporadic colorectal adenomas: a randomized placebo-controlled, double-blind trial. Cancer Prev Res. 2008;1:32–8.
86. Sporn MB, Hong WK. Concomitant DFMO and sulindac chemoprevention of colorectal adenomas: a major clinical advance. Nat Clin Pract Oncol. 2008;5:628–9.
87. Tuma R. Drugs to prevent colon cancer show promise, but hurdles remain for chemoprevention. J Natl Cancer Inst. 2008;100:764–6.
88. Tuynman JB, Vermeulen L, Boon EM, Kemper K, Zwinderman AH, Peppelenbosch MP, Richel DJ. Cyclooxygenase-2 inhibition inhibits c-Met kinase activity and Wnt activity in colon cancer. Cancer Res. 2008;68:1213–20.
89. Giardiello FM, Hamilton SR, Krush AJ, et al. Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. N Engl J Med. 1993;328:1313.
90. Stein U, Arlt F, Smith J, Sack U, Herrmann P, Walther W, Lemm M, Fichtner I, Shoemaker RH, Schlag PM. Intervening in β-catenin signaling by sulindac inhibits S100A4-dependent colon cancer metastasis. Neoplasia. 2011;13:131–44.
91. Sakaedaa Y, Hiroia M, Shimojimab T, Iguchic M, Kanegaec H, Ohmoria Y. Sulindac, a nonsteroidal anti-inflammatory drug, selectively inhibits interferon-γ-induced expression of the chemokine CXCL9 gene in mouse macrophages. Biochem Biophys Res Commun. 2006;350(2):339–44.
92. Reddy BS, Maruyama H, Kelloff G. Dose-related inhibition of colon carcinogenesis by dietary piroxicam, a nonsteroidal anti-inflammatory drug during different stages of rat colon tumor development. Cancer Res. 1987;47:5340–6.
93. Reddy BS, Nayin U, Tokumo K, Rigotry, Zang E, Kelloff G. Chemoprevention of colon carcinogenesis by concurrent administration of piroxicam, a nonsteroidal anti-inflammatory drug with D, L-a-difluoromethyI omithine, an ornithine decarboxylase inhibitor, in diet. Cancer Res. 1990;50:2562–8.
94. Pollard M, Luckert PH, Schirudt MA. The suppressive effect of piroxicam on autochthonous intestinal tumors in the rat. Cancer Lett. 1983;21:57–61.
95. Narisawa X, Sato M, Tani M, Kudo T, Takahashi T, Goto A. Inhibition of development of

methylnitrosourea-induced rat colon tumors by indomethacin. Cancer Res. 1981;41:1954–7.
96. Kudo T, Narisawa T, Abo S. Antitumor activity of indomethacin on methylazoxymethanol-induced large bowel tumors in rats. Gann. 1980;71:260–4.
97. Pollard M, Luckert PH. Effect of piroxicam on primary intestinal tumors induced in rats by N-methylnitrosourea. Cancer Lett. 1984;25:117–21.
98. Pollard M, Luckert PH. Prolonged antitumor effect of indomethacin on autochthonous intestinal tumors in rats. J Natl Cancer. 1983;70:1103–5.
99. Earnest DL, Hixson LJ, Alberts DS. Piroxicam and other cyclooxygenase inhibitors: potential for cancer chemoprevention. J Cell Biochem Suppl. 1992;16I:156–66.
100. Muller AJ, Scherle PA. Targeting the mechanisms of tumoral immune tolerance with small-molecule inhibitors. Nat Rev Cancer. 2006;6:613–25.
101. Lönnroth C, Andersson M, Arvidsson A, Nordgren, Brevinge H, Lagerstedt K, Lundholm K. Preoperative treatment with a non-steroidal anti-inflammatory drug (NSAID) increases tumor tissue infiltration of seemingly activated immune cells in colorectal cancer. Cancer Immun. 2008;8:5.
102. Reddy BS, Rao CV. Role of synthetic and naturally occurring cyclooxygenase inhibitors in colon cancer prevention. In: Harris RE, editor. COX-2 blockade in cancer prevention and therapy. Totowa: Humana Press; 2002. p. 71–83.
103. Reddy BS. Strategies for colon cancer prevention: combination of chemopreventive agents. Subcell Biochem. 2007;42:213–25.
104. Talmadgea JE, Hooda KC, Zobela LC, Shafera LR, Colesb M, Tothb B. Chemoprevention by cyclooxygenase-2 inhibition reduces immature myeloid suppressor cell expansion. Int Immunopharmacol. 2007;7(2):140–51.
105. Smalley W, Ray WA, Daugherty J, et al. Use of non-steroidal anti-inflammatory drugs and incidence of colorectal cancer: a population-based study. Arch Intern Med. 1999;2(152):161–6.
106. Arber N, Eagle CJ, Spicak J, et al. Celecoxib for the prevention of colorectal adenomatous polyps. N Engl J Med. 2006;9(355):885–95.
107. Bertagnolli MM, Eagle CJ, Zauber AG, Redston M, Solomon SD, Kim K, Tang J, et al. Celecoxib for the prevention of sporadic colorectal adenomas. N Engl J Med. 2006;9(355):873–84.
108. Rainsford KD. Anti-inflammatory drugs in the 21st century. Subcell Biochem. 2007;42:3–27.
109. Harris RE, Beebe-Donk J, Doss H, Burr-Doss D. Aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs in cancer prevention: a critical review of non-selective COX-2 blockade (review). Oncol Rep. 2005;4(13):559–83.
110. Couzin J. Withdrawal of Vioxx casts a shadow over COX-2 inhibitors. Science. 2004;306:384–5.
111. Mohammed A, Janakiram NB, Ely M, Lightfoot S, Steele VE, Rao CV. Licofelone, a novel dual COX-LOX inhibitor prevents progression of PanIN lesions to pancreatic carcinoma by targeting miRNAs and cancer stem cells in p48Cre/+-LSL-KrasG12D/+ transgenic mice. In: Proceedings of the 102nd annual meeting of the American Association for Cancer Research; 2011 Apr 2–6; Orlando: AACR; Cancer Res. 2011; vol. 71, p. 2839.
112. Mohammed A, Janakiram NB, Brewer M, Biddick L, Lightfoot S, Steele VE, et al. Targeting COX-LOX and EGFR pathways simultaneously by licofelone and gefitinib lead to complete blockade of progression of PanINs to pancreatic ductal adenocarcinoma. In: Proceedings of the 103rd annual meeting of the American Association for Cancer Research; 2012 Mar 31–Apr 4; Chicago: AACR; Cancer Res 2012; vol. 72, p. 1005.
113. LaRosa JC. What do the statins tell us? Am Heart J. 2002;144(Suppl):S21–6.
114. Poynter JN, Gruber SB, Higgins PD, Almog R, Bonner JD, Rennert HS, Low M, Greenson JK, Rennert G. Statins and the risk of colorectal cancer. N Engl J Med. 2005;352:2184–92.
115. Cui X, Xie Y, Chen M, Li J, Liao X, Shen J, et al. Statin use and risk of pancreatic cancer: a meta-analysis. Cancer Causes Control. 2012;3(7):1099–111.
116. Mohammed A, Qian L, Janakiram NB, Lightfoot S, Steele VE, Rao CV. Atorvastatin delays progression of pancreatic lesions to carcinoma by regulating PI3/AKT signaling in $p48^{Cre}$/LSL-$Kras^{G12D}$ mice. Int J Cancer. 2012;131:1951–62.
117. Haxhinasto S, Mathis D, Benoist C. The AKT-mTOR axis regulates de novo differentiation of CD4+Foxp3+ cells. J Exp Med. 2008;205:565–74.
118. Sauer S, Bruno L, Hertweck A, Finlay D, Leleu M, Spivakov M, et al. T cell receptor signaling controls Foxp3 expression via PI3K, Akt, and mTOR. Proc Natl Acad Sci U S A. 2008;105:7797–802.
119. Kusama T, Mukai M, Iwasaki T, et al. 3-hydroxy-3-methylglutaryl-coenzyme a reductase inhibitors reduce human pancreatic cancer cell invasion and metastasis. Gastroenterology. 2002;122:308–17.
120. Sun D, Fernandes G. Lovastatin inhibits bone marrow-derived dendritic cell maturation and up-regulates pro-inflammatory cytokine production. Cell Immunol. 2003;223:52–62.
121. Gruenbacher G, Gander H, Nussbaumer O, Nussbaumer W, Rahm A, Thurnher M. IL-2 co-stimulation enables statin-mediated activation of human NK cells, preferentially through a mechanism involving CD56+ dendritic cells. Cancer Res. 2010;70(23):9611–20.
122. Massaia M. Aminobisphosphonates, statins and the mevalonate pathway: a cross-road to fine-tune the activation of NK and Vγ9Vδ2 T cells. IBMS BoneKEy. 2012;9:11.
123. Cutts JL, Scallen TJ, Watson J, Bankhurst AD. Role of mevalonic acid in the regulation of natural killer cell cytotoxicity. J Cell Physiol. 1989;139:550–7.
124. Tanaka T, Porter CM, Horvath-Arcidiacono JA, Bloom ET. Lipophilic statins suppress cytotoxicity by freshly isolated natural killer cells through modulation of granule exocytosis. Int Immunol. 2007;19:163–73.

125. Raemer PC, Kohl K, Watzl C. Statins inhibit NK-cell cytotoxicity by interfering with LFA-1-mediated conjugate formation. Eur J Immunol. 2009;39:1456–65.
126. Hillyard DZ, Nutt CD, Thomson J, McDonald KJ, Wan RK, Cameron AJ, et al. Statins inhibit NK cell cytotoxicity by membrane raft depletion rather than inhibition of isoprenylation. Atherosclerosis. 2007;191:319–25.
127. Youssef S, Stuve O, Patarroyo JC, et al. The HMG-CoA reductase inhibitor, atorvastatin, promotes a Th2 bias and reverses paralysis in central nervous system autoimmune disease. Nature. 2002;420:78–84.
128. Rosenson RS, Tangney CC, Casey LC. Inhibition of proinflammatory cytokine production by pravastatin. Lancet. 1999;353:983–4.
129. Mausner-Fainberg K, Luboshits G, Mor A, Maysel-Auslender S, Rubinstein A, Keren G, George J. The effect of HMG-CoA reductase inhibitors on naturally occurring CD4+CD25+T cells. Atherosclerosis. 2007;197(2):829–39.
130. Veillard NR, Braunersreuther V, Arnaud C, Burger F, Pelli G, Steffens S, et al. Simvastatin modulates chemokine and chemokine receptor expression by geranylgeranyl isoprenoid pathway in human endothelial cells and macrophages. Atherosclerosis. 2006;188(1):51–8.
131. Ganesan A, Crum-Cianflone N, Higgins J, Qin J, Rehm C, Metcalf J, Brandt C, Vita J, Decker CF, Sklar P, et al. High dose atorvastatin decreases cellular markers of immune activation without affecting HIV-1 RNA levels: results of a double-blind randomized placebo controlled clinical trial. J Infect Dis. 2011;203:756–64.
132. Wang Y, Chang H, Zou J, Jin X, Qi Z. The effect of atorvastatin on mRNA levels of inflammatory genes expression in human peripheral blood lymphocytes by DNA microarray. Biomed Pharmacother. 2011;65:118–22.
133. Suzuki S, Tajima T, Sassa S, Kudo H, Okayasu I, Sakamoto S. Preventive effect of fluvastatin on ulcerative colitis-associated carcinogenesis in mice. Anticancer Res. 2006;6B(26):4223–8.
134. Yasui T, Maegawa M, Tomita J, Miyatani Y, Yamada M, Uemura H, Matsuzaki T, Kuwahara A, Kamada M, Tsuchiya N. Changes in serum cytokine concentrations during the menopausal transition. Maturitas. 2007;56:396–403.
135. Grip O, Janciauskiene S, Bredberg A. Use of atorvastatin as an anti-inflammatory treatment in Crohns disease. Br J Pharmacol. 2008;155:1085–92.
136. Grip O, Janciauskiene S, Lindgren S. Circulating monocytes and plasma inflammatory biomarkers in active Crohns disease: elevated oxidized low-density lipoprotein and the anti-inflammatory effect of atorvastatin. Inflamm Bowel Dis. 2004;10:193–200.
137. Grip O, Janciauskiene S. Atorvastatin reduces plasma levels of chemokine (CXCL10) in patients with Crohns disease. PLoS One. 2009;4:e5263.
138. Janakiram NB, Mohammed A, Bryant T, Zhang Y, Brewer M, Duff A, et al. Clinically relevant low dose combination of rosuvastatin and difluoromethylornithine provides effective Chemopreventive efficacy against AOM-induced colon cancers in F344 rats. American Association of cancer Research Annual Conference, Washington DC, 2013; Abstract # 186.
139. Polanczyk MJ, Carson BD, Subramanian S, et al. Cutting edge: estrogen drives expansion of the CD4+CD25+ regulatory T cell compartment. J Immunol. 2004;173:2227.
140. Polanczyk MJ, Hopke C, Vandenbark AA, Offner H. Estrogen-mediated immunomodulation involves reduced activation of effector T cells, potentiation of Treg cells, and enhanced expression of the PD-1 costimulatory pathway. J Neurosci Res. 2006;84:370.
141. Hanna N, Schneider M. Enhancement of tumor metastasis and suppression of NK cell activity by β-estradiol treatment. J Immunol. 1983;730:974–80.
142. Satake M, Sawai H, Go VL, Satake K, Reber HA, Hines OJ, Eibl G. Estrogen receptors in pancreatic tumors. Pancreas. 2006;33(2):119–27.
143. Iwao K, Miyoshi Y, Ooka M, et al. Quantitative analysis of estrogen receptor-α and -β messenger RNA expression in human pancreatic cancers by real-time polymerase chain reaction. Cancer Lett. 2001;170:91.
144. Maha R Boktour. Hormonal exposure and risk of pancreatic cancer in the United States. ProQuest. 2008;1–49.
145. Kozlowski JM, Fidler IJ, Campbell D, Xu Z-L, Kaighn EM, Hart IR. Metastatic behavior of human tumor cell lines grown in the nude mouse. Cancer Res. 1984;44:3522–9.
146. Janakiram NB, Mohammed A, Brewer M, Bryant T, Biddick L, Lightfoot S, et al. Raloxifene and antiestrogenic gonadorelin inhibits intestinal tumorigenesis by modulating immune cells and decreasing stem-like cells. Cancer Prev Res. 2014;7(3):300–9.
147. Pfeilschifter J, Koditz R, Pfohl M, Schatz H. Changes in proinflammatory cytokine activity after menopause. Endocr Rev. 2002;23:90–119.
148. Yasui T, Saijo A, Uemura H, Matsuzaki T, Tsuchiya N, Mitsutoshi Y, et al. Effects of oral and transdermal estrogen therapies on circulating cytokines and chemokines in postmenopausal women with hysterectomy. Eur J Endocrinol. 2009;161:267–73.
149. Wada-Hiraike O, Imamov O, Hiraike H, et al. Role of estrogen receptor β in colonic epithelium. Proc Natl Acad Sci. 2006;103:2959–64.
150. Giroux V, Lemay F, Bernatchez G, Yolaine R, Carrier JC. Estrogen receptor β deficiency enhances small intestinal tumorigenesis in ApcMin/+ mice. Int J Cancer. 2008;123:303–11.
151. Cho NL, Javid SH, Carothers AM, Redston M, Beragnolli MM. Estrogen receptors α and β are inhibitory modifiers of Apc-dependent tumorigenesis in the proximal colon of Min/+ mice. Cancer Res. 2007;67:2366–72.
152. Ray P, Ghosh SK, Zhang DH, Ray A. Repression of interleukin-6 gene expression by 17β-estradiol: inhibition of the DNA-binding activity of the transcription factors NF-IL6 and NF-κB by the estrogen receptor. FEBS Lett. 1997;409(1):79–85.

153. Ralston SH, Russell RG, Gowen M. Estrogen inhibits release of tumor necrosis factor from peripheral blood mononuclear cells in postmenopausal women. J Bone Miner Res. 1990;5:983–8.
154. Cvoro A, Tatomer D, Tee M-K, Zogovic T, Harris HA, Leitman DC. Selective estrogen receptor-β agonists repress transcription of proinflammatory genes. J Immunol. 2008;180(1):630–6.
155. Morois S, Fournier A, Clavel-Chapelon F, Mesrine S, Boutron-Ruault M-C. Menopausal hormone therapy and risks of colorectal adenomas and cancers in the French E3N prospective cohort: true associations or bias? Eur J Epidemiol. 2012;27(6):439–52.
156. Rao CV, Wang C-X, Simi B, et al. Enhancement of experimental colon cancer by genistein. Cancer Res. 1997;57:3717–22.
157. Speirs V, Malone C, Walton DS, Kerin MJ, Atkin SL. Increased expression of estrogen receptor β mRNA in tamoxifen-resistant breast cancer patients. Cancer Res. 1999;59:5421–4.
158. Janakiram NB, Steele VE, Rao CV. Estrogen receptor-beta as a potential target for colon cancer prevention: chemoprevention of azoxymethane-induced colon carcinogenesis by raloxifene in F344 rats. Cancer Prev Res. 2009;2(1):52–9.
159. Janakiram NB, Mohammed A, Misty B, Lightfoot S, Steele VE, Rao CV. Chemopreventive efficacy of raloxifene, bexarotene and their combination on the progression of AOM-induced colon adenomas to adenocarcinomas in F344 rats. Cancer Res. 2012;72(8 Suppl):Abstract nr 1633.
160. Janakiram NB, Mohammed A, Qian L, Venkateshwar M, Reddy MP, Steele VE, et al. Chemopreventive effects of selective estrogen receptor modulator (SERM) raloxifene on intestinal neoplasia of APCMin/+ mice. Cancer Res. 2011;71(8 Suppl):Abstract nr 831.
161. Mangelsdorf DJ, Ong ES, Dyck JA, Evans RM. Nuclear receptor that identifies a novel retinoic acid response pathway. Nature. 1990;345:224–9.
162. Lomo J, Smeland EB, Ulven S, et al. RAR-, not RXR, ligands inhibit cell activation and prevent apoptosis in B-lymphocytes. J Cell Physiol. 1998;175:68–77.
163. Sidell N, Kummer U, Aframian D, Thierfelder S. Retinoid regulation of interleukin-2 receptors on human T-cells. Cell Immunol. 1997;179:116–25.
164. Stephensen CB, Rasooly R, Jiang X, Ceddia MA, Weaver CT, Chandraratna RAS, Bucy RP. Vitamin A enhances in vitro Th2 development via retinoid X receptor pathway. J Immunol. 2002;168:4495.
165. Desreumaux P, Dubuquoy L, Nutten S, Peuchmaur M, Englaro W, Schoonjans K, et al. Attenuation of colon inflammation through activators of the retinoid X receptor (Rxr)/peroxisome proliferator–activated receptor γ (Pparγ) heterodimer. J Exp Med. 2001;193(7):827–38.
166. Janakiram NB, Mohammed A, Qian L, Choi C-I, Steele VE, Rao CV. Chemopreventive effects of RXR-selective rexinoid bexarotene on intestinal neoplasia of Apc(Min/+) mice. Neoplasia. 2012;14(2):159–68.
167. Liby K, Royce DB, Risingsong R, Williams CR, Maitra A, Hruban R, Sporn MB. Synthetic triterpenoids prolong survival in a transgenic mouse model of pancreatic cancer. Cancer Prev Res. 2010;3(11):1427–34.
168. Decensi A, Puntoni M, Goodwin P, Cazzaniga M, Gennari A, Bonanni B, Gandini S. Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis. Cancer Prev Res. 2010;3:1451–61.
169. Pearce EL, Walsh MC, Cejas PJ, Harms GM, Shen H, Wang L-S, et al. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. Nat Adv Publ. 2009;460:103–8.
170. Bodmer M, Becker C, Meier C, Jick SS, Meier CR. Use of metformin is not associated with a decreased risk of colorectal cancer: a case-control analysis. Cancer Epidemiol Biomark Prev. 2012;21:280–6.
171. Hosono K, Endo H, Takahashi H, Sugiyama M, Uchiyama T, Suzuki K, et al. Metformin suppresses azoxymethane-induced colorectal aberrant crypt foci by activating AMP-activated protein kinase. Mol Carcin. 2010;49(7):662–71.
172. Hosono K, Endo H, Takahashi H, Sugiyama M, Sakai E, Uchiyama T, et al. Metformin suppresses colorectal aberrant crypt foci in a short-term clinical trial. Cancer Prev Res. 2010;3(9):1077–83.
173. Tomimoto A, Endo H, Sugiyama M, et al. Metformin suppresses intestinal polyp growth in Apc(Min/+) mice. Cancer Sci. 2008;99:2136–41.
174. Buzzai M, Jones RG, Amaravadi RK, et al. Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. Cancer Res. 2007;67:6745–52.
175. Rao CV, Janakiram NB, Mohammed A, Madka V, Qian L, Brewer M, et al. Lack of chemopreventive effects of metformin in azoxymethane-induced rat colon carcinogenesis. Cancer Prev Res. 2011;4(10 Suppl):B42.
176. Mohammed A, Janakiram NB, Brewer M, Ritchie RL, Marya A, Lightfoot S, et al. Metformin prevents progression of pancreatic intraepithelial neoplasia to ductal adenocarcinoma by targeting cancer stem cells and mTOR signaling. Transl Oncol. 2013;6(6):649–59.
177. Sharma RA, McLelland HR, Hill KA, et al. Pharmacodynamic and pharmacokinetic study of oral Curcuma extract in patients with colorectal cancer. Clin Cancer Res. 2001;7:1894–900.
178. Lao CD, Ruffin 4th MT, Normolle D, Heath DD, Murray SI, Bailey JM, et al. Dose escalation of a curcuminoid formulation. BMC Complement Altern Med. 2006;6:10.
179. Cheng AL, Hsu CH, Lin JK, Hsu MM, Ho YF, Shen TS, et al. Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. Anticancer Res. 2001;21:2895–900.
180. Hanai H, Iida T, Takeuchi K, Watanabe F, Maruyama Y, Andoh A, et al. Curcumin maintenance therapy for ulcerative colitis: randomized, multicenter, double-blind, placebo-controlled trial. Clin Gastroenterol Hepatol. 2006;4(12):1502–6.

181. Holt PR, Katz S, Kirshoff R. Curcumin therapy in inflammatory bowel disease: a pilot study. Dig Dis Sci. 2005;50(11):2191–3.
182. Garcea G, Berry DP, Jones DJ, Singh R, Dennison AR, Farmer PB, et al. Consumption of the putative chemopreventive agent curcumin by cancer patients: assessment of curcumin levels in the colorectum and their pharmacodynamic consequences. Cancer Epidemiol Biomarkers Prev. 2005;14:120–5.
183. Dominiak K, McKinney J, Heilbrun LK, Sarkar FH. Critical need for clinical trials: an example of a pilot human intervention trial of a mixture of natural agents protecting lymphocytes against TNF-alpha induced activation of NF-kappaB. Pharm Res. 2010;27(6):1061–5.
184. Dileep KV, Tintu I, Sadasivan C. Molecular docking studies of curcumin analogs with phospholipase A2. Interdiscip Sci. 2011;3(3):189–97.
185. Carroll RE, Benya RV, Turgeon DK, Vareed S, Neuman M, Rodriguez L, et al. Phase IIa clinical trial of curcumin for the prevention of colorectal neoplasia. Cancer Prev Res. 2011;4(3):354–64.
186. Jagetia GC, Aggarwal BB. "Spicing up" of the immune system by curcumin. J Clin Immunol. 2007;27:19–35.
187. Churchill M, Chadburn A, Bilinski RT, Bertagnolli MM. Inhibition of intestinal tumors by curcumin is associated with changes in the intestinal immune cell profile. J Surg Res. 2000;89:169–75.
188. Varalakshmi A, Ali M, Pardhasaradhi BVV, Srivastava RM, Singh S, Khar A. Immunomodulatory effects of curcumin: in-vivo. Int Immunopharmacol. 2008;8:688–700.
189. Huang M-T, Deschner EE, Newmark HL, Wang Z-Y, Ferraro TA, Conney AH. Effect of dietary curcumin and ascorbyl palmitate on azoxymethane-induced colonic epithelial cell proliferation and focal areas of dysplasia. Cancer Lett. 1992;64:117–21.
190. Rao CV, Simi B, Reddy BS. Inhibition by dietary curcumin of azoxymethane-induced ornithine decarboxylase, tyrosine protein kinase, arachidonic acid metabolism and aberrant crypt foci formation in the rat colon. Carcinogenesis. 1993;14:2219–25.
191. Pereira MA, Grubbs DJ, Barnes LH, Li H, Olson GR, Eto I, et al. Effects of the phytochemicals, curcumin and quercetin, upon azoxymethane-induced colon cancer and 7,12-dimethylbenz[a]anthracene-induced mammary cancer in rats. Carcinogenesis. 1996;17:1305–11.
192. Rao CV, Rivenson A, Simi B, Reddy BS. Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound. Cancer Res. 1995;55:259–66.
193. Withers ST, Keasling JD. Review biosynthesis and engineering of isoprenoid small molecules. Appl Microbiol Biotechnol. 2007;73(5):980–90.
194. Janakiram NB, Indranie C, Malisetty SV, Jagan P, Steele VE, Rao CV. Chemoprevention of colon carcinogenesis by oleanolic acid and its analog in male F344 rats and modulation of COX-2 and apoptosis in human colon HT-29 cancer cells. Pharm Res. 2008;25(9):2151–7.
195. Suh N, Wang Y, Honda T, Gribble GW, Dmitrovsky E, Hickey WF, et al. A novel synthetic oleanane triterpenoid, 2-cyno-3,12-dioxoolean-1,9-dien-28-oic acid, with potent differentiating antiproliferative and anti-inflammatory activity. Cancer Res. 1999;59:336–41.
196. Kawamoori T, Tanaka T, Hara A, Yamahara J, Mori H. Modifying effects of naturally occurring products on the development of colonic aberrant crypt foci by azoxymethane in F344 rats. Cancer Res. 1995;55:1277–82.
197. Nagaraj S, Youn J-I, Weber H, Iclozan C, Lu L, Cotter MJ, et al. Anti-inflammatory triterpenoid blocks immune suppressive function of MDSCs and improves immune response in cancer. Clin Cancer Res. 2010;16(6):1812–23.
198. Bannenberg G, Arita M, Serhan CN. Endogenous receptor agonists: resolving inflammation. Sci World J. 2007;7:1440–62.
199. Janakiram NB, Rao CV. Role of lipoxins and resolvins as anti-inflammatory and proresolving mediators in colon cancer. Curr Mol Med. 2009;9(5):565–79.
200. Janakiram NB, Mohammed A, Rao CV. Role of lipoxins, resolvins, and other bioactive lipids in colon and pancreatic cancer. Cancer Metastasis Rev. 2011;30(3–4):507–23.
201. Yaqoob P, Newsholme EA, Calder PC. The effect of dietary lipid manipulation on rat lymphocyte subsets and proliferation. Immunology. 1994;82(4):603–10.
202. Puertollano MA, Algarra I, Ortega E, de Pablo MA, Alvarez de Cienfuegos G. Loss of natural killer cell activity after murine tumor transplantation appears as a consequence of dietary lipid administration. Anticancer Res. 2001;21:2697–702.
203. Sijben JW, Calder PC. Differential immunomodulation with long-chain n-3 PUFA in health and chronic disease. Proc Nutr Soc. 2007;66:237–59.
204. Yessoufou A, Ple A, Moutairou K, Hichami A, Khan NA. DHA reduces suppressive and migratory functions of Treg cells. J Lipid Res. 2009;12:2377–88.
205. Reddy BS. Omega-3 fatty acids in colorectal cancer prevention. Int J Cancer. 2004;112(1):1–7.
206. Woodworth HL, McCaskey SJ, Duriancik DM, Clinthorne JF, Langohr I, Gardner EM, Fenton JI. Dietary fish oil alters T lymphocyte cell populations and exacerbates disease in a mouse model of inflammatory colitis. Cancer Res. 2010;70(20):7960–9.
207. Berquin IM, Edwards IJ, Chen YQ. Multi-targeted therapy of cancer by omega-3 fatty acids. Cancer Lett. 2008;269:363–77.
208. Figler M, Gasztonyi B, Cseh J, et al. Association of n-3 and n-6 long-chain polyunsaturated fatty acids in plasma lipid classes with inflammatory bowel diseases. Br J Nutr. 2007;97:1154–61.
209. O'Sullivan M, O'Morain C. Nutrition in inflammatory bowel disease. Best Pract Res Clin Gastroenterol. 2006;20:561–73.
210. Mohammed A, Janakiram NB, Brewer M, Duff A, Lightfoot S, Brush RS, et al. Endogenous n-3 polyunsaturated fatty acids delay progression of pancreatic ductal adenocarcinoma in fat-1.P48$^{Cre/+}$-LSL-Kras$^{G12D/+}$ mice. Neoplasia. 2012;14:1249–59.

Immunology of Cutaneous Tumors and Immunotherapy for Melanoma

14

Amene Saghazadeh, Mahdia Saghazadeh, and Nima Rezaei

Contents

A. Saghazadeh, MD • M. Saghazadeh, MD
Department of Immunology, Molecular Immunology Research Center, School of Medicine, Tehran University of Medical Sciences, Tehran 14194, Iran
e-mail: A.Saghazadeh71@gmail.com; A-Saghazadeh@tums.ac.ir; mahdiasaghazadeh@gmail.com

N. Rezaei, MD, PhD (✉)
Research Center for Immunodeficiencies, Children's Medical Center, Pediatrics Center of Excellence, Tehran University of Medical Sciences, Tehran 14194, Iran

Department of Immunology, Molecular Immunology Research Center, School of Medicine, Tehran University of Medical Sciences, Tehran 14194, Iran

Universal Scientific Education and Research Network (USERN), Tehran, Iran
e-mail: Rezaei_nima@tums.ac.ir

14.1 Introduction

The skin-resident immune cells and relevant signaling pathways render the skin liable to the essential immunological functions. The skin immune system deregulation contributes to bridging the gap between the non-tumoral and tumoral skin. This issue is more complicated by the fact of the paradoxical behavior of the immune system in developing cancers. However, the relative clinical efficacy of current immunotherapies for melanoma skin cancers demands to make absolutely decisive immunotherapy-based interventions.

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_14, © Springer-Verlag Berlin Heidelberg 2015

There is a rapidly growing incidence rate of melanoma and non-melanoma skin cancers (NMSC) [1–3]. Nonmelanoma skin cancer (NMSC) was identified as the most common cancers in white-skinned populations with the high standardized incidence rate of 154 per 100,000 person-years (2000–2006) in the southwest of England and the age-adjusted incidence rate of approximately 693 per 100,000 persons in 2010 in the USA [4, 5]. Melanoma, the most lethal skin malignancy, is diagnosed as the third most common cancer in the USA (after male/female genital system cancers and lymphoma) with the frequency of 17 and 15 % in males and females, respectively, at the age of 15–29 years [6]. The American Joint Committee on Cancer (AJCC) Melanoma Staging Database anatomically classifies cutaneous melanoma based on the clinical and pathologic staging of tumor thickness (T), number of metastatic nodes (N), and site (M). According to this classification (TNM), disease stage is considered as the best prognostic factor of survival in patients with primary cutaneous melanoma in the way that 10-year survival rate rapidly declines from 93 % in melanoma patients at T1aN0M0 to 39 % in patients at T4bN0M0 stage [7, 8]. However, another prognostic model of 10-year metastasis including growth rate, mitotic rate, and sex factors is proposed for melanoma patients with thin lesions and at the high risk of metastasis [9].

Inevitably, the prophylactic settings, diagnostic guidelines, and cost-effective therapeutic approaches should be depicted while recognizing the genetic and environmental risk factors. Notwithstanding the therapeutic options include surgery, photodynamic therapy, systemic chemotherapy, immunotherapy, and combinational protocols are practically available, the permanently optimal efficacy has not been obtained yet [10–13].

14.2 Skin Immune System

On one hand, the simple epidermis, papillary and reticular dermis, and subcutaneous fat architectures superbly engineer the normal skin as the primary interface between the body and environment [14]. In a depth view, the skin's microvascular, cutaneous venules, small lymph capillaries, cutaneous appendages, and sweat glands as well as the cellular structures organize the normal human skin as the crucial multifunctional organ [14]. On the other hand, an immune response can be potentially elicited ubiquitously as the only necessary condition for that is antigen availability in the lymph organs in a dose- and time-dependent manner [15]. Hence, it is not surprising that the skin functions as an immune organ with respect to the variety of reasons including the presence of lymphoid organizations in addition to the various skin-resident immune cells [16–18]. However, it is surprising how this primary immune system demonstrates disregard of highly antigenic tumors. The underlying mechanisms could be justified on the grounds of "cancer immunoediting" hypothesis [19].

14.3 A Dual Perspective: Tumor Immunity

The "cancer immunoediting" hypothesis illustrates both beneficial and detrimental roles of the immune system and its immune cells in the tumor developing process [19–21]. In accordance with this model, the process of evading immune system takes place over the three key periods of elimination, equilibrium, and escape (three "E"s) known as the cancer immunosurveillance, cancer protection, and cancer progression stages, respectively [19]. The role of immune system in the protection against tumor development has led to a dramatic decrease of approximately 100 % in cancer immunosurveillance and cancer progression phases [22]. In a dual sequential process, the adaptive and innate immune cells including NK, MΦ, $CD4^+$, and $CD8^+$ T cells try to abolish tumoral cells and tumor formation during the elimination phase while protecting less immunogenic tumoral cells with the immune selection mechanism during the equilibrium phase [21, 20]. Hence, a list below comprising the main tumor-derived cells or factors involved in the immunology of skin tumorigenesis was compiled.

14.3.1 Tumor-Associated Macrophages (TAMs): Cause or Effect?

TAMs, the most common immune cell population within the tumor microenvironment, comprise "classically activated" M1- and "alternatively activated" M2-polarized macrophages. The tumor stage is the main determinant of the TAM phenotype [23]. M1 macrophages have the violent urge to produce an overwhelming inflammation stands in stark contrast to M2 macrophages which endeavor to moderate this aggressive behavior of M1 macrophages [24]. Both M1 and M2 macrophages accompany tumorigenesis during the early stages of tumor progression and the stages of tumor malignancy, respectively. M2 polarization encompasses the recruitment of peripheral monocytes by VEGF and differentiation into M2 macrophages by IL-4 and IL-10 [25, 26].

On one hand, inhibition of macrophage-derived NO synthesis takes part in tumor growth and delayed tumor rejection immune response; as a matter of fact, macrophage depletion disturbs immune response against highly antigenic UV-induced skin tumors [27]. On the other hand, macrophages constitute the principal fertile source of MMP-12 [28]. The expression of MMP-12 by keratinocytes and macrophage-like cells extracted from squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) and its association with tumor aggressiveness bespeak the pro-tumor role of macrophages [29]. According to the estimates of human skin cancers, either NMSC or melanoma, there was a correlation between the number of infiltrating TAMs and the invasion depth of tumor [30, 31]. It seems that the correlation between TAMs and invasion depth of human BCC is mediated by COX-2, which, in turn, might be activated by TAMs [30]. Likewise, the number of infiltrating $CD163^+$ TAMs may predict the survival of stage I/II melanomas [32]. It appears that the macrophage migration inhibitory factor (MIF) provides the defense mechanism against UVB-induced NMSC whereby VEGF and ensuing angiogenesis in company with the inflammatory response are upregulated [33]. Thus, MIF makes an impression on the initiation and progression of NMSCs following chronic UVB irradiation, due to a decrease of 45 % in the incident rate of UVB-induced NMSC in MIF-deficient mice [33].

As explained above, the "macrophage balance" hypothesis [24] remains constant in skin cancers. This dual and confusing behavior of TAMs is not improbable to be explained under the impression of their origins as well as their activating factors. For instance, macrophage chemotactic proteins (MCPs) resulted in melanoma clones with some paradoxical features including a twofold number of TAMs, more tumorigenic melanoma cells, and fewer response rates to IL-2 immunotherapy, whereas slower tumor growth rate, in comparison with normal melanoma clones [34]. The roles of TAMs have been comprehensively reviewed by several articles [35, 36].

As we are aware that the tumor microenvironment is dynamic as well as that TAMs belong to a heterogeneous constitution of surface markers [37, 38], the urgent need to exactly determine the "cause and effect" between two variables, the dynamicity of tumor microenvironment, and the dynamicity of markers expressing on TAMs during skin tumor progression as well as to draw the "cause-and-effect" diagram is felt.

14.3.2 Dendritic Cells (DCs): ADL Triangle

The triangle of "ADL" can be defined by three vertices of antigens, lymphocytes, and DCs; thereby, an immune response will be scattered over the area of this triangle dependent on its three sides [39]. A heterogeneous collection of up- and downregulatory immune responses have been attributed to a wide variety of DCs, which can be readily assigned to tolerogenic non-full mature and immunogenic full mature categories [40–42]. Langerhans cells in the skin act as APCs in the face of antigens and triggers such as local inflammation, pathogens, and tissue damage [43]. As a result of IL-10 effect, skin-resident Langerhans cells become capable of inducing Th2 cells, but not Th1 cells [44].

Indeed, it is not false to draw a positive feedback loop between immature DCs and IL-10, as DCs induce immunological tolerance via IL-10 production; IL-10 impedes the progress of DC maturation [43, 45].

The Th1 and Th2 patterns are perfectly fitted to govern responding and progressing melanoma metastases, respectively, by producing their specific cytokines, e.g., IL-2, IL-12, and IFN-γ for Th1 pattern and IL-10 for Th2 pattern [46]. This is a justifiable interpretation of the stimulation of both alloantigen- and melanoma antigen-specific anergy in $CD8^+$ CTLs by IL-10-treated DC which could be inversed by IL-2 [47]. Furthermore, IL-10 and IL-4 have been agreed on downregulation of DC-produced IL-12, whose stimulation is dependent on CD40 ligation [48]. It is interesting to compare rDCs and pDCs, derived from responding and progressing melanoma metastases. First, the CD86 expression is significantly higher in rDCs. Second, the DC-induced allogeneic T cell proliferation is significantly more in rM than pM [46].

The survival rate of patients with primary cutaneous melanoma was also delineated to be under the influence of peritumoral extent of mature DCs, which per se was positively correlated with lymphocyte infiltration [49].

14.3.3 Lymphocytes: Hero or Bystander or Antihero?

T cell subpopulations hold opposing functions categorized in one heterogeneous T cell population. In the light of evidence of skin tumorigenesis, γδ T cells stand guard on the body against tumor progression, whereas αβ T cells are more likely to behave as an antihero [50]. This finding is in parallel with the fact that natural killer T cells (NKTCs), which enormously express TCRαβ, appear to be potentially involved in the suppression of "tumor rejection" immune response to UV-induced skin carcinogenesis probably via a secretion wave of IL-4 [51]. A body of evidence emerged suggesting that skin tumors, either NMSC or primary cutaneous melanoma, may sometimes regress spontaneously [52–55]. The principal mechanisms including apoptosis of tumoral cells and infiltration of lymphocytes, especially $CD4^+$ T cells, underlie this process [55, 54]. Thus, spontaneous regression of skin tumors is known for its symphonious orchestration of two immunological processes.

The stimulatory and co-stimulatory signaling pathways are the precursors of T cell activation. The co-stimulatory signal is emitted dependent on the binding of CD28 to B7-1 (CD80) or B7-2 (CD86) mediated by APCs [56]. CTLA-4, known as CD152 and counter-receptor of B7 family, is believed to inhibit T cell activation and thereby to demonstrate disregard of recognized tumor antigens [57, 58]. Furthermore, IL-2 has also been known as one of the mediators, if not the only one, of the CD28-dependent CTLA-4 expression [59]. Overall, as well expected, an approximately 7-year follow-up study recently recommended that the therapeutic strategy with the design of ipilimumab, as the CTLA-4 blocker, in combination with IL-2 achieves a 17 % complete clinical response rate in patients with metastatic melanoma [60]. CTLA-4 has been proven as strategically vital in inducing and sustaining immunosuppression via influencing $Foxp3^+CD4^+$ regulatory T cells [61]. The local recurrence of cutaneous melanoma over 2 years led to substantial rise in the extent of $CD25^+FOXP3^+$ T regulatory cells, either in the tumor parenchyma or among melanoma cells or TILs [62].

Interestingly, the prognostic models of survival rate and sentinel lymph node (SLN) positivity in patients with primary cutaneous melanoma have been proposed [63, 64]. Accordingly, the patterns of tumor-infiltrating lymphocytes (TILs), e.g., brisk, nonbrisk, and absent, have been correlated with the survival rate of patients with primary cutaneous melanoma as the maximum and minimum 5-year survival of 77 and 37 % belong to the brisk and absent TIL categories, respectively [63]. Further, male melanoma patients with ulceration and increased Breslow thickness and without TILs pose a higher risk of SLN metastasis [64]. The aforementioned patterns of TILs were defined by Clark et al. [65] and can be definitely recommended due to

satisfactory agreement between observers (kappa value >0.6) [65, 66].

The role of B lymphocytes in recruitment of innate immunity makes impossible to ignore their importance in the epithelial carcinogenesis. It became perfectly plain where the recruitment of innate immune cells and subsequent chronic inflammation have been provided via adoptive transfer of B lymphocytes from HPV16 mice into T and B cell-deficient/HPV16 mice [67]. However, virtually no importance places per se on B cells in melanoma vaccination, which is fully assembled into T cell population [68]. Indeed, B cells act as bystanders and their aforementioned detrimental role is the bystander effect.

14.4 Tumor Antigens

Antigens, exogenous or endogenous substances, can evoke immune responses. Based on their expression on tumoral cells solely or both tumoral and normal cells, tumor antigens can be either specific or associated with tumors, respectively [69]. A hypothesis emerged suggesting that central and peripheral tolerance is an obstructive element of host immune responses against tumors due to recognizing tumor-associated antigens as "self" antigens [70, 42]. Based on the administration of antitumoral antibodies or specific tumoral antigens and/or nonspecific proinflammatory molecules or adjuvants, cancer immunotherapeutic strategies involve passive or active immunity, respectively [71]. Altogether, early recognition of all tumor antigens is essential for the design of cancer immunotherapy-based approaches.

14.4.1 Tumor Antigens of Melanoma

Cytotoxic T lymphocytes (CTLs) have been used to determine melanoma-specific antigens as well as melanoma-associated antigens such as MZ2E, MZ2D, gp100, gp75, CDK4, GAGE family, MAGE family, and MART-1 [72–81]. However, the central focus of debate surrounding melanoma antigen-targeted immunotherapies is on antigens with the highest expression in metastatic melanoma, e.g., tyrosinase, Melan-A/MART-1, and gp100 [82].

14.4.1.1 Vascular Endothelial Growth Factor (VEGF)

VEGF, also known as vascular permeability factor (*VPF*), gene encodes a signal protein affecting endothelial cells and mediating several functions, such as increasing vascular permeability; inducing angiogenesis, vasculogenesis, and endothelial cell growth; promoting cell migration; and inhibiting apoptosis (*NCBI* Gene Database). Upregulation of VEGF-A can be detectable in various inflammatory diseases including delayed-type hypersensitivity reactions (DTH) and rheumatoid arthritis as well as chronic inflammatory conditions in the skin [83]. Overexpression of both VEFG/VPF and their specific receptors at the neo-vessel formation sites led to recognition of VEGF/VPF as a proangiogenic factor [84]. The epidermis and keratinocyte-derived VEGF are known as the major productive sources for VEGF and angiogenic factor for the skin, respectively [85]. VEFG family directly induces angiogenesis and lymphangiogenesis, which are prerequisites for tumor enlargement and metastasis to distant sites.

The tumor is alive and kicking; there is a demand for the expression of VEGF due to genetic and epigenetic factors, e.g., lack of adequate amount of glucose and oxygen as well as *Ras* oncogene activation, UV radiation, TGF-α, and keratinocyte growth factor [86]. Together, VEGF-A can substantially contribute toward tumorigenesis through providing cancerous growth and inflammatory conditions.

Mouse skin carcinogenesis involves increase in mRNA expression and protein level of VEFG/VPF (VEFG-A) in a tumorigenicity-dependent manner [85, 87]. Further, it was suspected that VEGF is the key modulator in transition from epidermal hyperplasia to papilloma occurrence [85]. The minor problems of tumor-induced VEGF and subsequent angiogenesis can be solely solved. However, the main issue is caused by the major influence of VEGF-A on macrophages in the setting of skin carcinogenesis. The possible reasons include (a) the chemoattractive

effect of VEFG-A on macrophages due to the presence of VEGFR-1; (b) lower level of tumor progression, invasion, proliferation, and angiogenesis following macrophage depletion; and (c) macrophage-activated COX-2-dependent VEGF-A secretion [25, 30]. High stress levels accelerate the progression of SCC, which was escorted by higher immunosuppression and higher VEGF levels [88]. VEGF-C and VEGF-D, two structurally and functionally similar growth factors, lead to and accelerate the process of skin carcinogenesis in mice probably through mediating the proinflammatory microenvironment [89].

Indeed, like animal studies, VEGF expression is dependent on skin cancer tumorigenicity in humans. This notion is supported by more VEGF expression in SCC than BCC and significant positive correlation between the progression of melanoma and VEGF expression (distribution, intensity, and index) [90–92]. The roles of VEGF in NMSC are adopted to act in both autocrine and paracrine manner including directly affecting keratinocyte, tumoral cells and chemoattracting monocytes and macrophages [86]. However, Doppler ultrasound previously established the tumor blood flow in primary melanomas (thickness >1.2 mm) provoking to suggest the cutoff point for melanoma progression [93]. Recently, the three-dimensional model of melanoma firmly verified angiogenesis as a vital prerequisite in the process of melanoma dissemination [94]. These findings draw an interesting parallel with the fact of melanoma-derived proangiogenic cytokines, e.g., VEGF-A, FGF-2, TGF-1, PGF-1, and IL-8 (reviewed in [95]). Moreover, the VEGF genotypes were found to correlate with prognostic variables of cutaneous malignant melanoma, as the -1154 AA genotype was negatively associated with thickness of primary tumors in the vertical growth phase, whereas the GG genotype showed a positive correlation [96].

Undoubtedly, VEGF fuels skin tumorigenesis. Like a car, tumor progression without its fuel eventually stops. The VEGF-based anti-angiogenesis therapeutic approaches seem to be reasonably encouraging [97].

14.5 Immunosuppression and Skin Tumors

There is a devastating positive feedback loop between immunosuppression and tumorigenesis.

14.5.1 Skin Tumor-Induced Immunosuppression

Tumoral cells tend to progress steadily. The specific immune cells, relevant signaling pathways, and subsequent responses oppose to the progression plan of tumoral cells. Thus, the obvious alternative route is to suppress specific immune responses to tumoral antigens. All main immunosuppressive factors derived from skin tumors were previously mentioned under the "tumor immunity" heading.

14.5.2 Immunosuppression-Induced Skin Tumors

UV, the most important extrinsic risk factor for skin cancers, can induce specific immunological unresponsiveness, leading to specific immunosuppression, which, in turn, can cause tumorigenesis [98]. Moreover, it is found that long-term immunosuppressive therapeutic conditions in transplant recipients or in patients with bowel inflammatory disease (BID) lead to higher overall risk of developing malignant cancers particularly skin cancers [99–101].

The incidence of skin cancers, the second most common cancers in children, was correlated with immunosuppression, which was correlated with the time and stage of disease, age at transplantation, recipient sex, extent of sun exposure after and/or before transplantation, graft relation, and $CD4^+$ T cell lymphopenia [99, 102, 103]. Of those, a 42 % increase in the cumulative incident rate during a 20-year postrenal transplantation highlighted the impact of long-term immunosuppression on skin tumorigenesis [103]. Although various skin tumors can develop following organ transplantation, the most common ones are SCC

and BCC [99]. Interestingly, the incidence of SCC and BCC in recipients is respectively higher and lower compared to the general population. The underlying reason for this difference could be explained by the more powerful role of the immune system in the management of SCC rather than BCC [104]. In 2004 in the USA, the incidence rates of melanoma and NMSC in kidney recipients compared to the general population were fivefold and 20-fold, respectively [105]. Nonetheless, the aforementioned statistics were less and nonsignificant in the other cohort studies, which is partly due to registering recipients of different transplanted organs including the heart, pancreas, liver, and lungs [106, 107].

Although some primary predictive programs including patient education about the risk of skin cancers and the use of sunscreens as well as regular skin examinations are currently executed for high-risk patients, there are inevitable challenges to the existing aggressive early skin cancer therapeutic protocols such as electrodesiccation and curettage, excision, and radiotherapy [108]. Therefore, a special need for a bilateral alternative approach for tumorigenesis inhibition in unison with suppressing immunity exists [109].

Now that the possible pathway from UV irradiation to skin tumorigenesis is explained, the following paragraph tends to discuss the major mediators of this pathway, both concerning immunity and skin immune system.

14.6 Photoimmunology

As previously mentioned, the question of how the primary immune system demonstrates disregard of highly antigenic cancers was generally under consideration for all cancers; now photoimmunology needs to be addressed in order to answer a more specific question of how the primary skin immune system demonstrates disregard of UV-induced highly antigenic skin cancers.

UV radiations are categorized under three headings according to their wavelength. As we are not threatened by short-wavelength UVC due to complete filtration by the atmosphere, the main attention should be given to medium- and long-wavelength UV radiations (World Health Organization: http://www.who.int). The surface of the earth receives the solar radiation containing about 90–99 % long-wavelength UVA (315–400 nm) and 1–10 % medium-wavelength UVB (280–315 nm) [110].

UV radiation leads to the formation of a milieu with the antigen-presenting cell (APC) phenotype in the epidermis and dermis [111, 112]. The APC phenotype is mainly expressed by neutrophils, macrophages, and monocytes, of course, separately from Langerhans cells [111, 112]. The hairless mouse model proposes the association of UV radiation with skin carcinogenesis in a dose-, wavelength-, and time-dependent manner [113]. There is a twofold increase in the incidence rate of UV-induced NMSCs [114].

It is plausible to suppose that the immune system responses are selectively and maybe under the impression of UV irradiation. There are two reasons behind two main keywords of this statement, selectively and maybe. First, UV radiation alters some immune responses including tumor rejection, delayed hypersensitivity, and common hypersensitivity (CHS), while some others such as graft rejection and antibody production apparently remain intact [115]. Second, there are the proposed genetic models for susceptibility to UV-induced immunosuppression and the risk of skin carcinogenesis [116–118]. Local nonspecific and systemic specific models for UV-induced immunosuppression have been suggested. UV radiation hinders the immune effector functions inside the UV-irradiated skin and immune responses to antigens triggered outside the UV-irradiated skin within a critical time post-UV irradiation, known as the local nonspecific and systemic specific models, respectively [115, 119].

The primary translator of UVA radiation into intracellular pathways seems to be reactive oxygen species (ROSs) products rather than DAN damage as in UVB, while conceding the possibility of ROS-related point mutations, particularly tandem C → T mutations, for both UVA and UVB [110, 120, 121]. However, the pattern of form- and level-dependent acting makes ROSs permanently dance to a different tune. Generally, ROSs trigger the pathways of apoptosis and

transcriptional factors [122, 123]. The important signaling pathways under the impression of UV are briefly explained in the following.

14.6.1 BRAF-MAPK

Among all ROSs, UVA is more likely to generate singlet oxygen terminating in the activation of mitogen-activated protein kinases (MAPKs) [110]. Thus, the cardinal importance should be placed on the MAPK signaling cascade, whereby signals of vital cellular processes, e.g., proliferation, differentiation, and apoptosis, as well as the signals of mitogenic oncogenes are emitted [124].

The contribution of MAPK to melanoma tumorigenesis is not restricted to the evasion step, but to the melanoma initiation due to carrying BRAF mutations in approximately two-thirds of malignant melanomas [125]. It is recognized that ATP-competitive RAF inhibitors can inhibit MAPK pathway leading to the decline in progression of melanomas with BRAF mutation [126]. However, it is not easy to recognize that the same inhibitors can induce RAF-MEK-ERK pathway leading to the increase in the progression of melanomas with KRAS mutation or RAS/RAF wild type [126].

The MAPK signaling pathway with the escort of STAT-3 facilitates the BRAF mutant melanoma escape from the immune system by means of producing immunosuppressive cytokines, e.g., IL-6, IL-10, and VEGF [127].

There is a proposal concerning that MAPK pathway can be induced dependent on or independent of BRAF in melanoma progression. Its dependency has been proven by some animal studies demonstrating the inhibition of MAPK pathway by means of BRAF inhibitors [127]. Its independency has been suggested as the reason behind the crux of the matter, lack of long-term therapeutic responses to BRAF inhibitors in mutant malignant melanomas [128]. However, therapeutic response rates to the selectively BRAF or MEK inhibitors (e.g., vemurafenib, dabrafenib, or trametinib) in patients with malignant melanoma and BRAFV600E mutation have been proved to be generally satisfactory; the combinational strategy with both BRAF and MEK inhibitors produced more desirable clinical outcome [129–134]. Melanoma patients with BRAFV600E mutation treated with vemurafenib are more likely to contain mutations in RAS along with activation of MAPK pathway and ERK-mediated transcription, which, in turn, predisposes the patient to future SCC and keratoacanthomas [135]. Meanwhile, the therapeutic effect of BRAF inhibitors for melanoma exerted form is achieved by the elimination of immunosuppressive cytokines and enhancing infiltration by both $CD4^+$ and $CD8^+$ lymphocytes [136].

14.6.2 NF-κB

NF-κB pathway, induced by many tumor-induced stimuli, regulates the expression of genes controlling inflammation, cell proliferation, and apoptosis [123]. Proinflammatory cytokines induce the inhibition of the NF-κB pathway terminating the inflammation-related tumorigenesis [137]. In touch with skin tumors, both UVA and UVB radiation can activate NF-κB leading to the increase in the extent of proinflammatory cytokine, IL-6, in human keratinocytes [138]. Accordingly, loss of bcl-2 goes hand in hand with stimulation of Fas-l to witness the UV-induced apoptosis event [138].

14.7 Immunogenetics of Skin Tumors

Evolution is proceeding apace the possibility of genomic alterations contributing to tumorigenesis. These mutations consist of oncogenes with dominant gain of function and tumor-suppressor genes with recessive loss of function resulting in the perception that tumorigenesis is a sequential process involving many genomic mutations in cancer cells [139, 140]. Nevertheless, discoveries of these cancerous genes which yielded an invaluable insight into tumorigenesis cannot be denied, yet it is undeniable that this tumoral genetic model is strongly inattentive to the tumoral microenvironment [141, 142]. However, it has been established that the

dynamic tumor microenvironment and its interactions with genetically unstable primary tumoral cells are the main determinants of cancer tumor staging and malignancy progression [143, 144]. For instance, the HaCaT/HaCaT-ras human skin carcinogenesis model traced that there was no tumor progression from selectively recultured benign and malignant cell lines with H-ras status to late-stage malignant clones [145].

The primary causes of NMSC comprise high sun exposure, vitamin D deficiency, and ultraviolet (UV) overexposure [146, 147]. The highly antigenic UV-induced NMSCs including BCC, SCC, and UV-induced more lethal melanoma as well as UV-produced tandem mutations underlie the importance of acquiring and applying the immunogenetic knowledge of skin tumors [148–151].

14.7.1 p53

One of the mechanisms underlying UV-induced apoptosis is held by the expression of p53. The abnormally detectable accumulation of p53, a chiefly negative regulator of cell cycle and a normal tumor-suppressor protein, may contribute to the development of skin tumors including keratoacanthomas and solar keratoses [152, 153]. However, it is ambiguous to hypothesize the association between positive immunostaining and detectable mutations for p53 in patients with NMSC or melanoma [150, 152, 154, 155]. Hopefully, experimental studies indicated that sunscreens provide some degrees of protection against p53 mutations for UV-irradiated animals [156].

The expression of p63 along with REDD1, a direct transcriptional target of p53, is able to upregulate the production of ROSs [157]. The cultural model of human keratinocytes demonstrates that SCCs survive in a p63-dependent manner, which is also explained by the overexpression of p73 [158].

14.7.2 MMP

Matrix metalloproteinase-9 (MMP-9), mainly produced by neutrophils, macrophages, and mast cells, can shift to proangiogenic factors such as VEGF, ensuing an "angiogenic switch" during tumorigenesis [30, 159–161]. However, generally, the activated stromal cells attached to both animal and human cancer models provide the major source of MMPs [162]. MMPs make substantial contributions to skin tumorigenesis, particularly BCC and SCC [30, 163, 164, 165]. These contributions still remain outstanding in skin tumorigenesis induced by HPV8 and HPV16 [163, 166, 167]. Thus, it is not surprising that the genetic polymorphisms of MMPs could be associated with the risk of skin carcinogenesis (reviewed in [28, 168]).

14.8 Inflammation and Skin Cancers

Establishing the connection between inflammation and cancers was done over a long period of time. This connection was pioneered by Virchow's hypothesis based on the presence of leukocytes in malignant tissues in the nineteenth century [169]. However, cancer-related inflammation (CRI) is now recently established as the seventh hallmark of cancer after sustaining proliferative signaling, evading growth suppressors, activating invasion and metastasis, enabling replicative immortality, inducing angiogenesis, and resisting cell death [170, 171].

There is a chain reaction, known as the inflammatory cascade, between the immune cells in order to produce an inflammatory reaction, classified as either acute or chronic models. The activation energy of both models is determined by inflammatory conditions provided in the body including infections, chemical and physical circumstances, and autoimmune diseases [169, 172]. The inflammatory conditions, chiefly comprised of TAMs and T cells, may lead to increase in the promotion, progression, and metastasis risk of different malignancies [37, 169]. Inflammation serves as a trustee in the wound healing process. Inflammatory cells continue to serve their functions in chronic skin wounds providing chronic inflammation and ROS products, which, in turn, lead to the development and progression of skin cancers [173, 174].

Two possible intrinsic and extrinsic pathways connecting inflammation and cancer exist which are derived from oncogenes and the microenvironment, respectively [172]. Previously in this chapter, some inflammation-related compartments of the microenvironment of skin tumors were discussed; here, myeloid-derived suppressor cells and cytokines are briefly explained.

14.8.1 Myeloid-Derived Suppressor Cells (MDSCs)

The hypoxia-inducible factor (HIF) 1α-related hypoxia in the tumoral microenvironment makes tumor-derived MDSCs functionally different from peripheral-derived MDSCs [175]. Of those, the main differences are rapid differentiation into macrophages and inhibition of both antigen-specific and antigen-nonspecific T cells [175]. Tumor-derived soluble factors (TDSFs) including chemokines, cytokines, and growth factors allow MDSCs to accumulate at the tumor site in aid to the tumor escape [176].

The experimental melanoma model illustrates that the chronic inflammation-related MDSC-dependent immunosuppression is a simple statement of fact that tumor-derived MDSCs lead to a decrease in T cell proliferation and TCR ζ-chain expression in company with increases in the level of proinflammatory molecules, e.g., IL-1β, IFN-γ, and GM-CSF [177, 178].

14.8.2 Cytokines

UVB causes a temporary increase in IL-4 neutrophils resulting in Th2 responses [179]. This is justified by the observation of CD15 and CD11b markers on IL-4$^+$ cells and CD15$^+$ depletion-induced inhibition of Th2 responses [179]. However, protein levels of other cytokines including IL-6, IL-8, and TNF-α were also increased [179]. The role of IL-6 in aid to tumor cell proliferation is transmitted via STAT3 pathway [137]. UV-induced immunosuppression is mediated by the interaction between CD11b and its ligand, iC3b, which enforces the upregulation of an anti-inflammatory cytokine, IL-10, and downregulation of an anti-angiogenic cytokine, IL-12 [180]. The plan of IL-10 upregulation and IL-12 downregulation is initiated from the dermis and transmitted to the epidermis [181]. As TNF-α is localized in a pattern similar to iC3b which is the infiltrating site of CD11b monocytes/macrophages, a synergic effect between TNF-α and iC3b is present [180]. The proposed mechanism underlying the preventive effect of selenium against melanoma dissemination is based on the inhibition of IL-18 expression [182]. It is well understood that the proinflammatory cytokine, IL-17, contributes to skin tumorigenesis in a STAT3 pathway-dependent manner, which is induced by IL-23 [137, 183]. IL-17 is acknowledged due to its capability to provide an inflammatory condition via eliciting the proinflammatory cytokines including TNF-α, IL-1β, and IL-6 [137]. Apparently, IL-1α and TNF-α conduce to angiogenesis and tumorigenesis of human melanoma via increasing VEGF and IL-8 [31].

Here, the role of cytokines taking part in skin cancers is briefly described. Lin and Karin have reviewed the role of cytokines in detail [137].

Both therapeutic and preventive approaches could be designed in light of the current knowledge on the link between inflammation and cancer as well as underlying molecular mechanisms; it is not the time to reap the ripe fruits.

14.9 Immunotherapy for Melanoma

From the first description of melanoma provided by John Hunter in 1787 to the recent mutation discoveries owing to Watson and Crick's model of DNA in the current century, surgical therapeutic strategies have been refined, while notable advances in immunotherapeutic strategies for melanoma have been made [184]. Challenges in estimating the surgical margins and resistance to cytotoxic standard therapies, as well as understanding the main immunologic properties of melanoma skin cancer as an immunologic tumor, explain the utmost importance of immunotherapeutic strategies for melanoma [185, 186].

14.9.1 Adoptive (Passive) Immunotherapy

As previously mentioned, tumor-infiltrating lymphocytes (TILs) receive the focal attention in immune-based therapeutic approaches due to the involvement in the regression of metastatic melanoma. It is sufficient to have an overview of the literature demonstrating the substantially clinical benefit and response rate (RR) of combinational strategies of TILs and IL-2 for patients with metastatic melanoma [187–189]. Consequently, it was highlighted that the objective response rates directly reflect the impacts of transferred cell-related factors, such as the number of $CD8^+CD27^+$ cells and the telomerase length [189].

Moreover, it has been notched up to another success in patients with advanced melanoma who had received gene-modified TIL [190].

Lymphokine-activated killer cells (LAK), activated by IL-2 *in vitro*, can substantially abolish pulmonary melanoma metastases [191]. A 4-year follow-up study on metastatic melanoma patients spotted the statistically significant difference in objective response rate between patients receiving $gp100_{209–217}$ peptide-pulsed IL-2 and IL-2 alone. Interestingly, no significant difference in objective response rate was made between patients receiving IL-2 alone and different melanoma vaccines [192]. Overall, these lines of evidence convey two important notions. The first is that gp100 antigens stand actively to catch the undivided attention span in our melanoma immunotherapeutic approaches. The second is to identify the individual role of IL-2 by its outstanding influence in melanoma treatments.

14.9.2 Active Immunotherapy

14.9.2.1 Vaccination Therapy: A Surefire Therapy for Melanoma?

The field of cancer vaccination, an active cancer immunotherapy, is taken seriously as a therapeutic option, particularly in the last three decades predominantly for melanoma probably due to exhibiting all the main tumor immunologic features [186, 193, 194]. Overall, vaccines contain two compartments, antigens and adjuvants. The therapeutic efficacy of cancer vaccines can be explained by two main immunological events, which both of them are related to T cell population somehow: induction of tumor antigen-specific effector T cells and tumor antigen-specific memory T cells [68]. Even though the exact mechanism leading to the superiority of vaccines over other more successful procedures against tumors remains unknown, some convincing reasoning has been unveiled including the consideration of beneficial cancer vaccines as well as viral vaccines, easy administration of vaccines, and taking precedence of subjective endpoints over objective achievements [195].

Generally, autologous vaccines, made from tumoral cells derived from the patient's own tumor, constitute the major part of tumor vaccines [194]. The autologous whole-cell tumor vaccines, consisting of all the relevant tumor-associated antigens, have been considered for their clinical efficacy on the treatment of some cancers such as colorectal, melanoma, and renal cell cancer [196]. Choosing an autologous vaccine is challenged by the autologous tumor, which is occasionally the main challenge per se due to unresectable or unavailable melanoma lesions [197].

Allogeneic vaccines, made from intact and modified tumoral cells derived from the patient's tumor, can be efficiently administered to a subset of patients with similar tumor-associated antigens [194].

Melanoma Cell-Based Vaccines

Melacine, an allogeneic immunologic adjuvant, contains melanoma cell lines and an immunologic adjuvant, DETOX. The evidence emerged by phase I and phase II trials suggesting 15.5–47 % clinical benefits of Melacine [185, 198, 199]. The objective response rate of patients between 10 and 35 % resulted from Melacine in combination with IFN-α [185].

Melanoma Antigen-Specific Peptide-Based Vaccines

The MPS160 vaccine is a gp100-derived melanoma peptide which contains overlapping human leukemic antigen-restricted T cell epitopes.

Several therapeutic arms including MPS160 alone or in combination with various doses of GM-CSF have been administrated to patients with metastatic melanoma; nonetheless, there was no significant clinical efficacy at all [200]. However, that was noted for antitumor immune response of lymphocytes to gp100-based peptide vaccine in PBMCs derived from melanoma patients [201]; unfortunately, high levels of antitumor antigen-specific T cells do not guarantee the progression of melanoma. This fact was ascertained when there was no observation of any correlation between the levels of tumor antigen-reactive $CD8^+$ T cells and clinically tumor regression following modified gp100-based vaccinations [70]. On the other hand, a 5-year follow-up study of melanoma patients at stages IIb–IV compiled the recognized antitumor immune response rate to $gp100_{280}$ peptide-targeted vaccines, thereby the unchallenged overall survival of 75 % [202]. Just to complicate matters, there was no substantial immunological or clinical response in patients with metastatic melanoma who received plasmid DNA encoding the gp100 melanoma antigen [203].

The MART-1 and Melan-A/MART-1 DNA plasmid-based vaccines have failed to achieve desired antitumor immunological responses and subsequent clinical responses in patients with melanoma at stages IIb, III, and IV [204, 205]. As illustrated further in the chapter, it was not difficult to believe that these vaccines could be more efficient when combined with DCs [206]. It was gaining importance when exosome-based cell-free vaccines comprising tumor antigen-specific peptide-pulsed DCs had been remedied [207]. However, the immunological and clinical success rate has not been achieved by the endeavor of phase I clinical trial of autologous DC-derived exosome vaccination for metastatic melanoma patients at stages IIIb and IV [208].

IFN-α Adjuvant Therapy

IFN-α exerts antitumoral effects through direct induction of apoptosis in SCC [209]. To preserve the role of IFN-α in melanoma vaccines, it is sufficient to define its course of action in the "V" manner explaining the downregulation of IFN-α and upregulation of $CD8^+$ T cell-dependent antitumor immunity mediated by $CD4^+$ T cells and IFN-α, respectively [210]. Since a series of randomized-controlled trials (RCTs) considering the effect of IFN-α adjuvant treatment *vs.* observation for patients with high-risk malignant melanoma at different stages following nodal dissection surgery did not achieve statistically significant outcomes including overall survival (OS), distant-metastasis-free interval (DMFI), disease-free survival (DFS), and relapse-free survival (RFS) stand in stark contrast to another series of such studies [211–218]. The efficacy of low-dose IFN-α2a was evidenced for high-risk melanoma patients, of course, without clinical diagnosis of metastatic nodes [219]. In contrast, however, the RFS benefit was dependent upon the IFN-α2b dose, and it was declared independent of the presence of lymph nodes [220]. Consequently, the statistically significant RFS benefit of adjuvant pegylated IFN-α2b, consisted of IFN-α2b, in addition to polyethylene glycol molecules to make more stable adjuvant, was reaped for stage III melanoma [221]. The evidence does not support the clinical efficacy of low-dose recombinant IFN-γ for high-risk melanoma [218].

Interestingly, although a meta-analysis (2010) of 14 RCTs comparing the clinical benefit of IFN-α adjuvant treatment with observation or any other adjuvant treatments (GM2 vaccination [222]) recently demonstrates statistically significant improvement in DFS and OS of patients with high-risk cutaneous melanoma, no survival benefits accrued from a systematic review and a meta-analysis of 9 and 12 RCTs, respectively, both of which were published in 2003 [223–225].

Altogether, the clinical efficacy of adjuvant IFN for melanoma is dependent on the therapeutic dose, duration of therapy, and clinical stage of tumors, and it cannot be recommended yet, due to the long-running controversies on this issue.

Dendritic Cell-Based Vaccines

The DC-based vaccination was designed to induce specific antitumor responses, favorably tumor antigen-reactive $CD8^+$ CTLs, in aid to repress the melanoma progression.

A collection of clinical trials and experimental studies were delineated to investigate the clinical

responses and immunological responses to immunotherapy or immuno-gene therapy with DC-based vaccines, either DCs only or in combination with killed melanoma cells or specific tumor antigens selected according to the HLA haplotype among patients with melanoma, either at early or late stages. DCs could be derived from the bone marrow, $CD34^+$ hematopoietic progenitor cells (HPCs), and monocytes [226–236]. The immunological and clinical responses comprised the induction of specific delayed-type hypersensitivity (DTH) reactions, rising reactive $CD8^+$ CTLs, producing IFN-γ, regressing metastasis, and completely or partially controlling tumor progression. Accordingly, there is no doubt that clinical responses rely heavily on the postvaccination immune responses to melanoma-specific antigens; however, it was clearly obvious in the case of complete clinical response to MART-1 vaccines along with spreading immune responses to melanoma antigens except for MART-1 [233]. The follow-up study of peptide-pulsed CD34-derived DC vaccine on patients with metastatic melanoma provided the medians of 20 and 7 months for two clinical outcomes of overall survival and event-free survival, respectively [231]. No more significant efficacy was obtained for autologous peptide-pulsed DC vaccination compared to dacarbazine chemotherapy in melanoma patients at stage IV [237].

A comprehensive review study on 38 articles assessing the influence of different vaccine parameters of investigated DC-targeted vaccines on the clinical efficacy of patients with melanoma revealed that peptide antigens, adjuvants, and antigen-specific T cell responses exert significant effects on clinical responses, but not on objective responses [238]. The murine melanoma model indicated a vast mass of DCs in the T cell nest sites of lymph nodes post-SC DC vaccination, thereby a favorable antitumor immune response, which was against amassing DCs in the spleen post-IV DC vaccination [239]. This experiment and parallel jobs served to appreciate the importance that lies in the route of DC-based melanoma vaccine administration to induce antitumor immunological responses. Accordingly, the magnetic resonance imaging (MRI) could bring success in need of pursuing DCs in the body [240]. Also, the stark contrast in migration of DCs between the IV and SC administration of DC-based vaccines is similarly offered between immature and mature DCs, respectively [241].

Immuno-gene therapy with vaccine containing autologous DCs and tumor mRNA failed to achieve a significant response rate or clinical benefit among patients with malignant melanoma [229].

The possible mechanism underlying the clinical efficacy of GM-CSF-secreting melanoma vaccines is probably in relation to recruiting APCs, mainly DCs and macrophages, albeit along with increasing melanoma antigen-specific reactive $CD4^+$ and $CD8^+$ T cells (reviewed in [242]).

Generally, there were few and promising clinical trials on DCs up to the year 2000 [243], which quickly resulted in the great deal of attention to DC vaccination. However, to our knowledge, the DC-targeted vaccines for melanoma patients can be preferred to other treatments with a view of facilitating the treatment process, but not in the regard of overall clinical efficacy. This debate is open amid suggestions which may be constructive in the near future such as up- and/or downregulating expression of some target points including inflammatory cytokines/chemokines, potential immunosuppressor factors, and angiogenic factors in aid to antitumor response in *ex vivo* DC generation and subsequently *in vivo* application [244, 245].

14.10 Concluding Remarks

As some scholars believe that we are at the bottom of the ladder of "melanoma vaccination" [246], in this chapter, conflicting evidences were brought up, which made it confusing and ambiguous to decide whether the overall clinical benefit of melanoma vaccines can push scientists into aspiring them as the epigraph of current therapeutic approaches. Notwithstanding, this question mark has been established since the first melanoma vaccine was examined, and as well we know that the therapeutic window in patients with melanoma can accommodate vaccination. Even though sufficient ways and means are partway developed, the current traditional approaches should be used to the promised day of "melanoma vaccination."

References

1. Linos E, Swetter SM, Cockburn MG, Colditz GA, Clarke CA. Increasing burden of melanoma in the United States. J Invest Dermatol. 2009;129(7):1666–74.
2. Chen ST, Geller AC, Tsao H. Update on the epidemiology of melanoma. Curr Dermatol Rep. 2013;2(1):24–34.
3. Simard EP, Ward EM, Siegel R, Jemal A. Cancers with increasing incidence trends in the United States: 1999 through 2008. Cancer. 2012;62(2):118–28.
4. Lomas A, Leonardi-Bee J, Bath-Hextall F. A systematic review of worldwide incidence of nonmelanoma skin cancer. Br J Dermatol. 2012;166(5):1069–80.
5. Dacosta Byfield S, Chen D, Yim Y, Reyes C. Age distribution of patients with advanced non-melanoma skin cancer in the United States. Arch Dermatol Res. 2013;305:1–6.
6. Bleyer A, Viny A, Barr R. Cancer in 15- to 29-year-olds by primary site. Oncologist. 2006;11(6):590–601.
7. Balch CM, Gershenwald JE, Soong S-J, Thompson JF, Atkins MB, Byrd DR, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol. 2009;27(36):6199–206.
8. Francken A, Shaw H, Thompson J, Soong S-J, Accortt N, Azzola M, et al. The prognostic importance of tumor mitotic rate confirmed in 1317 patients with primary cutaneous melanoma and long follow-up. Ann Surg Oncol. 2004;11(4):426–33.
9. Gimotty PA, Guerry D, Ming ME, Elenitsas R, Xu X, Czerniecki B, et al. Thin primary cutaneous malignant melanoma: a prognostic tree for 10-year metastasis is more accurate than American Joint Committee on cancer staging. J Clin Oncol. 2004;22(18):3668–76.
10. Callahan MK, Postow MA, Wolchok JD. Immunomodulatory therapy for melanoma: ipilimumab and beyond. Clin Dermatol. 2013;31(2):191–9.
11. Raaijmakers MI, Rozati S, Goldinger SM, Widmer DS, Dummer R, Levesque MP. Melanoma immunotherapy: historical precedents, recent successes and future prospects. Immunotherapy. 2013;5(2):169–82.
12. Kostovic K, Pastar Z, Ceovic R, Mokos ZB, Buzina DS, Stanimirovic A. Photodynamic therapy in dermatology: current treatments and implications. Coll Antropol. 2012;36(4):1477–81.
13. Dubas LE, Ingraffea A. Nonmelanoma skin cancer. Facial Plast Surg Clin North Am. 2013;21(1):43–53.
14. Mihm MC, Soter NA, Dvorak HF, Austen KF. The structure of normal skin and the morphology of atopic eczema. J Invest Dermatol. 1976;67(3):305–12.
15. Zinkernagel RM, Ehl S, Aichele P, Oehen S, Kündig T, Hengartner H. Antigen localisation regulates immune responses in a dose- and time-dependent fashion: a geographical view of immune reactivity. Immunol Rev. 1997;156(1):199–209.
16. Bos JD, Zonneveld I, Das PK, Krieg SR, van der Loos CM, Kapsenberg ML. The skin immune system (SIS): distribution and immunophenotype of lymphocyte subpopulations in normal human skin. J Invest Dermatol. 1987;88(5):569–73.
17. Bos JD, Kapsenberg ML. The skin immune system: progress in cutaneous biology. Immunol Today. 1993;14(2):75–8.
18. Bos JD, Kapsenberg ML. The skin immune system its cellular constituents and their interactions. Immunol Today. 1986;7(7–8):235–40.
19. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3(11):991–8.
20. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science. 2011;331(6024):1565–70.
21. Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. Annu Rev Immunol. 2004;22(1):329–60.
22. Kim R, Emi M, Tanabe K. Cancer immunoediting from immune surveillance to immune escape. Immunology. 2007;121(1):1–14.
23. Murdoch C, Muthana M, Coffelt SB, Lewis CE. The role of myeloid cells in the promotion of tumour angiogenesis. Nat Rev Cancer. 2008;8(8):618–31.
24. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol. 2002;23(11):549–55.
25. Linde N, Lederle W, Depner S, van Rooijen N, Gutschalk CM, Mueller MM. Vascular endothelial growth factor-induced skin carcinogenesis depends on recruitment and alternative activation of macrophages. J Pathol. 2012;227(1):17–28.
26. De Palma M. Partners in crime: VEGF and IL-4 conscript tumour-promoting macrophages. J Pathol. 2012;227(1):4–7.
27. Yim C-Y, Bastian NR, Smith JC, Hibbs JB, Samlowski WE. Macrophage nitric oxide synthesis delays progression of ultraviolet light-induced murine skin cancers. Cancer Res. 1993;53(22):5507–11.
28. Fanjul-Fernández M, Folgueras AR, Cabrera S, López-Otín C. Matrix metalloproteinases: evolution, gene regulation and functional analysis in mouse models. Biochimica et Biophysica Acta (BBA) Mol Cell Res. 2010;1803(1):3–19.
29. Kerkelä E, Ala-aho R, Jeskanen L, Rechardt O, Grénman R, Shapiro SD, et al. Expression of human macrophage metalloelastase (MMP-12) by tumor cells in skin cancer. J Invest Dermatol. 2000;114(6):1113–9.
30. Tjiu J-W, Chen J-S, Shun C-T, Lin S-J, Liao Y-H, Chu C-Y, et al. Tumor-associated macrophage-induced invasion and angiogenesis of human basal cell carcinoma cells by cyclooxygenase-2 induction. J Invest Dermatol. 2008;129(4):1016–25.
31. Torisu H, Ono M, Kiryu H, Furue M, Ohmoto Y, Nakayama J, et al. Macrophage infiltration correlates with tumor stage and angiogenesis in human malignant

melanoma: possible involvement of TNFα and IL-1α. Int J Cancer. 2000;85(2):182–8.
32. Jensen TO, Schmidt H, Møller HJ, Høyer M, Maniecki MB, Sjoegren P, et al. Macrophage markers in serum and tumor have prognostic impact in American Joint Committee on cancer stage I/II melanoma. J Clin Oncol. 2009;27(20):3330–7.
33. Martin J, Duncan FJ, Keiser T, Shin S, Kusewitt DF, Oberyszyn T, et al. Macrophage migration inhibitory factor (MIF) plays a critical role in pathogenesis of ultraviolet-B (UVB) -induced nonmelanoma skin cancer (NMSC). FASEB J. 2009;23(3):720–30.
34. Bottazzi B, Walter S, Govoni D, Colotta F, Mantovani A. Monocyte chemotactic cytokine gene transfer modulates macrophage infiltration, growth, and susceptibility to IL-2 therapy of a murine melanoma. J Immunol. 1992;148(4):1280–5.
35. Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. Cancer Res. 2006;66(2):605–12.
36. Allavena P, Sica A, Solinas G, Porta C, Mantovani A. The inflammatory micro-environment in tumor progression: the role of tumor-associated macrophages. Crit Rev Oncol Hematol. 2008;66(1):1–9.
37. Pettersen JS, Fuentes-Duculan J, Suárez-Fariñas M, Pierson KC, Pitts-Kiefer A, Fan L, et al. Tumor-associated macrophages in the cutaneous SCC microenvironment are heterogeneously activated. J Invest Dermatol. 2011;131(6):1322–30.
38. Qian B-Z, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. Cell. 2010;141(1):39–51.
39. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature. 1998;392(6673):245–52.
40. Jonuleit H, Schmitt E, Steinbrink K, Enk AH. Dendritic cells as a tool to induce anergic and regulatory T cells. Trends Immunol. 2001;22(7):394–400.
41. Lutz MB, Schuler G. Immature, semi-mature and fully mature dendritic cells: which signals induce tolerance or immunity? Trends Immunol. 2002;23(9):445–9.
42. Steinman RM, Hawiger D, Nussenzweig MC Tolerogenic dendritic cells*. Ann Rev Immunol. 2003;21(1):685–711.
43. Banchereau J, Schuler-Thurner B, Palucka AK, Schuler G. Dendritic cells as vectors for therapy. Cell. 2001;106(3):271–4.
44. Enk AH, Angeloni VL, Udey MC, Katz SI. Inhibition of Langerhans cell antigen-presenting function by IL-10. A role for IL-10 in induction of tolerance. J Immunol. 1993;151(5):2390–8.
45. Steinbrink K, Wolfl M, Jonuleit H, Knop J, Enk AH. Induction of tolerance by IL-10-treated dendritic cells. J Immunol. 1997;159(10):4772–80.
46. Enk AH, Jonuleit H, Saloga J, Knop J. Dendritic cells as mediators of tumor-induced tolerance in metastatic melanoma. Int J Cancer. 1997;73(3):309–16.
47. Steinbrink K, Jonuleit H, Müller G, Schuler G, Knop J, Enk AH. Interleukin-10–treated human dendritic cells induce a melanoma-antigen–specific anergy in CD8+ T cells resulting in a failure to lyse tumor cells. Blood. 1999;93(5):1634–42.
48. Koch NF, Stanzl U, Jennewein P, Janke K, Heufler C, Kämpgen E, et al. High level IL-12 production by murine dendritic cells: upregulation via MHC class II and CD40 molecules and downregulation by IL-4 and IL-10. J Exp Med. 1996;184(2):74–6.
49. Ladányi A, Kiss J, Somlai B, Gilde K, Fejős Z, Mohos A, et al. Density of DC-LAMP+ mature dendritic cells in combination with activated T lymphocytes infiltrating primary cutaneous melanoma is a strong independent prognostic factor. Cancer Immunol Immunother. 2007;56(9):1459–69.
50. Girardi M, Glusac E, Filler RB, Roberts SJ, Propperova I, Lewis J, et al. The distinct contributions of murine T cell receptor (TCR)γδ+ and TCRαβ+ T cells to different stages of chemically induced skin cancer. J Exp Med. 2003;198(5):747–55.
51. Moodycliffe AM, Nghiem D, Clydesdale G, Ullrich SE Immune suppression and skin cancer development: regulation by NKT cells. Nat Immunol. 2000;1(6):521–5.
52. Marks R, Foley P, Goodman G, Hage BH, Selwood TS. Spontaneous remission of solar keratoses: the case for conservative management. Br J Dermatol. 1986;115(6):649–55.
53. McGovern VJ. Spontaneous regression of melanoma. Pathology. 1975;7(2):91–9.
54. Curson C, Weedon D. Spontaneous regression in basal cell carcinomas. J Cutan Pathol. 1979;6(5):432–7.
55. Halliday G, Patel A, Hunt M, Tefany F, Barnetson RC. Spontaneous regression of human melanoma/nonmelanoma skin cancer: association with infiltrating CD4+ T cells. World J Surg. 1995;19(3):352–8.
56. McCoy KD, Le Gros G. The role of CTLA-4 in the regulation of T cell immune responses. Immunol Biol. 1999;77(1):1–10.
57. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science. 1996;271(5256):1734–6.
58. Linsley PS, Brady W, Urnes M, Grosmaire LS, Damle NK, Ledbetter JA. CTLA-4 is a second receptor for the B cell activation antigen B7. J Exp Med. 1991;174(3):561–9.
59. Walunas TL, Lenschow DJ, Bakker CY, Linsley PS, Freeman GJ, Green JM, et al. CTLA-4 can function as a negative regulator of T cell activation. Immunity. 1994;1(5):405–13.
60. Prieto PA, Yang JC, Sherry RM, Hughes MS, Kammula US, White DE, et al. CTLA-4 blockade with ipilimumab: long-term follow-up of 177 patients with metastatic melanoma. Clin Cancer Res. 2012;18(7):2039–47.
61. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, et al. CTLA-4 control over Foxp3+ regulatory T cell function. Science. 2008;322(5899):271–5.

62. Miracco C, Mourmouras V, Biagioli M, Rubegni P, Mannucci S, Monciatti I, et al. Utility of tumour-infiltrating CD25+ FOXP3+ regulatory T cell evaluation in predicting local recurrence in vertical growth phase cutaneous melanoma. Oncol Rep. 2007;18(5):1115–22.
63. Clemente CG, Mihm MC, Bufalino R, Zurrida S, Collini P, Cascinelli N. Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. Cancer. 1996;77(7):1303–10.
64. Taylor RC, Patel A, Panageas KS, Busam KJ, Brady MS. Tumor-infiltrating lymphocytes predict sentinel lymph node positivity in patients with cutaneous melanoma. J Clin Oncol. 2007;25(7):869–75.
65. Clark WH, Elder DE, Guerry D, Braitman LE, Trock BJ, Schultz D, et al. Model predicting survival in stage I melanoma based on tumor progression. J Natl Cancer Inst. 1989;81(24):1893–904.
66. Busam KJ, Antonescu CR, Marghoob AA, Nehal KS, Sachs DL, Shia J, et al. Histologic classification of tumor-infiltrating lymphocytes in primary cutaneous malignant melanoma: a study of interobserver agreement. Am J Clin Pathol. 2001;115(6):856–60.
67. de Visser KE, Korets LV, Coussens LM. De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. Cancer Cell. 2005;7(5):411–23.
68. Banchereau J, Palucka AK. Dendritic cells as therapeutic vaccines against cancer. Nat Rev Immunol. 2005;5(4):296–306.
69. Boon T, van der Bruggen P. Human tumor antigens recognized by T lymphocytes. J Exp Med. 1996;183(3):725–9.
70. Rosenberg SA, Sherry RM, Morton KE, Scharfman WJ, Yang JC, Topalian SL, et al. Tumor progression can occur despite the induction of very high levels of self/tumor antigen-specific CD8+ T cells in patients with melanoma. J Immunol. 2005;175(9):6169–76.
71. Buonaguro L, Petrizzo A, Tornesello ML, Buonaguro FM. Translating tumor antigens into cancer vaccines. Clin Vaccine Immunol. 2011;18(1):23–34.
72. van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, et al. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science. 1991;254(5038):1643–7.
73. Bakker A, Schreurs M, de Boer AJ, Kawakami Y, Rosenberg SA, Adema GJ, et al. Melanocyte lineage-specific antigen gp100 is recognized by melanoma-derived tumor-infiltrating lymphocytes. J Exp Med. 1994;179(3):1005–9.
74. Gaugler B, Van den Eynde B, van der Bruggen P, Romero P, Gaforio JJ, De Plaen E, et al. Human gene MAGE-3 codes for an antigen recognized on a melanoma by autologous cytolytic T lymphocytes. J Exp Med. 1994;179(3):921–30.
75. Wolfel T, Hauer M, Schneider J, Serrano M, Wolfel C, Klehmann-Hieb E, et al. A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. Science. 1995;269(5228):1281–4.
76. Kawakami Y, Eliyahu S, Delgado CH, Robbins PF, Sakaguchi K, Appella E, et al. Identification of a human melanoma antigen recognized by tumor-infiltrating lymphocytes associated with in vivo tumor rejection. Proc Natl Acad Sci. 1994;91(14):6458–62.
77. Van den Eynde B, Peeters O, De Backer O, Gaugler B, Lucas S, Boon T. A new family of genes coding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma. J Exp Med. 1995;182(3):689–98.
78. Wang RF, Robbins PF, Kawakami Y, Kang XQ, Rosenberg SA. Identification of a gene encoding a melanoma tumor antigen recognized by HLA-A31-restricted tumor-infiltrating lymphocytes. J Exp Med. 1995;181(2):799–804.
79. Rivoltini L, Kawakami Y, Sakaguchi K, Southwood S, Sette A, Robbins PF, et al. Induction of tumor-reactive CTL from peripheral blood and tumor-infiltrating lymphocytes of melanoma patients by in vitro stimulation with an immunodominant peptide of the human melanoma antigen MART-1. J Immunol. 1995;154(5):2257–65.
80. Kawakami Y, Eliyahu S, Jennings C, Sakaguchi K, Kang X, Southwood S, et al. Recognition of multiple epitopes in the human melanoma antigen gp100 by tumor-infiltrating T lymphocytes associated with in vivo tumor regression. J Immunol. 1995;154(8):3961–8.
81. Chianese-Bullock KA, Pressley J, Garbee C, Hibbitts S, Murphy C, Yamshchikov G, et al. MAGE-A1-, MAGE-A10-, and gp100-derived peptides are immunogenic when combined with granulocyte-macrophage colony-stimulating factor and montanide ISA-51 adjuvant and administered as part of a multipeptide vaccine for melanoma. J Immunol. 2005;174(5):3080–6.
82. Boon T, Coulie PG, Eynde BJV, Bruggen P. Human T cell responses against melanoma. Annu Rev Immunol. 2006;24:175–208.
83. Kunstfeld R, Hirakawa S, Hong Y-K, Schacht V, Lange-Asschenfeldt B, Velasco P, et al. Induction of cutaneous delayed-type hypersensitivity reactions in VEGF-A transgenic mice results in chronic skin inflammation associated with persistent lymphatic hyperplasia. Blood. 2004;104(4):1048–57.
84. Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol. 1995;146(5):1029.
85. Larcher F, Murillas R, Bolontrade M, Conti CJ, Jorcano JL. VEGF/VPF overexpression in skin of transgenic mice induces angiogenesis, vascular hyperpermeability and accelerated tumor development. Oncogene. 1998;17(3):303.
86. Johnson KE, Wilgus TA. Multiple roles for VEGF in non-melanoma skin cancer: angiogenesis and beyond. J Skin Cancer. 2012;2012:6.

87. Larcher F, Robles AI, Duran H, Murillas R, Quintanilla M, Cano A, et al. Up-regulation of vascular endothelial growth factor/vascular permeability factor in mouse skin carcinogenesis correlates with malignant progression state and activated H-ras expression levels. Cancer Res. 1996;56(23):5391–6.
88. Dhabhar FS, Saul AN, Holmes TH, Daugherty C, Neri E, Tillie JM, et al. High-anxious individuals show increased chronic stress burden, decreased protective immunity, and increased cancer progression in a mouse model of squamous cell carcinoma. PLoS One. 2012;7(4):e33069.
89. Alitalo AK, Proulx ST, Karaman S, Aebischer D, Martino S, Jost M, et al. VEGF-C and VEGF-D blockade inhibits inflammatory skin carcinogenesis. Cancer Res. 2013;73(14):4212–21.
90. Rajabi P, Neshat A, Mokhtari M, Rajabi MA, Eftekhari M, Tavakoli P. The role of VEGF in melanoma progression. J Res Med Sci. 2012;17(6):534–9.
91. Bowden J, Brennan PA, Umar T, Cronin A. Expression of vascular endothelial growth factor in basal cell carcinoma and cutaneous squamous cell carcinoma of the head and neck. J Cutan Pathol. 2002;29(10):585–9.
92. Viac J, Palacio S, Schmitt D, Claudy A. Expression of vascular endothelial growth factor in normal epidermis, epithelial tumors and cultured keratinocytes. Arch Dermatol Res. 1997;289(3):158–63.
93. Srivastava A, Hughes LE, Woodcock JP, Shedden EJ. The significance of blood flow in cutaneous malignant melanoma demonstrated by Doppler flowmetry. Eur J Surg Oncol. 1986;12(1):13–8.
94. Gibot L, Galbraith T, Huot J, Auger F. Development of a tridimensional microvascularized human skin substitute to study melanoma biology. Clin Exp Metastasis. 2013;30(1):83–90.
95. Dewing D, Emmett M, Pritchard Jones R. The roles of angiogenesis in malignant melanoma: trends in basic science research over the last 100 years. ISRN Oncol. 2012;2012:546927.
96. Howell WM, Bateman AC, Turner SJ, Collins A, Theaker JM. Influence of vascular endothelial growth factor single nucleotide polymorphisms on tumour development in cutaneous malignant melanoma. Genes Immun. 2002;3(4):229–32.
97. Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J. Vascular-specific growth factors and blood vessel formation. Nature. 2000;407(6801):242–8.
98. Kripke ML. Immunological unresponsiveness induced by ultraviolet radiation. Immunol Rev. 1984;80(1):87–102.
99. Euvrard S, Kanitakis J, Claudy A. Skin cancers after organ transplantation. N Engl J Med. 2003;348(17):1681–91.
100. Long MD, Herfarth HH, Pipkin CA, Porter CQ, Sandler RS, Kappelman MD. Increased risk for non-melanoma skin cancer in patients with inflammatory bowel disease. Clin Gastroenterol Hepatol. 2010;8(3):268–74.
101. Singh S, Nagpal SJS, Murad MH, Yadav S, Kane SV, Pardi DS, et al. Inflammatory bowel disease is associated with an increased risk of melanoma: a systematic review and meta-analysis. Clin Gastroenterol Hepatol. 2014;12(2):210–8.
102. Penn I. De novo malignances in pediatric organ transplant recipients. Pediatr Transplant. 1998;2(1):56–63.
103. Bordea C, Wojnarowska F, Millard PR, Doll H, Welsh K, Morris PJ. Skin cancers in renal-transplant recipients occur more frequently than previously recognized in a temperate climate. Transplantation. 2004;77(4):574–9.
104. Boukamp P. Non-melanoma skin cancer: what drives tumor development and progression? Carcinogenesis. 2005;26(10):1657–67.
105. Kasiske BL, Snyder JJ, Gilbertson DT, Wang C. Cancer after kidney transplantation in the United States. Am J Transplant. 2004;4(6):905–13.
106. Adami J, Gabel H, Lindelof B, Ekstrom K, Rydh B, Glimelius B, et al. Cancer risk following organ transplantation: a nationwide cohort study in Sweden. Br J Cancer. 2003;89(7):1221–7.
107. Lindelöf B, Sigurgeirsson B, Gäbel H, Stern RS. Incidence of skin cancer in 5356 patients following organ transplantation. Br J Dermatol. 2000;143(3):513–9.
108. Francis S, Berg D. Reducing skin malignancy risk in organ transplant recipients. Skin Ther Lett. 2013;18(1):1–3.
109. Guba M, Graeb C, Jauch K-W, Geissler EK. Pro- and anti-cancer effects of immunosuppressive agents used in organ transplantation. Transplantation. 2004;77(12):1777–82.
110. Bachelor MA, Bowden GT. UVA-mediated activation of signaling pathways involved in skin tumor promotion and progression. Semin Cancer Biol. 2004;14(2):131–8.
111. Meunier L, Bata-Csorgo Z, Cooper KD. In human dermis, ultraviolet radiation induces expansion of a CD36+; CD11b+; CD1− macrophage subset by infiltration and proliferation; CD1+; langerhans-like dendritic antigen-presenting cells are concomitantly depleted. J Invest Dermatol. 1995;105(6):782–8.
112. Cooper KD, Duraiswamy N, Hammerberg C. Neutrophils, differentiated macrophages, and monocyte/macrophage antigen presenting cells infiltrate murine epidermis after UV injury. J Invest Dermatol. 1993;101(2):155–63.
113. de Gruijl FR, Forbes PD. UV-induced skin cancer in a hairless mouse model. Bioessays. 1995;17(7):651–60.
114. Grossman D, Leffell DJ. The molecular basis of nonmelanoma skin cancer: new understanding. Arch Dermatol. 1997;133(10):1263–70.
115. Kripke ML. Ultraviolet radiation and immunology: something new under the sun-presidential address. Cancer Res. 1994;54(23):6102–5.
116. Clemens K, Churchill G, Bhatt N, Richardson K, Noonan F. Genetic control of susceptibility to

UV-induced immunosuppression by interacting quantitative trait loci. Genes Immun. 1999;1(4):251–9.
117. Noonan FP, Hoffman HA. Control of UVB immunosuppression in the mouse by autosomal and sex-linked genes. Immunogenetics. 1994;40(4):247–56.
118. Noonan FP, Muller HK, Fears TR, Kusewitt DF, Johnson TM, De Fabo EC. Mice with genetically determined high susceptibility to ultraviolet (UV)-induced immunosuppression show enhanced UV carcinogenesis. J Invest Dermatol. 2003;121(5):1175–81.
119. Nishigori C, Yarosh D, Donawho C, Kripke M. The immune system in ultraviolet carcinogenesis. J Invest Dermatol Symp Proc. 1996;1(2):143–6.
120. de Gruijl FR. Photocarcinogenesis: UVA vs. UVB radiation. Skin Pharmacol Appl Skin Physiol. 2002;15(5):316–20.
121. Feig DI, Reid TM, Loeb LA. Reactive oxygen species in tumorigenesis. Cancer Res. 1994;54(7 Supplement):1890s–4.
122. Matés JM, Sánchez-Jiménez FM. Role of reactive oxygen species in apoptosis: implications for cancer therapy. Int J Biochem Cell Biol. 2000;32(2):157–70.
123. Waris G, Ahsan H. Reactive oxygen species: role in the development of cancer and various chronic conditions. J Carcinog. 2006;5(1):14.
124. Seger R, Krebs EG. The MAPK signaling cascade. FASEB J. 1995;9(9):726–35.
125. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. Nature. 2002;417(6892):949–54.
126. Hatzivassiliou G, Song K, Yen I, Brandhuber BJ, Anderson DJ, Alvarado R, et al. RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. Nature. 2010;464(7287):431–5.
127. Sumimoto H, Imabayashi F, Iwata T, Kawakami Y. The BRAF-MAPK signaling pathway is essential for cancer-immune evasion in human melanoma cells. J Exp Med. 2006;203(7):1651–6.
128. Alcalá AM, Flaherty KT. BRAF inhibitors for the treatment of metastatic melanoma: clinical trials and mechanisms of resistance. Clin Cancer Res. 2012;18(1):33–9.
129. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med. 2011;364(26):2507–16.
130. Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med. 2010;363(9):809–19.
131. Sosman JA, Kim KB, Schuchter L, Gonzalez R, Pavlick AC, Weber JS, et al. Survival in BRAF V600–mutant advanced melanoma treated with vemurafenib. N Engl J Med. 2012;366(8):707–14.
132. Flaherty KT, Robert C, Hersey P, Nathan P, Garbe C, Milhem M, et al. Improved survival with MEK inhibition in BRAF-mutated melanoma. N Engl J Med. 2012;367(2):107–14.
133. Flaherty KT, Infante JR, Daud A, Gonzalez R, Kefford RF, Sosman J, et al. Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. N Engl J Med. 2012;367(18):1694–703.
134. Hauschild A, Grob J-J, Demidov LV, Jouary T, Gutzmer R, Millward M, et al. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. Lancet. 2012;380(9839):358–65.
135. Su F, Viros A, Milagre C, Trunzer K, Bollag G, Spleiss O, et al. RAS mutations in cutaneous squamous-cell carcinomas in patients treated with BRAF inhibitors. N Engl J Med. 2012;366(3):207–15.
136. Wilmott JS, Long GV, Howle JR, Haydu LE, Sharma RN, Thompson JF, et al. Selective BRAF inhibitors induce marked T-cell infiltration into human metastatic melanoma. Clin Cancer Res. 2012;18(5):1386–94.
137. Lin W-W, Karin M. A cytokine-mediated link between innate immunity, inflammation, and cancer. J Clin Invest. 2007;117(5):1175–83.
138. Xia J, Song X, Bi Z, Chu W, Wan Y. UV-induced NF-κB activation and expression of IL-6 is attenuated by (−)-epigallocatechin-3-gallate in cultured human keratinocytes in vitro. Int J Mol Med. 2005;16(5):943–50.
139. Hanahan D, Weinberg R. The hallmarks of cancer. Cell. 2000;100(1):57–70.
140. Heng HHQ, Stevens JB, Bremer SW, Ye KJ, Liu G, Ye CJ. The evolutionary mechanism of cancer. J Cell Biochem. 2010;109(6):1072–84.
141. Mueller MM, Fusenig NE. Friends or foes – bipolar effects of the tumour stroma in cancer. Nat Rev Cancer. 2004;4(11):839–49.
142. Tlsty TD, Coussens LM. Tumor stroma and regulation of cancer development. Annu Rev Pathol Mech Dis. 2006;1:119–50.
143. Gupta GP, Massagué J. Cancer metastasis: building a framework. Cell. 2006;127(4):679–95.
144. Mueller MM, Fusenig NE. Tumor-stroma interactions directing phenotype and progression of epithelial skin tumor cells. Differentiation. 2002;70(9–10):486–97.
145. Mueller MM, Peter W, Mappes M, Huelsen A, Steinbauer H, Boukamp P, et al. Tumor progression of skin carcinoma cells in vivo promoted by clonal selection, mutagenesis, and autocrine growth regulation by granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. Am J Pathol. 2001;159(4):1567–79.
146. Leccia MT. Skin, sun exposure and vitamin D: facts and controversies. Ann Dermatol Venereol. 2013;140(3):176–82.
147. Chen AC, Halliday GM, Damian DL. Non-melanoma skin cancer: carcinogenesis and chemoprevention. Pathology. 2013;45(3):331–41.
148. Kripke ML. Effects of UV radiation on tumor immunity. J Natl Cancer Inst. 1990;82(17):1392–6.
149. De Fabo EC, Noonan FP. Mechanism of immune suppression by ultraviolet irradiation in vivo. I. Evidence for the existence of a unique photoreceptor in skin and its role in photoimmunology. J Exp Med. 1983;158(1):84–98.

150. Greenblatt M, Bennett W, Hollstein M, Harris C. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res. 1994;54(18):4855–78.
151. Armstrong BK, Kricker A. The epidemiology of UV induced skin cancer. J Photobiol B Biol. 2001;63(1–3):8–18.
152. McGregor JM, Yu CCW, Dublin EA, Levison DA, MacDonald DM. Aberrant expression of p53 tumour-suppressor protein in non-melanoma skin cancer. Br J Dermatol. 1992;127(5):463–9.
153. Levine AJ, Momand J, Finlay CA. The p53 tumour suppressor gene. Nature. 1991;351(6326):453–6.
154. Campbell C, Quinn AG, Angus B, Rees JL. The relation between p53 mutation and p53 immunostaining in non-melanoma skin cancer. Br J Dermatol. 1993;129(3):235–41.
155. Kubo Y, Urano Y, Yoshimoto K, Iwahana H, Fukuhara K, Arase S, et al. p53 gene mutations in human skin cancers and precancerous lesions: comparison with immunohistochemical analysis. J Invest Dermatol. 1994;102(4):440–4.
156. Ananthaswamy HN, Loughlin SM, Cox P, Evans RL, Ullrich SE, Kripke ML. Sunlight and skin cancer: inhibition of p53 mutations in UV-irradiated mouse skin by sunscreens. Nat Med. 1997;3(5):510–4.
157. Ellisen LW, Ramsayer KD, Johannessen CM, Yang A, Beppu H, Minda K, et al. REDD1, a developmentally regulated transcriptional target of p63 and p53, links p63 to regulation of reactive oxygen species. Mol Cell. 2002;10(5):995–1005.
158. DeYoung MP, Johannessen CM, Leong C-O, Faquin W, Rocco JW, Ellisen LW. Tumor-specific p73 up-regulation mediates p63 dependence in squamous cell carcinoma. Cancer Res. 2006;66(19):9362–8.
159. Bergers G, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K, et al. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol. 2000;2(10):737–44.
160. Folkman J. Role of angiogenesis in tumor growth and metastasis. Semin Oncol. 2002;29(6 Suppl 16):15–8.
161. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med. 1995;1(1):27–30.
162. de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. Nat Rev Cancer. 2006;6(1):24–37.
163. Akgül B, Pfefferle R, Marcuzzi GP, Zigrino P, Krieg T, Pfister H, et al. Expression of matrix metalloproteinase (MMP)-2, MMP-9, MMP-13, and MT1-MMP in skin tumors of human papillomavirus type 8 transgenic mice. Exp Dermatol. 2006;15(1):35–42.
164. Kerkelä E, Saarialho-Kere U. Matrix metalloproteinases in tumor progression: focus on basal and squamous cell skin cancer. Exp Dermatol. 2003;12(2):109–25.
165. Zhang G, Luo X, Sumithran E, Pua VSC, Barnetson RSC, Halliday GM, et al. Squamous cell carcinoma growth in mice and in culture is regulated by c-Jun and its control of matrix metalloproteinase-2 and -9 expression. Oncogene. 2006;25(55):7260–6.
166. Akgül B, García-Escudero R, Ghali L, Pfister HJ, Fuchs PG, Navsaria H, et al. The E7 protein of cutaneous human papillomavirus type 8 causes invasion of human keratinocytes into the dermis in organotypic cultures of skin. Cancer Res. 2005;65(6):2216–23.
167. Coussens LM, Tinkle CL, Hanahan D, Werb Z. MMP-9 supplied by bone marrow–derived cells contributes to skin carcinogenesis. Cell. 2000;103(3):481–90.
168. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer. 2002;2(3):161–74.
169. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001;357(9255):539–45.
170. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis. 2009;30(7):1073–81.
171. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74.
172. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008;454(7203):436–44.
173. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420(6917):860–7.
174. Schäfer M, Werner S. Cancer as an overhealing wound: an old hypothesis revisited. Nat Rev Mol Cell Biol. 2008;9(8):628–38.
175. Corzo CA, Condamine T, Lu L, Cotter MJ, Youn J-I, Cheng P, et al. HIF-1α regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. J Exp Med. 2010;207(11):2439–53.
176. Dolcetti L, Marigo I, Mantelli B, Peranzoni E, Zanovello P, Bronte V. Myeloid-derived suppressor cell role in tumor-related inflammation. Cancer Lett. 2008;267(2):216–25.
177. Meyer C, Sevko A, Ramacher M, Bazhin AV, Falk CS, Osen W, et al. Chronic inflammation promotes myeloid-derived suppressor cell activation blocking antitumor immunity in transgenic mouse melanoma model. Proc Natl Acad Sci. 2011;108(41):17111–6.
178. Bunt SK, Sinha P, Clements VK, Leips J, Ostrand-Rosenberg S. Inflammation induces myeloid-derived suppressor cells that facilitate tumor progression. J Immunol. 2006;176(1):284–90.
179. Teunissen MBM, Piskin G, Nuzzo S, Sylva-Steenland RMR, de Rie MA, Bos JD. Ultraviolet B radiation induces a transient appearance of IL-4+ neutrophils, which support the development of Th2 responses. J Immunol. 2002;168(8):3732–9.
180. Yoshida Y, Kang K, Berger M, Chen G, Gilliam AC, Moser A, et al. Monocyte induction of IL-10 and down-regulation of IL-12 by iC3b deposited in ultraviolet-exposed human skin. J Immunol. 1998;161(11):5873–9.
181. Kang K, Gilliam AC, Chen G, Tootell E, Cooper KD. In human skin, UVB initiates early induction of IL-10 over IL-12 preferentially in the expanding dermal monocytic/macrophagic population. J Invest Dermatol. 1998;111(1):31–8.

182. Song H, Kim J, Lee HK, Park HJ, Nam J, Park GB, et al. Selenium inhibits migration of murine melanoma cells via down-modulation of IL-18 expression. Int Immunopharmacol. 2011;11(12):2208–13.
183. Wang L, Yi T, Zhang W, Pardoll DM, Yu H. IL-17 enhances tumor development in carcinogen-induced skin cancer. Cancer Res. 2010;70(24):10112–20.
184. Lee C, Collichio F, Ollila D, Moschos S. Historical review of melanoma treatment and outcomes. Clin Dermatol. 2013;31(2):141–7.
185. Vaishampayan U, Abrams J, Darrah D, Jones V, Mitchell MS. Active immunotherapy of metastatic melanoma with allogeneic melanoma lysates and interferon α. Clin Cancer Res. 2002;8(12):3696–701.
186. Mukherji B. Immunology of melanoma. Clin Dermatol. 2013;31(2):156–65.
187. Rosenberg SA, Packard BS, Aebersold PM, Solomon D, Topalian SL, Toy ST, et al. Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report. N Engl J Med. 1988;319(25):1676–80.
188. Rosenberg SA, Yannelli JR, Yang JC, Topalian SL, Schwartzentruber DJ, Weber JS, et al. Treatment of patients with metastatic melanoma with autologous tumor-infiltrating lymphocytes and interleukin 2. J Natl Cancer Inst. 1994;86(15):1159–66.
189. Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res. 2011;17(13):4550–7.
190. Rosenberg SA, Aebersold P, Cornetta K, Kasid A, Morgan RA, Moen R, et al. Gene transfer into humans – immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. N Engl J Med. 1990;323(9):570–8.
191. Mazumder A, Rosenberg SA. Successful immunotherapy of natural killer-resistant established pulmonary melanoma metastases by the intravenous adoptive transfer of syngeneic lymphocytes activated in vitro by interleukin 2. J Exp Med. 1984;159(2):495–507.
192. Smith FO, Downey SG, Klapper JA, Yang JC, Sherry RM, Royal RE, et al. Treatment of metastatic melanoma using interleukin-2 alone or in conjunction with vaccines. Clin Cancer Res. 2008;14(17):5610–8.
193. Chan AD, Morton DL. Active immunotherapy with allogeneic tumor cell vaccines: present status. Semin Oncol. 1998;25(6):611–22.
194. Sondak VK, Sabel MS, Mulé JJ. Allogeneic and autologous melanoma vaccines: where have we been and where are we going? Clin Cancer Res. 2006;12(7):2337s–41.
195. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. Nat Med. 2004;10(9):909–15.
196. Gruijl T, den Eertwegh AM, Pinedo H, Scheper R. Whole-cell cancer vaccination: from autologous to allogeneic tumor- and dendritic cell-based vaccines. Cancer Immunol Immunother. 2008;57(10):1569–77.
197. Yannelli JR, Wroblewski JM. On the road to a tumor cell vaccine: 20 years of cellular immunotherapy. Vaccine. 2004;23(1):97–113.
198. Mitchell MS, Kan-Mitchell J, Kempf RA, Harel W, Shau H, Lind S. Active specific immunotherapy for melanoma: phase I trial of allogeneic lysates and a novel adjuvant. Cancer Res. 1988;48(20):5883–93.
199. Mitchell MS, Harel W, Kempf RA, Hu E, Kan-Mitchell J, Boswell WD, et al. Active-specific immunotherapy for melanoma. J Clin Oncol. 1990;8(5):856–69.
200. Melanoma Study Group of the Mayo Clinic Cancer Center, Celis E. Overlapping human leukocyte antigen class I/II binding peptide vaccine for the treatment of patients with stage IV melanoma. Cancer. 2007;110(1):203–14.
201. Salgaller ML, Marincola FM, Cormier JN, Rosenberg SA. Immunization against epitopes in the human melanoma antigen gp100 following patient immunization with synthetic peptides. Cancer Res. 1996;56(20):4749–57.
202. Slingluff CL, Yamshchikov G, Neese P, Galavotti H, Eastham S, Engelhard VH, et al. Phase I trial of a melanoma vaccine with gp100280–288 peptide and tetanus helper peptide in adjuvant: immunologic and clinical outcomes. Clin Cancer Res. 2001;7(10):3012–24.
203. Rosenberg SA, Yang JC, Sherry RM, Hwu P, Topalian SL, Schwartzentruber DJ, et al. Inability to immunize patients with metastatic melanoma using plasmid DNA encoding the gp100 melanoma-melanocyte antigen. Hum Gene Ther. 2003;14(8):709–14.
204. Triozzi PL, Aldrich W, Allen KO, Carlisle RR, LoBuglio AF, Conry RM. Phase I study of a plasmid DNA vaccine encoding MART-1 in patients with resected melanoma at risk for relapse. J Immunother. 2005;28(4):382–8.
205. Weber J, Boswell W, Smith J, Hersh E, Snively J, Diaz M, et al. Phase 1 trial of intranodal injection of a Melan-A/MART-1 DNA plasmid vaccine in patients with stage IV melanoma. J Immunother. 2008;31(2):215–23.
206. Timmerman MDJM, Levy MDR. Dendritic cell vaccines for cancer immunotherapy. Ann Rev Med. 1999;50(1):507–29.
207. Zitvogel L, Regnault A, Lozier A, Wolfers J, Flament C, Tenza D, et al. Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell derived exosomes. Nat Med. 1998;4(5):594–600.
208. Escudier B, Dorval T, Chaput N, André F, Caby M-P, Novault S, et al. Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: results of the first phase I clinical trial. J Transl Med. 2005;3(1):10.
209. Rodríguez-Villanueva J, McDonnell TJ. Induction of apoptotic cell death in non-melanoma skin cancer by interferon-α. Int J Cancer. 1995;61(1):110–4.

210. Steitz J, Brück J, Lenz J, Knop J, Tüting T. Depletion of CD25+ CD4+ T cells and treatment with tyrosinase-related protein 2-transduced dendritic cells enhance the interferon α-induced, CD8+ T-cell-dependent immune defense of B16 melanoma. Cancer Res. 2001;61(24):8643–6.
211. Eggermont AMM, Suciu S, MacKie R, Ruka W, Testori A, Kruit W, et al. Post-surgery adjuvant therapy with intermediate doses of interferon alfa 2b versus observation in patients with stage IIb/III melanoma (EORTC 18952): randomised controlled trial. Lancet. 2005;366(9492):1189–96.
212. Garbe C, Radny P, Linse R, Dummer R, Gutzmer R, Ulrich J, et al. Adjuvant low-dose interferon α2a with or without dacarbazine compared with surgery alone: a prospective-randomized phase III DeCOG trial in melanoma patients with regional lymph node metastasis. Ann Oncol. 2008;19(6):1195–201.
213. Creagan ET, Dalton RJ, Ahmann DL, Jung SH, Morton RF, Langdon RM, et al. Randomized, surgical adjuvant clinical trial of recombinant interferon alfa-2a in selected patients with malignant melanoma. J Clin Oncol. 1995;13(11):2776–83.
214. Pehamberger H, Soyer HP, Steiner A, Kofler R, Binder M, Mischer P, et al. Adjuvant interferon alfa-2a treatment in resected primary stage II cutaneous melanoma. Austrian malignant Melanoma Cooperative Group. J Clin Oncol. 1998;16(4):1425–9.
215. Hancock BW, Wheatley K, Harris S, Ives N, Harrison G, Horsman JM, et al. Adjuvant interferon in high-risk melanoma: the AIM HIGH study – United Kingdom coordinating committee on cancer research randomized study of adjuvant low-dose extended-duration interferon alfa-2a in high-risk resected malignant melanoma. J Clin Oncol. 2004;22(1):53–61.
216. Cameron DA, Cornbleet MC, Mackie RM, Hunter JA, Gore M, Hancock B, et al. Adjuvant interferon alpha 2b in high risk melanoma – the Scottish study. Br J Cancer. 2001;84(9):1146–9.
217. Cascinelli N, Belli F, MacKie RM, Santinami M, Bufalino R, Morabito A. Effect of long-term adjuvant therapy with interferon alpha-2a in patients with regional node metastases from cutaneous melanoma: a randomised trial. Lancet. 2001;358(9285):866–9.
218. Kleeberg UR, Suciu S, Brocker EB, Ruiter DJ, Chartier C, Lienard D, et al. Final results of the EORTC 18871/DKG 80-1 randomised phase III trial. rIFN-alpha2b versus rIFN-gamma versus ISCADOR M versus observation after surgery in melanoma patients with either high-risk primary (thickness >3 mm) or regional lymph node metastasis. Eur J Cancer. 2004;40(3):390–402.
219. Grob JJ, Dreno B, de la Salmoniere P, Delaunay M, Cupissol D, Guillot B, et al. Randomised trial of interferon?-2a as adjuvant therapy in resected primary melanoma thicker than 1.5mm without clinically detectable node metastases. Lancet. 1998;351(9120):1905–10.
220. Kirkwood JM, Ibrahim JG, Sondak VK, Richards J, Flaherty LE, Ernstoff MS, et al. High- and low-dose interferon alfa-2b in high-risk melanoma: first analysis of intergroup trial E1690/S9111/C9190. J Clin Oncol. 2000;18(12):2444–58.
221. Eggermont AMM, Suciu S, Santinami M, Testori A, Kruit WHJ, Marsden J, et al. Adjuvant therapy with pegylated interferon alfa-2b versus observation alone in resected stage III melanoma: final results of EORTC 18991, a randomised phase III trial. Lancet. 2008;372(9633):117–26.
222. Kirkwood JM, Ibrahim J, Lawson DH, Atkins MB, Agarwala SS, Collins K, et al. High-dose interferon alfa-2b does not diminish antibody response to GM2 vaccination in patients with resected melanoma: results of the multicenter eastern cooperative oncology group phase II trial E2696. J Clin Oncol. 2001;19(5):1430–6.
223. Mocellin S, Pasquali S, Rossi CR, Nitti D. Interferon alpha adjuvant therapy in patients with high-risk melanoma: a systematic review and meta-analysis. J Natl Cancer Inst. 2010;102(7):493–501.
224. Wheatley K, Ives N, Hancock B, Gore M, Eggermont A, Suciu S. Does adjuvant interferon-α for high-risk melanoma provide a worthwhile benefit? A meta-analysis of the randomised trials. Cancer Treat Rev. 2003;29(4):241–52.
225. Lens MB, Dawes M. Interferon alfa therapy for malignant melanoma: a systematic review of randomized controlled trials. J Clin Oncol. 2002;20(7):1818–25.
226. Mackensen A, Herbst B, Chen J-L, Köhler G, Noppen C, Herr W, et al. Phase I study in melanoma patients of a vaccine with peptide-pulsed dendritic cells generated in vitro from CD34+ hematopoietic progenitor cells. Int J Cancer. 2000;86(3):385–92.
227. Paczesny S, Shi H, Saito H, Mannoni P, Fay J, Banchereau J, et al. Measuring melanoma-specific cytotoxic T lymphocytes elicited by dendritic cell vaccines with a tumor inhibition assay in vitro. J Immunother. 2005;28(2):148–57.
228. Schuler-Thurner B, Dieckmann D, Keikavoussi P, Bender A, Maczek C, Jonuleit H, et al. Mage-3 and influenza-matrix peptide-specific cytotoxic T cells are inducible in terminal stage HLA-A2.1+ melanoma patients by mature monocyte-derived dendritic cells. J Immunol. 2000;165(6):3492–6.
229. Kyte JA, Mu L, Aamdal S, Kvalheim G, Dueland S, Hauser M, et al. Phase I/II trial of melanoma therapy with dendritic cells transfected with autologous tumor-mRNA. Cancer Gene Ther. 2006;13(10):905–18.
230. O'Rourke ME, Johnson M, Lanagan C, See J, Yang J, Bell J, et al. Durable complete clinical responses in a phase I/II trial using an autologous melanoma cell/dendritic cell vaccine. Cancer Immunol Immunother. 2003;52(6):387–95.
231. Fay J, Palucka AK, Paczesny S, Dhodapkar M, Johnston D, Burkeholder S, et al. Long-term outcomes in patients with metastatic melanoma

vaccinated with melanoma peptide-pulsed CD34+ progenitor-derived dendritic cells. Cancer Immunol Immunother. 2006;55(10):1209–18.
232. Banchereau J, Palucka AK, Dhodapkar M, Burkeholder S, Taquet N, Rolland A, et al. Immune and clinical responses in patients with metastatic melanoma to CD34+ progenitor-derived dendritic cell vaccine. Cancer Res. 2001;61(17):6451–8.
233. Butterfield LH, Ribas A, Dissette VB, Amarnani SN, Vu HT, Oseguera D, et al. Determinant spreading associated with clinical response in dendritic cell-based immunotherapy for malignant melanoma. Clin Cancer Res. 2003;9(3):998–1008.
234. Celluzzi CM, Mayordomo JI, Storkus WJ, Lotze MT, Falo LD. Peptide-pulsed dendritic cells induce antigen-specific CTL-mediated protective tumor immunity. J Exp Med. 1996;183(1):283–7.
235. Thurner B, Haendle I, Röder C, Dieckmann D, Keikavoussi P, Jonuleit H, et al. Vaccination with mage-3a1 peptide–pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. J Exp Med. 1999;190(11):1669–78.
236. Berard F, Blanco P, Davoust J, Neidhart-Berard E-M, Nouri-Shirazi M, Taquet N, et al. Cross-priming of naive Cd8 T cells against melanoma antigens using dendritic cells loaded with killed allogeneic melanoma cells. J Exp Med. 2000;192(11):1535–44.
237. Schadendorf D, Ugurel S, Schuler-Thurner B, Nestle FO, Enk A, Bröcker EB, et al. Dacarbazine (DTIC) versus vaccination with autologous peptide-pulsed dendritic cells (DC) in first-line treatment of patients with metastatic melanoma: a randomized phase III trial of the DC study group of the DeCOG. Ann Oncol. 2006;17(4):563–70.
238. Engell-Noerregaard L, Hansen T, Andersen M, Thor Straten P, Svane I. Review of clinical studies on dendritic cell-based vaccination of patients with malignant melanoma: assessment of correlation between clinical response and vaccine parameters. Cancer Immunol Immunother. 2009;58(1):1–14.
239. Eggert AAO, Schreurs MWJ, Boerman OC, Oyen WJC, de Boer AJ, Punt CJA, et al. Biodistribution and vaccine efficiency of murine dendritic cells are dependent on the route of administration. Cancer Res. 1999;59(14):3340–5.
240. de Vries IJM, Lesterhuis WJ, Barentsz JO, Verdijk P, van Krieken JH, Boerman OC, et al. Magnetic resonance tracking of dendritic cells in melanoma patients for monitoring of cellular therapy. Nat Biotechnol. 2005;23(11):1407–13.
241. de Vries IJM, Krooshoop DJEB, Scharenborg NM, Lesterhuis WJ, Diepstra JHS, van Muijen GNP, et al. Effective migration of antigen-pulsed dendritic cells to lymph nodes in melanoma patients is determined by their maturation state. Cancer Res. 2003;63(1):12–7.
242. Dranoff G. GM-CSF-secreting melanoma vaccines. Oncogene. 2003;22(20):3188–92.
243. Fong L, Engleman EG. Dendritic cells in cancer immunotherapy. Ann Rev Immunol. 2000;18(1):245–73.
244. Aarntzen EHJG, Figdor CG, Adema GJ, Punt CJA, Vries IJM. Dendritic cell vaccination and immune monitoring. Cancer Immunol Immunother. 2008;57(10):1559–68.
245. Lesterhuis WJ, Aarntzen EHJG, De Vries IJM, Schuurhuis DH, Figdor CG, Adema GJ, et al. Dendritic cell vaccines in melanoma: from promise to proof? Crit Rev Oncol Hematol. 2008;66(2):118–34.
246. Yang JC. Melanoma vaccines. Cancer J. 2011;17(5):277–82.

15 Immunopathology of Head and Neck Tumors and Immunotherapy of Squamous Cell Carcinoma

Xu Qian, Andreas M. Kaufmann, and Andreas E. Albers

Contents

X. Qian, MD • A.E. Albers, MD, PhD (✉)
Department of Otolaryngology and Head and Neck Surgery, Campus Benjamin Franklin, Charité-Universitätsmedizin Berlin,
Hindenburgdamm 30, 12200 Berlin, Germany
e-mail: qian_michelle2014@163.com; andreas.albers@charite.de

A.M. Kaufmann, PhD
Department of Gynecology, Campus Benjamin Franklin and Campus Mitte, Charité-Universitätsmedizin Berlin,
Hindenburgdamm 30, 12200 Berlin, Germany
e-mail: andreas.kaufmann@charite.de

15.1 Introduction

Squamous cell carcinoma of the head and neck (HNSCC) is the sixth most common type of malignancy with over 650,000 new cases and 350,000 deaths worldwide each year [1]. Current treatments for HNSCC including surgery, radiation, and chemotherapy are of limited efficiency in preventing local and regional recurrences and distant metastases. Despite recent therapeutic improvements, long-term survival rates in patients with advanced-stage HNSCC have not significantly increased in the past 30 years [1].

Molecular studies suggest that HNSCC is either caused by spontaneous accumulation of multiple epigenetic and genetic alterations modulated by genetic predisposition and chronic inflammation and enhanced by environmental influences such as tobacco and alcohol abuse or by infection with oncogenic human papillomavirus (HPV) [1]. In recent years, the incidence of HPV-related head and neck cancer is rising and shows a distinctly different biology from head and neck cancer caused by other etiologies. Thus, two main etiologies can be defined: tumors induced by toxic substances or by the activity of the viral oncogenes of HPV. Both etiologies involve a multistep process and result in alterations affecting two large groups of genes: oncogenes and tumor suppressor genes. In addition, there is the notion of immune selection. This is related to major histocompatibility complex (MHC)-involved immune response mechanisms.

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_15,

For example, HPV16 E7 contributed to reduced MHC class I expression in HPV-associated malignancies [2]. MHC class II deficiency patients are at a risk of oral HPV infection [3]. These observations are important because they show that immune recognition is relevant and probably a force driving selection in the tumor toward immune-resistant variants. It is therefore essential to develop a deeper understanding of the biology of this disease for more effective alternative therapies such as immunotherapy.

Host immune reactions against tumors have long been a subject of laboratory and clinical investigations. On one hand, it has been shown that a naturally induced T cell response recognizing HNSCC-derived antigens exists that could potentially target and kill tumor cells. On the other hand, this process also exerts an immune selection pressure on tumor cells. Surviving tumor cells show a variety of mechanisms to escape immunosurveillance [4]. In this respect, immunological approaches promoting effective host responses and reversing immunosuppression would hopefully lead to successful immunotherapy against tumors.

Table 15.1 Studies of immunotherapy in HNSCC

Approach of immunotherapy	Model	Reference
Alpha-galactosylceramide-pulsed antigen-presenting cells	Human	[93]
Anti-CD3/CD28 monoclonal antibody	Human	[94]
CSC-based vaccination	*In vivo, in vitro*	[89, 95]
DNA-Hsp65 (a DNA vaccine)	Human	[96]
Epstein-Barr virus-associated AdE1-LMP poly vaccine	Human	[97]
Interferon-alpha	Human	[98]
Interleukin-12	Human	[99]
Interleukin-2	Human	[100]
Irradiated autologous tumor cells + granulocyte-macrophage colony-stimulating factor	Human	[101]
IRX-2 (cytokines)	Human	[102]
Invariant natural killer T cells	*In vitro*	[93]
Virus-modified autologous tumor cell vaccine	Human	[103]
Wild-type p53 peptide	*In vitro*	[104]

The identification and characterization of a variety of human tumor antigens with possible use for immunotherapy and immunomonitoring [5] and expectations triggered by successful *in vitro* tests and therapeutic results in animal models have led to rapid translation of these experimental findings into clinical testing. A rather large number of patients with various types of malignant diseases including HNSCC have been enrolled in clinical trials of T cell-based immunotherapy (Table 15.1). In many cases tumor antigen-specific CTL immune responses could be achieved and successfully monitored, but unfortunately these findings did not correlate with clinical responses [6]. True clinical responses attributable to immunotherapy have been sparse so far. Recently, it was reported that anti-CTLA4 (CTL-associated antigen-4) antibody ipilimumab achieved a significant increase in overall survival for patients with metastatic melanoma [7]. In a recent clinical trial reported by Schaefer et al., the functional status but not the frequency or phenotype of melanoma peptide-specific $CD8^+$ T cells correlated with survival to the multi-epitope peptide-based vaccine [8]. This discrepancy has initiated investigations into mechanisms underlying the failure of tumor antigen-specific CTLs to control tumor growth in cancer patients, especially those treated with immunotherapies. The mechanisms responsible for this impairment may vary depending on the nature of the tumor milieu and manifestation in the tumor microenvironment as well as in the periphery [9, 10].

In the past decade, tumor heterogeneity has been redefined by the hypothesis of cancer stem(-like) cells (CSCs). As a subpopulation of tumor cells, CSCs have been identified in many types of solid tumors including HNSCC. CSCs present stem cell characteristics with the ability to generate tumor, develop metastasis, and display increased resistance against current modes of therapies. This might explain the phenomenon that the response to immunotherapy may be delayed by a period of apparent tumor growth or the final failure of treatment. Therefore, the underlying mechanisms of interaction between CSC and the host immune system may present

a therapeutic challenge. Recent developments in immunotherapy may allow identification and targeting of CSC specifically.

In this chapter, the mechanisms of tumor-mediated interference with the host immune system and CTLs, in particular, concerning HNSCC will be summarized. Tumor-escape mechanisms from the immune system at the tumor site and in the periphery will be discussed and finally strategies to redirect the immune system to a more effective antitumor response.

15.2 Immune Responses in HNSCC

Effective antitumor responses in individuals with HNSCC depend on the ability of immune cells to recognize and eliminate tumor cells. These tumor antigen-specific T cells like $CD8^+$ CTLs or $CD4^+$ T-helper lymphocytes (Th) are known to be present in the peripheral circulation and the tumor site of patients with HNSCC [9–14]. Staining T cells with a tetrameric peptide-MHC complex monitored by multicolor flow cytometry were developed and frequently used for directed identification and phenotyping of antigen-specific T cells. These engineered tetrameric peptide-MHC complexes were able to bind more than one T cell receptor (TCR) on a specific T cell. These stainings are combined with T cell markers (CD3, 4, 8, etc.) and if desired with makers for the differentiation (CCR7, CD45RO, CD45RA, etc.) and the functional (CD107a, IFN-gamma, TNF-alpha, perforin, granzyme B, etc.) or dysfunctional (annexin, 7-AAD, CD3-zeta-chain, etc.) status of these cells [15–18].

15.2.1 Wt p53-Specific T Cells

Overexpression and/or accumulation of mutated p53 protein was reported in the majority of human cancers, including HNSCC. Therefore, T cells reactive against p53-derived epitopes by human leukocyte antigen (HLA) class I and II were investigated and described in various studies.

Wild-type (wt) sequence p53 peptides like other tumor epitopes are processed and presented to the host immune cells either directly by the tumor cells or by professional antigen-presenting cells (APCs) such as dendritic cells (DCs). This results in an increased number of wt p53 peptide-specific T cells and, in some instances, p53-specific antibodies [19–21].

Wt p53 epitope-specific T cells were reported significantly higher in the peripheral circulation and enriched in tumor-infiltrating lymphocytes (TILs) of patients with head and neck cancer than that in normal donors [9, 21]. These findings demonstrated that wt p53 epitope-specific T cells were not only present in the peripheral circulation but also at the tumor site or in tumor-involved lymph nodes. Interestingly, the presence and frequency of wt p53 epitope-specific effector T cells among TILs did not correlate with tumor stage. This implies that the frequency of $tetramer^+$/$CD8^+$ effector cells alone has no effect on tumor progression. In one of the studies performed by the authors, two patients with sufficient numbers of TILs were available to test *in vitro* responsiveness after polyclonal stimulation with anti-CD3 monoclonal antibody (mAb). Only a low interferon (IFN)-γ expression of the $CD3^+$/$CD8^+$ T cells could be measured indicating a poor responsiveness to this stimulus. At the same time, a significantly increased number of regulatory T cells (Tregs) were found at the tumor site compared to the periphery [9]. It has been well accepted that the presence and accumulation of Tregs inhibits T cell responses *in vivo* and may be responsible, in part, for the downregulation of antitumor immune responses in patients with head and neck cancer [22]. Tregs are likely to mediate suppressive effects directed at self-reactive T cells [23]. This immunosuppressive mechanism may be particularly relevant to T cells that recognize self-peptides such as wt p53 epitopes; thus they are likely to be tolerized, especially at the sites of their accumulation in tumor tissues. Other data also confirm the depressed functionality or even spontaneous apoptosis of $CD8^+$ tumor-specific T lymphocytes [10].

Although CTLs are considered to play the primary role in tumor eradication, it is also

hypothesized that the participation of tumor antigen-specific $CD4^+$Th cells may be required for optimal antitumor effects by generating and maintaining antitumor immune responses through interactions with CTLs and other cells [24, 25]. Current evidence indicates that $CD4^+$ Th cells play an important role in generating and maintaining antitumor immune responses [26–28]. Chikamatsu et al. demonstrated the identification and ability of anti-wt $p53_{110–124}$ $CD4^+$ T cells to enhance the *ex vivo* generation and antitumor functions of $CD8^+$ effector cells [14]. Later, Ito et al. reported that the presence of anti-$p53_{25–35}$ $CD4^+$ Th cells was shown to enhance the *in vitro* generation/expansion of HLA-A2-restricted, anti-wt $p53_{264–272}$ $CD8^+$ T cells, which from one donor were initially "nonresponsive" to the wt $p53_{264–272}$ peptide [29]. Recently, Chikamatsu et al. demonstrated that wt $p53_{108–122}$ and $p53_{153–166}$ peptides stimulate both Th1- and Th2-type $CD4^+$ cell responses in patients with head and neck cancer [30]. These results suggest that future vaccination strategies targeting tumor cells should incorporate helper and cytotoxic T cell-defined epitopes [29].

15.2.2 HPV-Derived Antigen-Specific T Cells

HPV-related HNSCC defines a different entity when compared to HPV-unrelated HNSCC. Therefore, immune responses to a persistent HPV infection were explored recently. Virus-derived antigens are considered superior targets for T cells than tumor-associated self-antigens because they have higher affinity to MHC and are more immunogenic [31]. HPV-encoded oncogenic proteins, such as E6 and E7, are promising tumor-specific antigens in addition to the fact that they are considered obligatory for tumor growth. In HNSCC, two studies showed an increased frequency of $CD8^+$ T lymphocytes directed against HPV E7 epitopes, documenting a natural immune response [11, 32]. These HPV-specific T cells were able to recognize and kill a naturally HPV-16-transformed HNSCC cell line after IFN-γ treatment that enhanced antigen processing and presentation by the tumor cells. Further phenotypic characterization of the HPV-specific T cells revealed an increase in terminally differentiated/lytic T cells ($CD8^+CD45RA^+CCR7^-$). This population was also characterized by a high frequency of staining for the degranulation marker CD107a in E7 tetramer$^+$ T cells, compared with bulk $CD8^+$ T cells, consistent with their terminally differentiated lytic, degranulated status. These cells may account for the unsuccessful antiviral immune response [24] to these tumors, indicating that incomplete activation of tumor-specific T cells or suboptimal target recognition may enable tumor progression *in vivo*. On the other hand, if such T cells could be adequately activated and expanded, these cells could provide a valuable source of effectors for cancer vaccination. Williams et al. used a mouse model to investigate whether HPV-specific immune mechanisms can result in tumor clearance [33]. They found an *in vivo* antigen-specific antitumor response that is generated against HPV^+-transformed cells and that this response requires $CD4^+$ and $CD8^+$ cells to mount this antitumor response.

15.3 Mechanisms of Tumor Immune Evasion

15.3.1 Suppression of T Cells in the Cancer-Bearing Host

Several mechanisms by which tumors escape from the host immune system have been identified in patients with head and neck cancer. One of them is the induction of apoptosis by Fas/FasL signaling pathways in effector T cells [34]. It was shown that a proportion of $CD3^+/Fas^+$ T cells in the peripheral circulation of tumor patients were in the process of apoptosis. This indicates that the Fas/FasL pathway is involved in spontaneous apoptosis of circulating CD95 (Fas^+) T lymphocytes [24]. Hoffmann et al. showed that Fas/FasL interactions might lead to increased turnover of T cells in the circulation and, consequently, to reduced immune competence in patients with HNSCC [13]. This may be explained by an imbalance in the absolute count of T-lymphocyte

subsets and an overall decreased absolute T cell count in patients not treated with cytotoxic agents [35, 36]. The rapid turnover mostly affects T cells with effector phenotype [37] that also show defects in signaling [24]. Preferentially tumor-specific T cells are affected by apoptosis indicating a tumor-related effect [10]. This observation can be explained further by the analysis of TCR Vbeta profiles of $CD8^+$ T cells in patients with HNSCC that were altered relative to normal controls. This may reflect increased apoptosis of expanded or tumor-contracted $CD8^+$ T cells, which define the TCR Vbeta profile of antigen-responsive T cell populations in patients with cancer [12]. Reports on T cell apoptosis at the tumor site and in the peripheral circulation [38] support these observations and suggest that induced death of TILs, generally considered to represent tumor-associated antigen-specific effector cells, is driven by the tumor or tumor-derived factors.

Tregs were also reported to be involved in Fas/FasL-mediated apoptosis. FasL is upregulated exclusively on Tregs isolated from patients with no evidence of disease after receiving cancer therapy [39]. These FasL-expressing Tregs are resistant to apoptosis themselves, but strongly suppress and kill $CD8^+$ effector cells, adverting the cancer community that traditional cancer therapy might contribute to tumor progression by collaborating with the peripheral tolerance process. In addition, Tregs in patients with HNSCC kill $CD4^+$ T effector cells via granzyme B in the presence of IL-2 [39].

Signaling defects in the TCR as well as nuclear factor (NF)-β activation pathways in TILs have been described in comparison to T cells infiltrating inflammatory noncancerous sites. These defects appear to be responsible for their loss of function [40]. Patients with tumors infiltrated by TILs expressing normal levels of CD3-zeta chain were found to have a better 5-year survival than those showing loss of CD3-zeta-chain expression [40, 41]. This protein is a signal adaptor of the T cell receptor and only when present the T cell can be activated. A high rate of apoptosis in TILs is considered to be a factor for poor prognosis [42].

Taken together it appears that apoptosis of lymphocytes in the periphery as well as at the tumor site leads to rapid and selective tumor-specific lymphocyte turnover followed by a loss of effector cells and thus failure to control tumor growth in cancer patients.

15.3.2 Role of Regulatory T Cells

By the identification of the expression of interleukin (IL)-2 receptor α and the forkhead-box transcription factor (Foxp3) as an essential transcription factor, $CD4^+$ Tregs have been characterized as a distinct lineage of T cells [43]. It has been documented in the past that Treg frequency increases in peripheral blood, lymph nodes, and tumors of patients with several types of cancer [44], including HNSCC [45, 46]. This correlates with HNSCC tumor progression [47, 48], but their relationship to patient prognosis was not established [49, 50]. Suppressor capacity and suppressor phenotype of Tregs isolated from HNSCC cancer patients are significantly increased in comparison to Tregs isolated from healthy subjects [45, 46], suggesting that enhanced function and survival of suppressor cells might constitute one of the mechanisms responsible for the immunosuppression of adaptive and innate immunity in these patients. Tregs could suppress the activity of $CD4^+$ and $CD8^+$ effector T cells, decrease antigen presentation, and promote the immunosuppressive functions of DCs, monocytes, and macrophages [51]. The blockade of Tregs was found to improve tumor immunosurveillance in a variety of tumor models [52–55]. Removing Tregs has also been shown to increase tumor immunity elicited by vaccination [56–58].

Thus, one therapeutic possibility for restoration of antitumor immunity in patients with cancer is to eliminate tumor antigen (TA)-specific Tregs and to boost simultaneously TA-specific T helper and CTL responses. The fact that Tregs and activated T effector cells share receptors and common metabolites in their differentiation, function, survival, and expansion (i.e., IL-2) suggests that regulation of the effector and suppressor compartment is

dichotomic. Thus, one new challenge in modern immunotherapy is to understand the signaling pathways which command the interplay of effector and suppressor responses in physiologic conditions and in inflammation. A detailed knowledge of these pathways might enable us to design immunotherapeutic strategies that selectively promote expansion, survival, and function of effector T cells or Treg responses in pathologies where one of the two compartments is in disadvantage resulting in autoreactive killer responses in the absence of Tregs or in immunosuppression in the case of an excessive Treg response. Revert immunosuppression in cancer to antitumor immunity is essential to increase the quality and success rate of traditional cancer therapy as well as the response to tumor vaccines.

15.3.3 Tumor Immune Escape

Collectively, downregulation of MHC class I or II and co-stimulatory molecules, which compromised tumor-associated antigen processing and presentation, and overexpression of immunosuppressive molecules (i.e., TGF-beta, PGE2, IL-4, IL-10) lead to a selection pressure on tumor cells [59–61]. This selection process and the resulting immune escape variants in the tumor indicate that an effective CTL response must have taken place during the development of the malignancy. The CTL-mediated cytolysis of immunogenic tumor cells is the driving force of the selection process toward CTL-resistant tumor cell variants. The immune-evaded tumor cells have several features making them resistant to further natural CTL attack.

With regard to the two different etiologies of HNSCC, more detailed studies are needed to investigate if this dysregulation can be observed in tumors with both etiologies. Whether this is a general phenomenon, as reported in other tumor types without viral etiologies, or if it is due to HPV-specific factors, as suggested in HPV6- and HPV11-associated laryngeal papilloma [62], remains to be clarified. However, it is becoming evident that virally induced tumors succeed in escaping the host immune system [63].

15.4 Reversing Immune Escape

Current immunotherapy is insufficient by the fact that immunosuppressive mechanisms are pronounced and relevant effector cells are suppressed in patients with HNSCC. Thus, enhancing the specific antitumor immune response and reversing the tumor-mediated immunosuppression is the primary goal of immunotherapy. One approach is the development of prophylactic HPV vaccines to prevent formation of malignancies or therapeutic HPV vaccines to treat patients in combination with other therapies [64]. Two studies have investigated if an endogenous T cell immunity to HPV-encoded oncogenes *E6* and *E7* in HNSCC patients exists [11, 65]. This group of T cells would have the potential to specifically identify and target the tumor upon appropriate activation. Therefore, these cells are a critical prerequisite for the development of vaccine-based strategies for enhancing antitumor immunity in patients with HPV^+ tumors. Indeed, in both studies it was found that infection with HPV16 (as compared to uninfected control individuals) significantly alters the frequency and functional capacity of virus-specific T cells in HNSCC patients. In addition to the presence of HPV-specific effector T cells, successful tumor elimination requires that HPV-infected tumor cells function as appropriate targets for cytotoxic T-lymphocyte recognition and elimination. Immunohistochemistry of $HPV16^+$ HNSCC tumors showed that the antigen-processing machinery components are downregulated in tumors compared to adjacent normal squamous epithelium [11]. Thus, immunity to HPV16 E7 is associated with the presence of HPV16 infection and presentation of E7-derived peptides on HNSCC cells, suggesting immune escape mechanisms comparable to cervical cancers [66]. These findings suggest that development of E7-specific immunotherapy in HPV-related HNSCC should be combined with strategies to enhance the antigen-processing machinery component expression and function [11]. Patients with HPV-unrelated HNSCC have a high incidence of p53 mutations. A number of p53-derived epitopes that can be used for the design

of vaccines have been identified [67, 68]. Since mutations in the p53 sequence are frequent, epitopes incorporating these mutations would have to be tailored specifically to each patient. Therefore, epitopes composed of the wt sequence are especially attractive, since they are shared among the same HLA type, and are therefore not patient specific.

Except specific immune stimulation of cytolytic $CD8^+$ T cells, nonspecific immune activation on MHC class I or II molecules, lymphocytes, and NK cells is also aimed to reverse the suppressive effects [69–71]. Downregulation of dysfunction of antigen-processing machinery (APM) components by the tumor may disturb both the induction of tumor-specific T cells in the initial phase of the immune response and subsequently during the effector phase the proper recognition of the tumor. This effect is augmented by absence or reduced expression of MHC class I molecules on the cellular surface. These cells are considered to have a more aggressive phenotype [72] which may also be the result of immunoselection of tumor cells able to evade the immunosurveillance. The result can be seen by a low number of tumor-infiltrating lymphocytes and ineffective generation, activation, or even enhanced apoptosis of tumor-specific T cells [9, 10]. In experimental systems, incubation of HNSCC cell lines with IFN-γ was able to restore T cell recognition and killing [11, 62]. These preliminary data should inspire more basic and clinical research to better understand and further refine and develop such adjuvant strategies for clinical application. From the current point of view, it seems indispensable to combine APM and MHC class I restoration with induction of tumor-specific immune responses.

Tumors can also interfere with the immune system by the production and release of numerous factors that modulate functions of immune cells or directly induce apoptosis. These factors take action in the tumor microenvironment and beyond. Accumulation of Treg cells in the tumor microenvironment could suppress effector T cell responses. Pharmacologic inhibitors can be used to eliminate Treg cells and reiterate antitumor functions of effector T cells [73, 74]. Studies showed that myeloid-derived suppressor cells (MDSCs) play a role in suppressing immune responses in conjunction with Tregs in HNSCC [75, 76]. Chikamatsu et al. reported that $CD14^+$ HLA-DR- MDSCs contribute to immune suppression in HNSCC [76]. These MDSCs expressed a higher level of CD86 and PD-L1. Blocking CD86 or PD-L1 could partially restore T cell proliferation and IFN-γ production. Chemotherapeutic drugs such as 5-fluorouracil (5-FU) or gemcitabine are reported selectively cytotoxic on MDSCs, whereas no significant effect on T cells, NK cells, and DC cells was observed. The elimination of MDSC by 5-FU increased IFN-γ production by tumor-specific $CD8^+$ T cells infiltrating the tumor and promoted T cell-dependent antitumor responses *in vivo* [77].

15.5 T Cell Therapies Directed to Cancer Stem Cells

In previous *in vitro* studies, we have shown that a small population of the HNSCC tumor cells known as CSCs exhibited properties like self-renewal, invasion capacity, and epithelial-mesenchymal transition (EMT) [78]. Later, in formalin-fixed paraffin-embedded HSNCC tissues, CSC populations were found related to poor differentiation of tumors and high nodal status. Compared to primary tumors, the proportion of aldehyde dehydrogenase-1 (ALDH1)-expressing CSCs was significantly increased in the corresponding metastases [79]. These findings suggested that CSCs were able to complete the metastatic cascade. Studies from other groups addressed the radio- and chemoresistance of CSCs and their ability to initiate tumor in HNSCC [80, 81]. Due to these properties, CSCs have been moved into the focus of targeted therapies.

Due to a relative resistance of the CSCs, established therapeutic modalities such as radiation and chemotherapy more preferentially kill the bulk of the tumor; hence, it is being envisioned that targeting CSCs with these therapies may decrease the frequency of recurrences and enhance the patient's long-term survival.

Therefore, the development of strategies that target the CSC subpopulation directly is highly desirable. Since radio- and chemotherapies have already been optimized toward the limits of clinical benefit and yet tolerable side effects, a very attractive alternative approach of specifically targeting CSCs is to develop antitumor T cell vaccines. One of the possible reasons that these therapies lacked efficacy in past clinical trials could be attributed to the fact that rather bulk tumor than CSCs has been targeted. This may change with the identification of tumor-specific epitopes derived from CSC markers. One such a CSC model target for head and neck cancer and others is a recently described CD8-defined T cell epitope of ALDH1 [82]. Examples of other such CD8-defined T cell epitopes are available for prostate stem cell antigen [83]. Less well-defined approaches include the development of a CSC-dendritic cell vaccine [84]. Studies in animal models for prostate cancer and malignant glioma demonstrated the potential of different vaccination strategies (DCs, gene gun, and virus) targeting CSCs in cancer immunotherapy [85, 86]. It was recently suggested that stemness-related proteins expressed in CSCs might also be a source for tumor antigens. Tumor types most dependent on CSC for their growth kinetics were named to be the best suited for approaches targeting stem cell genes [87].

The authors recently reported that in HNSCC cell lines, $ALDH^+$ CSC populations were less sensitive to MHC class I-restricted alloantigen-specific $CD8^+$ CTL lysis as compared to matched monolayer-derived cells. When treated with IFN-γ, its sensitivity was upregulated. $ALDH^{high}$-expressing CSCs presented more sensitivity than the $ALDH^{low}$ CSCs toward $CD8^+$ CTL killing [88]. Visus et al. investigated the ability of ALDH1-specific $CD8^+$ T cells to eliminate $ALDH^{bright}$ CSCs and control tumor growth and metastases. They found that $ALDH^{bright}$ CSCs isolated from HLA-$A2^+$ human HNSCC were tumorigenic in immunodeficient mice and could be recognized by ALDH1-specific $CD8^+$ T cells *in vitro*. The antitumor activity of adoptive immunotherapy with ALDH1-specific CTLs *in vitro* and *in vivo* showed its effect on the inhibition of tumor growth and metastases or prolonged survival of xenograft-bearing immunodeficient mice. Ning et al. successfully demonstrated the vaccination effects after inoculation of $ALDH^+$ CSC populations from mouse models of squamous cell carcinoma and melanoma into different syngeneic immunocompetent hosts [89]. They found that CTLs generated from peripheral blood mononuclear cells or splenocytes harvested from CSC-vaccinated hosts were capable of killing CSCs *in vitro*.

Therefore, the classification of conclusive CSC markers followed by the identification of defined T cell-recognized CSC epitopes in the future may lead to the clinical application of anti-CSC vaccination strategies. Whether targeted therapies directed against stem cell-associated signaling pathways, which may be activated in CSCs, will be of clinical use or be limited by undesirable side effects *in vivo* remains unresolved so far. It will be necessary to carefully monitor the effects of CSC-based vaccines on normal stem cells.

15.6 Current Vaccination Strategies

HPV-associated HNSCC may be prevented by the existing prophylactic HPV vaccines or treated by vaccines designed to induce an appropriate antitumor immune response against HPV-specific tumor antigens [11, 12]. Prophylactic HPV vaccines have been developed via virus-neutralizing antibodies targeting the L1 capsid antigen. However, this capsid protein is not expressed in persistently HPV-infected basal epithelial cells and transformed cells in infected mucosa and is therefore useless for therapeutic vaccination. Accordingly, prophylactic vaccines have not shown any therapeutic activity [90]. Thus, vaccines with therapeutic potential must target the two HPV oncogenic proteins, E6 and E7, as antigens that are important in the induction and maintenance of cellular transformation and are co-expressed in the majority of HPV-associated carcinomas. Peptide vaccination in patients with cervical cancer as well as HNSCC is still under investigation but generally has failed so far.

p53 may serve as a model antigen for the development of broadly applicable antitumor vaccines in HNSCC. *In vitro* stimulation of $CD8^+$ T cells with wt p53 peptide-pulsed autologous DCs can be used to induce either HLA-A2-restricted, wt $p53_{149–157}$ and/or wt $p53_{264–272}$ peptide-specific responses from epitope-specific precursors. Interestingly, using these single epitopes, wt p53 peptide-specific $CD8^+$ T cells were generated in only a third of healthy donors or subjects with cancer [21]. Others have reported comparable findings [91, 92]. The limited responsiveness of healthy donors may be explained by negative thymic selection of T cells with receptors specific for self-antigens. It can be expected that especially T cells with high-affinity receptors are eradicated. The observed limited responsiveness to HLA class I-restricted wt p53 peptides among HLA class I-compatible healthy donors and subjects with cancer suggests that multiple wt p53 peptides are needed in order to maximize donor responsiveness. The underlying causes can only be suspected and may partly be due to the mechanisms of tumor immune evasion discussed above. Since it may prove difficult to determine the responsiveness pre-vaccination in an individual case, a vaccine consisting of more than one epitope may be the more promising approach.

Despite the current immune-based therapy for HNSCC, which is using bulk tumor masses with heterogeneous populations of cancer cells as a source of antigen either to generate effector T cells or to prime DC vaccines, the inability to target CSCs may be a significant factor for treatment failures. Therefore, CSC-targeted vaccine-based immunotherapy should be included in the current investigation.

15.7 Concluding Remarks

The rapid progress in understanding the molecular biology of malignancies of HNSCC has not been matched by impressive progress in cancer therapies. Therapeutic strategies should consider that HNSCC has at least two distinct etiologies with HPV-related and HPV-unrelated cancer. Both etiologies will differ significantly in the antigenic makeup of the tumor cells based on the presence of self or viral antigens, respectively. As a result, immunotherapeutic approaches should aim at induction of adequate antigen processing and presentation by the tumor cells to become visible for the immune system as target. Furthermore, tumor-induced immune dysregulation should be redirected in favor of tumor rejection, and finally an adequate stimulation of effector T cells capable of *in vivo* expansion and survival in the tumor microenvironment is thought critical for improving clinical results. In addition, by the identification of the human CSC compartment, studies indicated that CSCs could potentially be specifically identified and targeted. It will be necessary to develop new strategies to target the CSCs to improve the outcome of those individuals with HNSCC.

References

1. Leemans CR, Braakhuis BJ, Brakenhoff RH. The molecular biology of head and neck cancer. Nat Rev Cancer. 2011;11(1):9–22.
2. Bottley G, Watherston OG, Hiew YL, Norrild B, Cook GP, Blair GE. High-risk human papillomavirus E7 expression reduces cell-surface MHC class I molecules and increases susceptibility to natural killer cells. Oncogene. 2008;27(12):1794–9.
3. Guirat-Dhouib N, Baccar Y, Mustapha IB, Ouederni M, Chouaibi S, El Fekih N, et al. Oral HPV infection and MHC class II deficiency (a study of two cases with atypical outcome). Clin Mol Allergy. 2012;10(1):6.
4. Duray A, Demoulin S, Hubert P, Delvenne P, Saussez S. Immune suppression in head and neck cancers: a review. Clin Dev Immunol. 2010;2010:701657.
5. Novellino L, Castelli C, Parmiani G. A listing of human tumor antigens recognized by T cells: March 2004 update. Cancer Immunol Immunother. 2005;54(3):187–207.
6. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. Nat Med. 2004;10(9):909–15.
7. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711–23.
8. Schaefer C, Butterfield LH, Lee S, Kim GG, Visus C, Albers A, et al. Function but not phenotype of melanoma peptide-specific CD8(+) T cells correlate with survival in a multiepitope peptide vaccine trial (ECOG 1696). Int J Cancer. 2012;131(4):874–84.

9. Albers AE, Ferris RL, Kim GG, Chikamatsu K, DeLeo AB, Whiteside TL. Immune responses to p53 in patients with cancer: enrichment in tetramer+ p53 peptide-specific T cells and regulatory T cells at tumor sites. Cancer Immunol Immunother. 2005;54(11):1072–81.
10. Albers AE, Schaefer C, Visus C, Gooding W, DeLeo AB, Whiteside TL. Spontaneous apoptosis of tumor-specific tetramer+ CD8+ T lymphocytes in the peripheral circulation of patients with head and neck cancer. Head Neck. 2009;31(6):773–81.
11. Albers A, Abe K, Hunt J, Wang J, Lopez-Albaitero A, Schaefer C, et al. Antitumor activity of human papillomavirus type 16 E7-specific T cells against virally infected squamous cell carcinoma of the head and neck. Cancer Res. 2005;65(23):11146–55.
12. Albers AE, Visus C, Tsukishiro T, Ferris RL, Gooding W, Whiteside TL, et al. Alterations in the T-cell receptor variable beta gene-restricted profile of CD8+ T lymphocytes in the peripheral circulation of patients with squamous cell carcinoma of the head and neck. Clin Cancer Res. 2006;12(8):2394–403.
13. Hoffmann TK, Dworacki G, Tsukihiro T, Meidenbauer N, Gooding W, Johnson JT, et al. Spontaneous apoptosis of circulating T lymphocytes in patients with head and neck cancer and its clinical importance. Clin Cancer Res. 2002;8(8):2553–62.
14. Chikamatsu K, Albers A, Stanson J, Kwok WW, Appella E, Whiteside TL, et al. P53(110-124)-specific human CD4+ T-helper cells enhance in vitro generation and antitumor function of tumor-reactive CD8+ T cells. Cancer Res. 2003;63(13):3675–81.
15. Whiteside TL, Schuler P, Schilling B. Induced and natural regulatory T cells in human cancer. Expert Opin Biol Ther. 2012;12(10):1383–97.
16. Lanzavecchia A, Sallusto F. Progressive differentiation and selection of the fittest in the immune response. Nat Rev Immunol. 2002;2(12):982–7.
17. Czystowska M, Gooding W, Szczepanski MJ, Lopez-Abaitero A, Ferris RL, Johnson JT, et al. The immune signature of CD8(+)CCR7(+) T cells in the peripheral circulation associates with disease recurrence in patients with HNSCC. Clin Cancer Res. 2013;19(4):889–99.
18. Watanabe M, Kono K, Kawaguchi Y, Mizukami Y, Mimura K, Maruyama T, et al. Interleukin-21 can efficiently restore impaired antibody-dependent cell-mediated cytotoxicity in patients with oesophageal squamous cell carcinoma. Br J Cancer. 2010;102(3):520–9.
19. Chikamatsu K, Nakano K, Storkus WJ, Appella E, Lotze MT, Whiteside TL, et al. Generation of anti-p53 cytotoxic T lymphocytes from human peripheral blood using autologous dendritic cells. Clin Cancer Res. 1999;5(6):1281–8.
20. Soussi T. The humoral response to the tumor-suppressor gene-product p53 in human cancer: implications for diagnosis and therapy. Immunol Today. 1996;17(8):354–6.
21. Hoffmann TK, Nakano K, Elder EM, Dworacki G, Finkelstein SD, Appella E, et al. Generation of T cells specific for the wild-type sequence p53(264-272) peptide in cancer patients: implications for immunoselection of epitope loss variants. J Immunol. 2000;165(10):5938–44.
22. Schaefer C, Kim GG, Albers A, Hoermann K, Myers EN, Whiteside TL. Characteristics of CD4+CD25+ regulatory T cells in the peripheral circulation of patients with head and neck cancer. Br J Cancer. 2005;92(5):913–20.
23. Shimizu J, Yamazaki S, Sakaguchi S. Induction of tumor immunity by removing CD25+CD4+ T cells: a common basis between tumor immunity and autoimmunity. J Immunol. 1999;163(10):5211–8.
24. Kuss I, Donnenberg AD, Gooding W, Whiteside TL. Effector CD8+CD45RO-CD27-T cells have signalling defects in patients with squamous cell carcinoma of the head and neck. Br J Cancer. 2003;88(2):223–30.
25. Hung K, Hayashi R, Lafond-Walker A, Lowenstein C, Pardoll D, Levitsky H. The central role of CD4(+) T cells in the antitumor immune response. J Exp Med. 1998;188(12):2357–68.
26. Knutson KL, Disis ML. Tumor antigen-specific T helper cells in cancer immunity and immunotherapy. Cancer Immunol Immunother. 2005;54(8):721–8.
27. Wang RF. The role of MHC class II-restricted tumor antigens and CD4+ T cells in antitumor immunity. Trends Immunol. 2001;22(5):269–76.
28. Chikamatsu K, Sakakura K, Yamamoto T, Furuya N, Whiteside TL, Masuyama K. CD4+ T helper responses in squamous cell carcinoma of the head and neck. Oral Oncol. 2008;44(9):870–7.
29. Ito D, Albers A, Zhao YX, Visus C, Appella E, Whiteside TL, et al. The wild-type sequence (wt) p53(25-35) peptide induces HLA-DR7 and HLA-DR11-restricted CD4+ Th cells capable of enhancing the ex vivo expansion and function of anti-wt p53(264-272) peptide CD8+ T cells. J Immunol. 2006;177(10):6795–803.
30. Chikamatsu K, Sakakura K, Takahashi G, Okamoto A, Furuya N, Whiteside TL, et al. CD4+ T cell responses to HLA-DP5-restricted wild-type sequence p53 peptides in patients with head and neck cancer. Cancer Immunol Immunother. 2009;58(9):1441–8.
31. Bhat P, Mattarollo SR, Gosmann C, Frazer IH, Leggatt GR. Regulation of immune responses to HPV infection and during HPV-directed immunotherapy. Immunol Rev. 2011;239(1):85–98.
32. Hoffmann TK, Loftus DJ, Nakano K, Maeurer MJ, Chikamatsu K, Appella E, et al. The ability of variant peptides to reverse the nonresponsiveness of T lymphocytes to the wild-type sequence p53(264-272) epitope. J Immunol. 2002;168(3):1338–47.
33. Williams R, Lee DW, Elzey BD, Anderson ME, Hostager BS, Lee JH. Preclinical models of HPV+ and HPV- HNSCC in mice: an immune clearance of HPV+ HNSCC. Head Neck. 2009;31(7):911–8.
34. Whiteside TL. Tumor-induced death of immune cells: its mechanisms and consequences. Semin Cancer Biol. 2002;12(1):43–50.

35. Kuss I, Hathaway B, Ferris RL, Gooding W, Whiteside TL. Decreased absolute counts of T lymphocyte subsets and their relation to disease in squamous cell carcinoma of the head and neck. Clin Cancer Res. 2004;10(11):3755–62.
36. Kuss I, Hathaway B, Ferris RL, Gooding W, Whiteside TL. Imbalance in absolute counts of T lymphocyte subsets in patients with head and neck cancer and its relation to disease. Adv Otorhinolaryngol. 2005;62:161–72.
37. Tsukishiro T, Donnenberg AD, Whiteside TL. Rapid turnover of the CD8(+)CD28(−) T-cell subset of effector cells in the circulation of patients with head and neck cancer. Cancer Immunol Immunother. 2003;52(10):599–607.
38. Reichert TE, Strauss L, Wagner EM, Gooding W, Whiteside TL. Signaling abnormalities, apoptosis, and reduced proliferation of circulating and tumor-infiltrating lymphocytes in patients with oral carcinoma. Clin Cancer Res. 2002;8(10):3137–45.
39. Strauss L, Bergmann C, Whiteside TL. Human circulating CD4+ CD25highFoxp3+ regulatory T cells kill autologous CD8+ but not CD4+ responder cells by Fas-mediated apoptosis. J Immunol. 2009;182(3):1469–80.
40. Whiteside TL. Signaling defects in T lymphocytes of patients with malignancy. Cancer Immunol Immunother. 1999;48(7):346–52.
41. Reichert TE, Day R, Wagner EM, Whiteside TL. Absent or low expression of the zeta chain in T cells at the tumor site correlates with poor survival in patients with oral carcinoma. Cancer Res. 1998;58(23):5344–7.
42. Okada K, Komuta K, Hashimoto S, Matsuzaki S, Kanematsu T, Koji T. Frequency of apoptosis of tumor-infiltrating lymphocytes induced by fas counterattack in human colorectal carcinoma and its correlation with prognosis. Clin Cancer Res. 2000;6(9):3560–4.
43. Fontenot JD, Rasmussen JP, Gavin MA, Rudensky AY. A function for interleukin 2 in Foxp3-expressing regulatory T cells. Nat Immunol. 2005;6(11):1142–51.
44. Liyanage UK, Moore TT, Joo HG, Tanaka Y, Herrmann V, Doherty G, et al. Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of patients with pancreas or breast adenocarcinoma. J Immunol. 2002;169(5):2756–61.
45. Strauss L, Bergmann C, Gooding W, Johnson JT, Whiteside TL. The frequency and suppressor function of CD4+CD25highFoxp3+ T cells in the circulation of patients with squamous cell carcinoma of the head and neck. Clin Cancer Res. 2007;13(21):6301–11.
46. Strauss L, Bergmann C, Szczepanski M, Gooding W, Johnson JT, Whiteside TL. A unique subset of CD4+CD25highFoxp3+ T cells secreting interleukin-10 and transforming growth factor-beta1 mediates suppression in the tumor microenvironment. Clin Cancer Res. 2007;13(15 Pt 1):4345–54.
47. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med. 2004;10(9):942–9.
48. Boucek J, Mrkvan T, Chovanec M, Kuchar M, Betka J, Boucek V, et al. Regulatory T cells and their prognostic value for patients with squamous cell carcinoma of the head and neck. J Cell Mol Med. 2010;14(1–2):426–33.
49. Badoual C, Hans S, Rodriguez J, Peyrard S, Klein C, Agueznay Nel H, et al. Prognostic value of tumor-infiltrating CD4+ T-cell subpopulations in head and neck cancers. Clin Cancer Res. 2006;12(2):465–72.
50. Loose D, Signore A, Bonanno E, Vermeersch H, Dierckx R, Deron P, et al. Prognostic value of CD25 expression on lymphocytes and tumor cells in squamous-cell carcinoma of the head and neck. Cancer Biother Radiopharm. 2008;23(1):25–33.
51. Alhamarneh O, Amarnath SM, Stafford ND, Greenman J. Regulatory T cells: what role do they play in antitumor immunity in patients with head and neck cancer? Head Neck. 2008;30(2):251–61.
52. Golgher D, Jones E, Powrie F, Elliott T, Gallimore A. Depletion of CD25+ regulatory cells uncovers immune responses to shared murine tumor rejection antigens. Eur J Immunol. 2002;32(11):3267–75.
53. Jones E, Dahm-Vicker M, Simon AK, Green A, Powrie F, Cerundolo V, et al. Depletion of CD25+ regulatory cells results in suppression of melanoma growth and induction of autoreactivity in mice. Cancer Immun. 2002;2:1.
54. Knutson KL, Dang Y, Lu H, Lukas J, Almand B, Gad E, et al. IL-2 immunotoxin therapy modulates tumor-associated regulatory T cells and leads to lasting immune-mediated rejection of breast cancers in neu-transgenic mice. J Immunol. 2006;177(1):84–91.
55. Ge Y, Domschke C, Stoiber N, Schott S, Heil J, Rom J, et al. Metronomic cyclophosphamide treatment in metastasized breast cancer patients: immunological effects and clinical outcome. Cancer Immunol Immunother. 2012;61(3):353–62.
56. Gritzapis AD, Voutsas IF, Baxevanis CN. Ontak reduces the immunosuppressive tumor environment and enhances successful therapeutic vaccination in HER-2/neu-tolerant mice. Cancer Immunol Immunother. 2012;61(3):397–407.
57. Litzinger MT, Fernando R, Curiel TJ, Grosenbach DW, Schlom J, Palena C. IL-2 immunotoxin denileukin diftitox reduces regulatory T cells and enhances vaccine-mediated T-cell immunity. Blood. 2007;110(9):3192–201.
58. Sutmuller RP, van Duivenvoorde LM, van Elsas A, Schumacher TN, Wildenberg ME, Allison JP, et al. Synergism of cytotoxic T lymphocyte-associated antigen 4 blockade and depletion of CD25(+) regulatory T cells in antitumor therapy reveals alternative pathways for suppression of autoreactive cytotoxic T lymphocyte responses. J Exp Med. 2001;194(6):823–32.

59. Ferris RL, Whiteside TL, Ferrone S. Immune escape associated with functional defects in antigen-processing machinery in head and neck cancer. Clin Cancer Res. 2006;12(13):3890–5.
60. Wang S, Chen L. T lymphocyte co-signaling pathways of the B7-CD28 family. Cell Mol Immunol. 2004;1(1):37–42.
61. Marincola FM, Jaffee EM, Hicklin DJ, Ferrone S. Escape of human solid tumors from T-cell recognition: molecular mechanisms and functional significance. Adv Immunol. 2000;74:181–273.
62. Vambutas A, DeVoti J, Pinn W, Steinberg BM, Bonagura VR. Interaction of human papillomavirus type 11 E7 protein with TAP-1 results in the reduction of ATP-dependent peptide transport. Clin Immunol. 2001;101(1):94–9.
63. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3(11):991–8.
64. Albers AE, Kaufmann AM. Therapeutic human papillomavirus vaccination. Public Health Genomics. 2009;12(5–6):331–42.
65. Hoffmann TK, Arsov C, Schirlau K, Bas M, Friebe-Hoffmann U, Klussmann JP, et al. T cells specific for HPV16 E7 epitopes in patients with squamous cell carcinoma of the oropharynx. Int J Cancer. 2006;118(8):1984–91.
66. Ritz U, Momburg F, Pilch H, Huber C, Maeurer MJ, Seliger B. Deficient expression of components of the MHC class I antigen processing machinery in human cervical carcinoma. Int J Oncol. 2001;19(6):1211–20.
67. DeLeo AB, Whiteside TL. Development of multi-epitope vaccines targeting wild-type sequence p53 peptides. Expert Rev Vaccines. 2008;7(7):1031–40.
68. Sakakura K, Chikamatsu K, Furuya N, Appella E, Whiteside TL, Deleo AB. Toward the development of multi-epitope p53 cancer vaccines: an in vitro assessment of CD8(+) T cell responses to HLA class I-restricted wild-type sequence p53 peptides. Clin Immunol. 2007;125(1):43–51.
69. Whiteside TL. Immune modulation of T-cell and NK (natural killer) cell activities by TEXs (tumour-derived exosomes). Biochem Soc Trans. 2013;41(1):245–51.
70. Baskic D, Vujanovic L, Arsenijevic N, Whiteside TL, Myers EN, Vujanovic NL. Suppression of natural killer-cell and dendritic-cell apoptotic tumoricidal activity in patients with head and neck cancer. Head Neck. 2013;35(3):388–98.
71. Whiteside TL. Inhibiting the inhibitors: evaluating agents targeting cancer immunosuppression. Expert Opin Biol Ther. 2010;10(7):1019–35.
72. Seliger B, Maeurer MJ, Ferrone S. Antigen-processing machinery breakdown and tumor growth. Immunol Today. 2000;21(9):455–64.
73. Whiteside TL. Disarming suppressor cells to improve immunotherapy. Cancer Immunol Immunother. 2012;61(2):283–8.
74. Sistigu A, Viaud S, Chaput N, Bracci L, Proietti E, Zitvogel L. Immunomodulatory effects of cyclophosphamide and implementations for vaccine design. Semin Immunopathol. 2011;33(4):369–83.
75. Diaz-Montero CM, Salem ML, Nishimura MI, Garrett-Mayer E, Cole DJ, Montero AJ. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. Cancer Immunol Immunother. 2009;58(1):49–59.
76. Chikamatsu K, Sakakura K, Toyoda M, Takahashi K, Yamamoto T, Masuyama K. Immunosuppressive activity of CD14+ HLA-DR- cells in squamous cell carcinoma of the head and neck. Cancer Sci. 2012;103(6):976–83.
77. Vincent J, Mignot G, Chalmin F, Ladoire S, Bruchard M, Chevriaux A, et al. 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. Cancer Res. 2010;70(8):3052–61.
78. Chen C, Wei Y, Hummel M, Hoffmann TK, Gross M, Kaufmann AM, et al. Evidence for epithelial-mesenchymal transition in cancer stem cells of head and neck squamous cell carcinoma. PLoS One. 2011;6(1):e16466.
79. Qian X, Wagner S, Ma C, Klussmann JP, Hummel M, Kaufmann AM, et al. ALDH1-positive cancer stem-like cells are enriched in nodal metastases of oropharyngeal squamous cell carcinoma independent of HPV status. Oncol Rep. 2013;29(5):1777–84.
80. Chen YC, Chang CJ, Hsu HS, Chen YW, Tai LK, Tseng LM, et al. Inhibition of tumorigenicity and enhancement of radiochemosensitivity in head and neck squamous cell cancer-derived ALDH1-positive cells by knockdown of Bmi-1. Oral Oncol. 2010;46(3):158–65.
81. Chen YC, Chen YW, Hsu HS, Tseng LM, Huang PI, Lu KH, et al. Aldehyde dehydrogenase 1 is a putative marker for cancer stem cells in head and neck squamous cancer. Biochem Biophys Res Commun. 2009;385(3):307–13.
82. Visus C, Ito D, Amoscato A, Maciejewska-Franczak M, Abdelsalem A, Dhir R, et al. Identification of human aldehyde dehydrogenase 1 family member A1 as a novel CD8+ T-cell-defined tumor antigen in squamous cell carcinoma of the head and neck. Cancer Res. 2007;67(21):10538–45.
83. Kiessling A, Schmitz M, Stevanovic S, Weigle B, Holig K, Fussel M, et al. Prostate stem cell antigen: identification of immunogenic peptides and assessment of reactive CD8+ T cells in prostate cancer patients. Int J Cancer. 2002;102(4):390–7.
84. Xu Q, Liu G, Yuan X, Xu M, Wang H, Ji J, et al. Antigen-specific T-cell response from dendritic cell vaccination using cancer stem-like cell-associated antigens. Stem Cells. 2009;27(8):1734–40.
85. Garcia-Hernandez Mde L, Gray A, Hubby B, Klinger OJ, Kast WM. Prostate stem cell antigen vaccination induces a long-term protective immune

response against prostate cancer in the absence of autoimmunity. Cancer Res. 2008;68(3):861–9.
86. Pellegatta S, Poliani PL, Corno D, Menghi F, Ghielmetti F, Suarez-Merino B, et al. Neurospheres enriched in cancer stem-like cells are highly effective in eliciting a dendritic cell-mediated immune response against malignant gliomas. Cancer Res. 2006;66(21):10247–52.
87. Dhodapkar MV. Immunity to stemness genes in human cancer. Curr Opin Immunol. 2010;22(2):245–50.
88. Liao T, Kaufmann AM, Qian X, Sangvatanakul V, Chen C, Kube T, et al. Susceptibility to cytotoxic T cell lysis of cancer stem cells derived from cervical and head and neck tumor cell lines. J Cancer Res Clin Oncol. 2013;139(1):159–70.
89. Ning N, Pan Q, Zheng F, Teitz-Tennenbaum S, Egenti M, Yet J, et al. Cancer stem cell vaccination confers significant antitumor immunity. Cancer Res. 2012;72(7):1853–64.
90. Hildesheim A, Herrero R, Wacholder S, Rodriguez AC, Solomon D, Bratti MC, et al. Effect of human papillomavirus 16/18 L1 viruslike particle vaccine among young women with preexisting infection: a randomized trial. JAMA. 2007;298(7):743–53.
91. Nikitina EY, Clark JI, Van Beynen J, Chada S, Virmani AK, Carbone DP, et al. Dendritic cells transduced with full-length wild-type p53 generate antitumor cytotoxic T lymphocytes from peripheral blood of cancer patients. Clin Cancer Res. 2001;7(1):127–35.
92. Azuma K, Shichijo S, Maeda Y, Nakatsura T, Nonaka Y, Fujii T, et al. Mutated p53 gene encodes a nonmutated epitope recognized by HLA-B*4601-restricted and tumor cell-reactive CTLs at tumor site. Cancer Res. 2003;63(4):854–8.
93. Molling JW, Moreno M, de Groot J, van der Vliet HJ, von Blomberg BM, van den Eertwegh AJ, et al. Chronically stimulated mouse invariant NKT cell lines have a preserved capacity to enhance protection against experimental tumor metastases. Immunol Lett. 2008;118(1):36–43.
94. Shibuya TY, Wei WZ, Zormeier M, Ensley J, Sakr W, Mathog RH, et al. Anti-CD3/anti-CD28 bead stimulation overcomes CD3 unresponsiveness in patients with head and neck squamous cell carcinoma. Arch Otolaryngol Head Neck Surg. 2000;126(4):473–9.
95. Visus C, Wang Y, Lozano-Leon A, Ferris RL, Silver S, Szczepanski MJ, et al. Targeting ALDH(bright) human carcinoma-initiating cells with ALDH1A1-specific CD8(+) T cells. Clin Cancer Res. 2011;17(19):6174–84.
96. Victora GD, Socorro-Silva A, Volsi EC, Abdallah K, Lima FD, Smith RB, et al. Immune response to vaccination with DNA-Hsp65 in a phase I clinical trial with head and neck cancer patients. Cancer Gene Ther. 2009;16(7):598–608.
97. Smith C, Tsang J, Beagley L, Chua D, Lee V, Li V, et al. Effective treatment of metastatic forms of Epstein-Barr virus-associated nasopharyngeal carcinoma with a novel adenovirus-based adoptive immunotherapy. Cancer Res. 2012;72(5):1116–25.
98. Seixas-Silva Jr JA, Richards T, Khuri FR, Wieand HS, Kim E, Murphy B, et al. Phase 2 bioadjuvant study of interferon alfa-2a, isotretinoin, and vitamin E in locally advanced squamous cell carcinoma of the head and neck: long-term follow-up. Arch Otolaryngol Head Neck Surg. 2005;131(4):304–7.
99. van Herpen CM, van der Voort R, van der Laak JA, Klasen IS, de Graaf AO, van Kempen LC, et al. Intratumoral rhIL-12 administration in head and neck squamous cell carcinoma patients induces B cell activation. Int J Cancer. 2008;123(10):2354–61.
100. De Stefani A, Forni G, Ragona R, Cavallo G, Bussi M, Usai A, et al. Improved survival with perilymphatic interleukin 2 in patients with resectable squamous cell carcinoma of the oral cavity and oropharynx. Cancer. 2002;95(1):90–7.
101. To WC, Wood BG, Krauss JC, Strome M, Esclamado RM, Lavertu P, et al. Systemic adoptive T-cell immunotherapy in recurrent and metastatic carcinoma of the head and neck: a phase 1 study. Arch Otolaryngol Head Neck Surg. 2000;126(10):1225–31.
102. Freeman SM, Franco JL, Kenady DE, Baltzer L, Roth Z, Brandwein HJ, et al. A phase 1 safety study of an IRX-2 regimen in patients with squamous cell carcinoma of the head and neck. Am J Clin Oncol. 2011;34(2):173–8.
103. Karcher J, Dyckhoff G, Beckhove P, Reisser C, Brysch M, Ziouta Y, et al. Antitumor vaccination in patients with head and neck squamous cell carcinomas with autologous virus-modified tumor cells. Cancer Res. 2004;64(21):8057–61.
104. Kurosaki M, Horiguchi S, Yamasaki K, Uchida Y, Motohashi S, Nakayama T, et al. Migration and immunological reaction after the administration of alphaGalCer-pulsed antigen-presenting cells into the submucosa of patients with head and neck cancer. Cancer Immunol Immunother. 2011;60(2):207–15.

16 Immunotherapy and Immunosurveillance of Oral Cancers: Perspectives of Plasma Medicine and Mistletoe

Hans-Robert Metelmann, Thomas von Woedtke, Kai Masur, Peter Hyckel, and Fred Podmelle

Contents

H.-R. Metelmann, MD, DDS, PhD (✉)
F. Podmelle, MD, DDS
Department of Oral and Maxillo-Facial Surgery/Plastic Surgery, Greifswald University, Ferdinand-Sauerbruch-Strasse DZ, Greifswald 17475, Germany
e-mail: metelman@uni-greifswald.de

T. von Woedtke, PhD
Department of Plasma Medicine, Leibniz Institute for Plasma Science and Technology, INP Greifswald e.V., Felix-Hausdorff-Str. 2, Greifswald 17489, Germany

K. Masur, PhD
ZIKplasmatis, Leibniz Institute for Plasma Science and Technology, INP Greifswald e.V., Felix-Hausdorff-Str. 2, Greifswald 17489, Germany

P. Hyckel, PhD em, MD, DMD
Department of Oral and Maxillo-Facial Surgery/Plastic Surgery, Jena University, Erlanger Allee 101, Jena 07749, Germany

16.1 Introduction

The development of oral cancer and the process of malignant cell transformation have to be considered in context with immunosuppressive mechanisms and relevant effector cells suppressed in their function [1]. Therefore, one of the objectives of immunological interventions is activation of effective mechanisms such as maturation of dendritic cells (DCs) [2, 3]. There is some evidence that mistletoe lectins stimulate maturation and activation of DCs [4, 5], possibly inducing cytotoxic effects [6]. Lymph node metastasis is seen in accordance with the inability of DCs due to the undifferentiated tumor to maturate, hence providing a peripheric kind of tolerance. PHA reactivity is serving as an important factor for DC maturation [7, 8] and monocytes which are considered the early stages of DCs and stimulated to maturize by transducing factors. In general, mistletoe therapy in oncology has to be considered in a very careful approach [9]. The cytostatic effect of mistletoe lectins in individually cultivated oral cavity carcinoma stem cells is well documented since long time ago [10]; however, it needs some differentiation [11]. A systemic review on controlled clinical trials using mistletoe in cancer has been published by Kienle and co-workers [12]. There are some data showing that extracts from Viscum and Crataegus are cytotoxic against larynx cancer cells [13], demonstrating the influence of complimentary *Viscum album* administration on

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_16, © Springer-Verlag Berlin Heidelberg 2015

microcirculation and immune system of ear, nose, and throat carcinoma patients treated with radiation and chemotherapy [14]. Steuer-Vogt and co-workers [15] have published a randomized controlled clinical trial, concerning the effect of an adjuvant mistletoe treatment program in resected head and neck cancer patients. However, there is only a single report in the literature that is presenting a trapping effect of mistletoe injections on an advanced oral cavity carcinoma of a patient that did not receive any other tumor treatment at all [16].

16.2 Trapping an Advanced Squamous Cell Carcinoma of the Tongue by Continuously Repeated Peritumoral Injection of Mistletoe Preparation

A 66-year-old Caucasian man presented to his doctor in May 2007 with the complaints of itching and burning at the lower tongue while drinking juices, attacks of night sweat, and signs of listlessness. Bad smell of the breath was present (foetor ex ore).

The local examination and MRI scan showed an exophytic tumor at the base of the tongue with palpable and massively enlarged lymph nodes on both sides of the neck (Fig. 16.1). Histological examination of the tumor area revealed a poorly differentiated squamous cell carcinoma with a low tendency of keratosis. Summarizing all the findings demonstrated a carcinoma of the oropharynx and hypopharynx at the dorsal border of the right side of the tongue in stage $cT_{4a–b}$, cN_{2b}, cM_0.

The patient refused any kind of surgery or radiotherapy, fearing the side effects and complications. Following a series of consultations with several oncologists and head and neck surgeons, the patient finally agreed to take a treatment protocol combining peritumoral injections of a mistletoe preparation (abnobaVISCUM Abietis 0.2 mg) every 2 weeks, applied by a specialist in maxillofacial surgery at the university hospital in combination with injection of additive components (Silicea Comp-Wala) applied by a medical practitioner and specialist in advanced cancer treatment. This fourth-line treatment (being surgery, radiotherapy, and cytostatic chemotherapy, the three main lines of cancer therapy in the head and neck area) was started in November 2007.

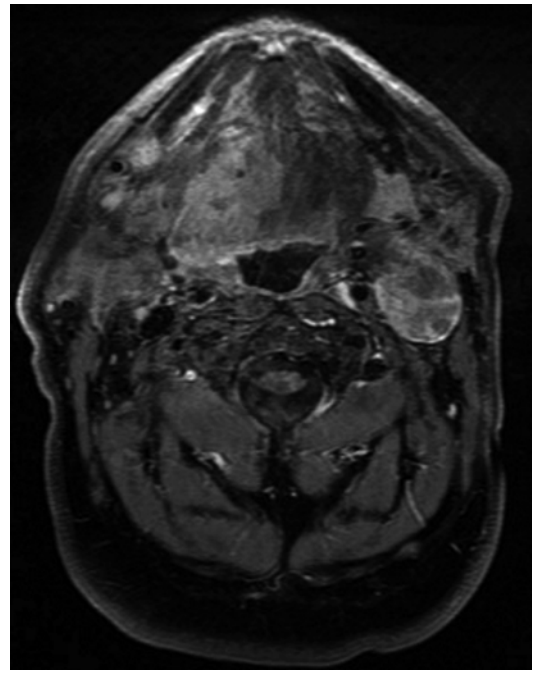


Fig. 16.1 MRI scan, horizontal layer at the level of the basis of the tongue, presenting the tumor area at the right site and a group of lymph nodes at the left side of the hypopharynx (May 2008)

As an absolutely surprising result and against the expectations of the treating medical doctors but fully in line with the conviction of the patient, the tumor has stopped its growth since November 2007 and presented reduction of size. Comparing the MRI of May 2008 and January 2009 (Fig. 16.2), lymph node metastasis at the neck site does not show any progressive disease, and even the primary tumor at the tongue is becoming smaller. The histological examination of the primary tumor (May 2009) is presenting squamous carcinoma cells imbedded in major epithelial cells together with some spots of keratinizations and surrounded by a local inflammatory reaction (Fig. 16.3). The general condition of the patient at this time is excellent. There is no weight loss. The patient is continuously active in his normal

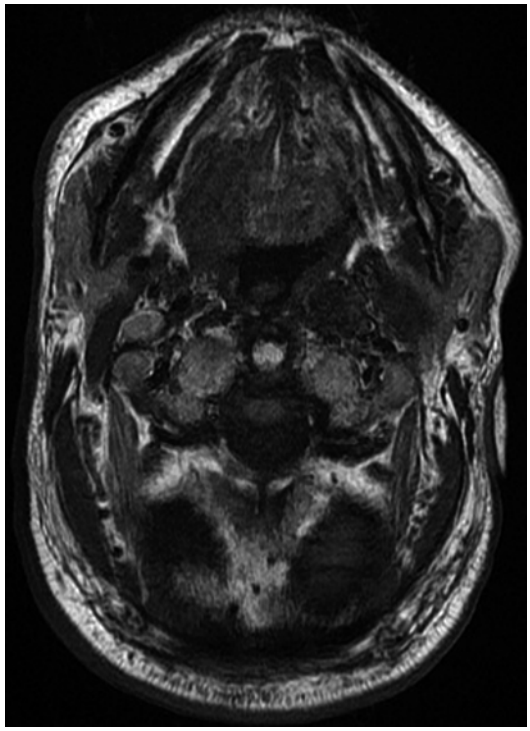


Fig. 16.2 MRI scan, horizontal layer at the level of the basis of the tongue, presenting the tumor area at the right side and a group of lymph nodes at the left side of the hypopharynx with signs of fibrosis of tumor and lymph node area (January 2009)

social life. The situation might be described from a clinical point of view as trapping of a tumor disease by nothing else than peritumoral injections of mistletoe preparations.

With the tumor of the tongue still in partial remission, the patient is presenting in June 2009 a second malignant tumor, now located within the right kidney. Retrospective investigation of previous MRT imaging is revealing that in May 2007, there was already a tiny irregularity visible at the site of the now second tumor that remains without histological specification, since the patient is refusing taking a biopsy. Further treatment follows continuously the protocol of peritumor injections of mistletoe preparations intraorally. By applying this method, the tumor of the tongue is still under control. The second tumor in the area of the kidney not treated by peritumor injections is rapidly growing, limiting the survival of the patient.

The impact observed in this case seems to be caused by a concentration of mistletoe lectins in the tumor periphery. Braun and co-workers [17] have published that mistletoe lectins in low doses do not provoke and increase secretion of TNF alpha and IL6. However, cytotoxic concentrations however do exactly this on a very significant level, leading to the assumption that placing

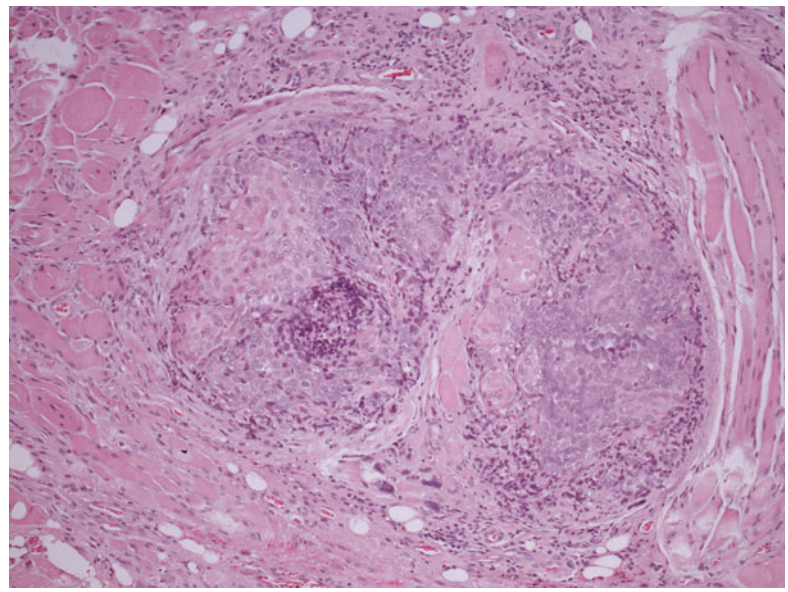


Fig. 16.3 Histological result of mistletoe injection (HE staining)

the mistletoe lectins peritumorally has provoked exactly this cytotoxic effect. A peritumoral concentration of mistletoe lectins is not achievable by application p.o., because the substances are not stable and absorb badly within the gastrointestinal system [18].

Since the development of immature DCs is bound to P13K/Akt expression, it is interesting to raise the question whether the instillation of mistletoe preparations makes sense in highly differentiated small tumors in terms of prophylactic support, since in cases of a non-disturbed immune system, additional maturation of the DCs might lead to a limited surgical treatment, due to the treatment recommendations in context with PHA reactivity published by Hyckel and co-workers [19]. This case study impressively supports the hypothesis of immunosurveillance [20, 21]. Mistletoe leads to the upregulation of IL12 and TNF alpha in macrophages [22]. IL12 and TNF alpha expressions are characteristic for the classically activated or M1 tumor-related macrophage (TAM) related to tumor rejection [23–25]. In contrast, tumor progression is associated with the occurrence of M2 macrophages. The immunosuppressive reactivity of M2 macrophages corresponds to a reduced response to mitogens, e.g., PHA [19]. The hypothesis of the therapeutic effect of mistletoe: application of a switch from tolerogen M2 to proinflammatory M1 macrophages is induced [26] by peritumoral mistletoe. As a result of M1 activation, there is a maturation of DCs leading to tumor antigen presentation and subsequently to a suppression of tumor growth and metastasis (Fig. 16.4). In conclusion, the impact of mistletoe extracts injected in the periphery of the tumor might be explained as the activation of macrophage polarization (M1). Maturation of DCs is going along with an induced cytotoxicity and the performance of antigen presenting cells.

As part of the interactions between epithelial cells and mesenchymal cells in the periphery of the tumor, the ongoing growth of tumor is limited. Lymph node metastasis maturation of DCs is cutting of further spread of carcinoma cells that is otherwise only possible under the influence of immature DCs, known as tolerance in the periphery [27]. Since lymph node metastases

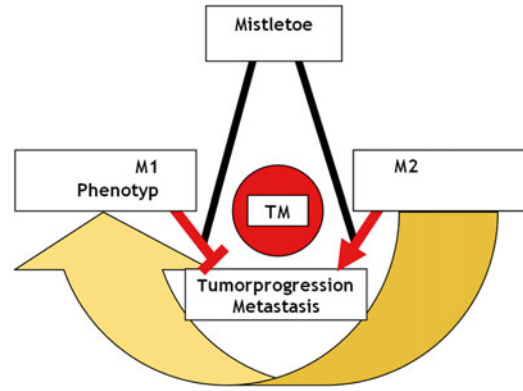


Fig. 16.4 A hypothetical pattern of change of immunophenotype forced by the treatment

limit themselves by central necrosis, additionally leading to the reduction of PHA reactivity (lectin activity) [19], further development of lymph node metastasis has a poor basis [28].

Concerning immunology and immunotherapy of oral cavity carcinoma, there is so far no clinical basis for standard interventions with the aim to inhibit tumor growth. Hoffmann and Schuler have reviewed current studies in patients with head and neck squamous cell carcinoma including vaccination, virus injection, and stimulation of epidermal growth factor receptors [1]. The authors conclude that some vaccination strategies are regarded as most promising or are resulting in a positive clinical response of vaccinated patients.

However, there is a new promising technology on the horizon in order to manipulate cell activities. Physical cold atmospheric-pressure plasmas (partially ionized gases close to body temperature) are able to influence cells on a molecular level. Bekeschus and co-workers used an argon-based plasma to investigate the plasma effects on immune cells isolated from human blood and found a treatment time-dependent increase in apoptotic cells [29]. The plasma device named kINPen (neoplas GmbH, Greifswald, Germany) is a bullet-type atmospheric-pressure argon plasma jet in MHz operating regime [30]. By applying this argon plasma, Bekeschus and co-workers showed that PHA-pretreated human PBMCs display a significant higher survival rate than non-stimulated immune cells – while surviving cells still displayed

unchanged ability to proliferate [29]. However, there was also a cell-type-specific difference between the investigated PBMC subtypes. This is in accordance with another study using a different type of plasma source developed at the INP Greifswald, Germany. Haertel and co-workers [31] also found different sensitivities of plasma-treated mononuclear cells isolated from rat spleen (without PHA stimulation). For this study, surface dielectric barrier discharge plasma using atmospheric air as working gas has been used. It has to be clarified in future studies whether these changes can be used to generate regulatory T cells to sensitize immune cells or to modify homing of lymphocytes [31]. On the other hand, Schmidt and co-workers showed that human skin cell activity was also altered on a transcriptomic level in a treatment time- and incubation time-dependent manner, when treated with an argon plasma generated by the plasma source kINPen [32]. Another study also using the kINPen device showed similar effects on human cancer cells. Partecke and co-workers have investigated the effect of argon plasma on the human metastatic pancreatic cancer cell line Colo-357 in an *in vivo* tumor chorioallantoic membrane (TUM-CAM) assay. TUNEL staining showed plasma-induced apoptosis up to a depth of tissue penetration of 48.8 ± 12.3 µm [33]. Therefore, all studies proofed a possible application of different physical cold atmospheric-pressure plasmas – either working with air or noble gases – in order to manipulate the fate of different types of cells.

16.3 Concluding Remarks

Continuously repeated peritumoral injection of mistletoe preparation is a novel method in the immunotherapy of oral cancers; nonetheless, further studies are mandated to explore its various applications.

References

1. Hoffmann TK, Schuler PJ. Antigen-specific immunotherapy in head and neck cancer. Adv Cell Mol Otolaryngol. 2013;1:21758.
2. Meissner M, Reichert TE, Kunkel M, Gooding W, Whiteside TL, Ferrone S, et al. Defects in the human leukocyte antigen class I antigen processing machinery in head and neck squamous cell carcinoma: association with clinical outcome. Clin Cancer Res. 2005;11(7):2552–60.
3. Hoffmann TK, Muller-Berghaus J, Ferris RL, Johnson JT, Storkus WJ, Whiteside TL. Alterations in the frequency of dendritic cell subsets in the peripheral circulation of patients with squamous cell carcinomas of the head and neck. Clin Cancer Res. 2002;8(6):1787–93.
4. Elluru SR, van Huyen JP, Delignat S, Kazatchikine MD, Friboulet A, Kaveri SV, et al. Induction of maturation and activation of human dendritic cells: a mechanism underlying the beneficial effect of Viscum album as complimentary therapy in cancer. BMC Cancer. 2008;8:161.
5. Stein GM, Buessing A, Schietzel M. Stimulation of the maturation of dendritic cells in vitro by a fermented mistletoe extract. Anticancer Res. 2002;22(6C):4215–9.
6. Eggenschwiler J, von Balthazar L, Stritt B, Pruntsch D, Ramos M, Urech K, Rist L, Simoes-Wuest AP, Viviani A. Mistletoe lectin is not the only cytotoxic component in fermented preparations of Viscum album from white fir (Abies pectinata). BMC Complement Altern Med. 2007;10(7):14.
7. Schuett C, Pfueller U, Siegl E, Walzel H, Franz H. Selective killing of human monocytes by an immunotoxin containing partially denatured mistletoe lectin I. Int J Immunopharmacol. 1989;11(8):977–80.
8. Schultze JL, Stettin A, Berg PA. Demonstration of specifically sensitized lymphocytes in patients treated with an aqueous mistletoe extract (Viscum album L.). Klin Wochenschr. 1991;69(9):397–403.
9. Horneber MA, Bueschel G, Huber R, Linde K, Rostock M. Mistletoe therapy in oncology. Cochrane Database Syst Rev. 2008;16(2):CD003297.
10. Metelmann HR, Wartenberg E, Kindermann H, Platz H, Scheffler A. Direct antineoplastic activity of different mistletoe preparations against oral cancer cells in vitro. Proc AACR. 1992;33:224.
11. Scheer R, Metelmann HR, Errenst M, Scheffler A. Influence of the host tree on the content and the pattern of active principles of mistletoe preparations and consequences for therapy. Eur J Pharm Sci. 1994;2:115.
12. Kienle GS, Berrino F, Buessing A, Portalupi E, Rosenzweig S, Kiene H. Mistletoe in cancer – a systematic review on controlled clinical trials. Eur J Med Res. 2003;8(3):109–19.
13. Saenz MT, Ahumada MC, Garcia MD. Extracts from Viscum and Crataegus are cytotoxic against larynx cancer cells. Z Naturforsch C. 1997;52:42–4.
14. Klopp R, Schmidt W, Werner E, Werner M, Niemer W, Beuth J. Influence of complementary Viscum album (Iscador®) administration on microcirculation and immune system of ear, nose and throat carcinoma patients treated with radiation and chemotherapy. Anticancer Res. 2005;25:601–10.

15. Steuer-Vogt MK, Bonkowsky V, Ambrosch P, Scholz M, Neiss A, Strutz J, Hennig M, Lenarz T, Arnold W. The effect of an adjuvant mistletoe treatment programme in resected head and neck cancer patients: a randomised controlled clinical trial. Eur J Cancer. 2001;37(1):23–31.
16. Metelmann HR, Hyckel P, Podmelle F. Oral cancer treatment and immune targets – a role for dendritic cells? J Cranio Maxillofac Surg. 2012;40(2):103–4.
17. Braun JM, Blackwell CC, Weir DM, Beuth J. Cytokine release of whole blood from adult female donors challenged with mistletoe lectin-1 standardised mistletoe extract and E. coli endotoxin or phytohaemagglutinin (PHA). Anticancer Res. 2003;23(2B):1349–52.
18. Lyu SY, Park WB. Transport of mistletoe lectin by M cells in human intestinal follicle-associated epithelium (FAE) in vitro. Arch Pharm Res. 2008;31(12): 1613–21.
19. Hyckel P, Metzner G, Mueller P, Quade R. Prognostic relevance of histologic and immunologic parameters as a contribution to the data on individualized treatment plans for carcinoma of the mouth. Dtsch Z Mund Kiefer Gesichtschir. 1985;9(6):475–80.
20. Swann JB, Smyth MJJ. Immune surveillance of tumors. Clin Invest. 2007;117(5):1137–46.
21. Reiman JM, Kmieciak M, Manjili MH, Knutson K. Tumor immunoediting and immunosculpting pathways to cancer progression. Semin Cancer Biol. 2007;17(4):275–87.
22. Heinzerling L, von Baehr V, Liebenthal C, von Baehr R, Volk HD. Immunologic effector mechanisms of a standardized mistletoe extract on the function of human monocytes and lymphocytes in vitro, ex vivo, and in vivo. J Clin Immunol. 2006;26(4):347–59.
23. Ostrand-Rosenberg S. Immune surveillance: a balance between protumor and antitumor immunity. Curr Opin Genet Dev. 2008;18(1):11–8.
24. Mantovani A, Sica A. Macrophages, innate immunity and cancer: balance, tolerance, and diversity. Curr Opin Immunol. 2010;22(2):231–7.
25. DeNardo DG, Andreu P, Coussens LM. Interactions between lymphocytes and myeloid cells regulate pro- versus anti-tumor immunity. Cancer Metastasis Rev. 2010;29(2):309–16.
26. Hajtó T, Fodor K, Perjési P, Németh P. Difficulties and perspectives of immunomodulatory therapy with mistletoe lectins and standardized mistletoe extracts in evidence-based medicine. Evid Based Complement Alternat Med. 2011;298972.
27. Steinmann RM, Inaba K. Myeloid dendritic cells. J Leukoc Biol. 1999;66(2):205–8.
28. Melief CJ. Cancer immunotherapy by dendritic cells. Immunity. 2008;29(3):372–83.
29. Bekeschus S, Masur K, Kolata J, Wende K, Schmidt A, Bundscherer L, Barton A, Kramer A, Broeker B, Weltmann KD. Human mononuclear cell survival and proliferation in modulated by cold atmospheric plasma jet. Plasma Proc Polym. 2013;10:706–13.
30. Weltmann KD, Kindel E, Brandenburg R, Meyer C, Bussiahn R, Wilke C, von Woedtke T. Atmospheric pressure plasma jet for medical therapy: plasma parameters and risk estimation. Contrib Plasma Phys. 2009;49(9):631–40.
31. Haertel B, Volkmann F, von Woedtke T, Lindequist U. Differential sensitivity of lymphocyte subpopulations to non-thermal atmospheric-pressure plasma. Immunobiology. 2012;217(6):628–33.
32. Schmidt A, Wende K, Bekeschus S, Bundscherer L, Barton A, Ottmueller K, Weltmann KD, Masur K. Non-thermal plasma treatment is associated with changes in transcriptome and stress-induced gene profile of human epithelial skin cells. Free Radic Res. 2013;47:577–92.
33. Partecke LI, Evert K, Haugk J, Doering F, Normann L, Diedrich S, Weiss FU, Evert M, Huebner NO, Heidecke CD, Kramer A, Bussiahn R, Weltmann KD, Pati O, Bender C, von Bernstorff W. Tissue Tolerable Plasma (TTP) induces apoptosis in the human pancreatic cancer cells in vitro and in vivo. BMC Cancer. 2012;12:473.

Immunopathology of Bone and Connective Tissue Cancers and Immunotherapy of Sarcomas

17

Julie A. Alosi, Scott W. Baldwin, Jacqueline Oxenberg, and Joseph J. Skitzki

Contents

J.A. Alosi, MD • S.W. Baldwin, MD
Department of Surgical Oncology, Roswell Park Cancer Institute, Buffalo, NY 14263, USA
e-mail: scott.w.baldwin@outlook.com

J. Oxenberg, DO
Department of Surgical Oncology, Roswell Park Cancer Institute, Buffalo, NY 14263, USA

Department of Surgical Oncology, Geisinger Wyoming Valley Hospital, Wilkes-Barre, PA 18702, USA
e-mail: jackieofrg@yahoo.com

J.J. Skitzki, MD (✉)
Department of Surgical Oncology, Roswell Park Cancer Institute, Buffalo, NY 14263, USA

Department of Immunology, Roswell Park Cancer Institute, Buffalo, NY 14263, USA
e-mail: joseph.skitzki@roswellpark.org

17.1 Introduction

Despite being grouped together and labeled "sarcomas," bone and connective tissue cancers represent a diverse, heterogeneous collection of tumor types. Their clinical behavior in terms of aggressiveness, risk for metastases, and response to therapies are equally diverse. Common to all sarcomas, however, is their potential ability to be recognized by the immune system. The principle of immune-mediated antitumor responses has long been recognized, and the current field has evolved to leverage the immune system in both preclinical and clinical settings. Novel approaches to sarcoma treatment that utilize the immune system are actively being explored.

17.2 Coley's Toxin and Toll-Like Receptors

William B. Coley is referred to as the "Father of Immunotherapy" for his pioneering and innovative treatment of sarcoma. Observing that patients with metastatic bone sarcoma had few treatment options, he scoured the literature and found a

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_17,

connection between bacterial infection and tumor regression. In particular, he noted that high fever due to erysipelas infection was followed by an unexpected regression of tumor burden in several patients with sarcoma. Convinced that severe infection could induce regression of cancer, in 1891 he injected patients with streptococcal organisms in order to induce a tumor response. Although two of his first three patients died of sequelae from infection, there was noted shrinkage of their malignant tumors [1]. By 1893 Coley had treated ten patients with a heat-killed streptococcal organism combined with a second organism: *Serratia marcescens*. This combination became known as Coley's toxin [2]. Over the next 20 years, Coley's toxin was used to treat patients with inoperable sarcomas. Treatment with Coley's toxin grew out of favor due to unpredictable side effects and toxicities. His work was criticized as inconsistent with the use of different bacterial preparations and poor documentation and patient follow-up. Notably, over a thousand patients treated with Coley's toxin demonstrated near-complete regression in almost half of the cases [3]. Despite these results, the use of Coley's toxin was abandoned, and the use of radiation and chemotherapy became more prominent for the treatment of sarcomas as they had predictable and controllable toxicities.

Concerning the mechanism of action, Coley's toxin contained unmethylated deoxycytidyl-deoxyguanosine dinucleotides (CpGs), lipoteichoic acid, and bacterial lipopolysaccharide (LPS), all of which have been shown to stimulate Toll-like receptors (TLRs) [4, 5]. TLRs are a type of binding pattern recognition receptor. As membrane proteins expressed on immune cells such as macrophages and dendritic cells (DCs), they recognize structurally conserved molecules typically derived from microbes. These molecules, collectively referred to as pathogen-associated molecular patterns (PAMPs), include bacterial lipopolysaccharide (LPS), flagellin, and unmethylated CpG DNA from bacteria and some viruses [6]. Additionally, TLRs recognize a variety of molecules released from stressed and/or dying cells referred to as damage-associated molecular patterns (DAMPs). DAMPs include heat shock proteins (HSPs), fibronectin, and extracellular membrane proteins [6].

TLR signaling activates the innate as well as the adaptive immune system. Activation results in an inflammatory cascade with cytokine secretion and immune responses including dendritic cell (DC) maturation, antigen presentation, and $CD8^+$ T-cell cytotoxicity [7]. These responses not only impede microbial infection but are also capable of generating an antitumor immune response [8]. Tumor cells evolve to evade immune detection and immune-mediated clearance. TLR activation of downstream immunologic processes may overcome tumor-induced immune tolerance and was the likely mechanism of Coley's toxin success.

In response to TLR activation, macrophages secrete chemokines and proinflammatory cytokines including TNF-α and interleukin-1β, triggering local and systemic inflammatory responses [9]. Dendritic cells secrete IL-12 and mature into antigen-presenting cells as part of the adaptive immune response [10]. Dendritic cells present antigens to lymphocytes as part of the adaptive immune response but also are capable of activating cancer-specific natural killer (NK) and NKT cells [11].

TLR expression, however, is not limited to immune cell subsets as tumor cells express TLRs and activation may lead to tumor growth and immune evasion. Therefore, TLRs have the dual potential to mediate both anti- and pro-tumorigenic pathways [12]. Activation of TLRs on tumor cells can lead to upregulation of NF-κB signaling and antiapoptotic proteins. DAMP activation of TLRs on tumor cells leads to signaling cascades associated with the release of pro-angiogenic mediators, tumor-promoting growth factors, cytokines, and chemokines which recruit cellular inhibitors of the immune response [13].

TLRs are expressed on a variety of cancer types and the behavior of TLR subtypes vary in different settings. Activation of one TLR on a particular tumor type may induce cell death, whereas stimulation of the same TLR on a different tumor type might promote survival and/or induce proliferation. Identifying the optimal TLR agonist for cancer immunotherapy is not straight-

Table 17.1 Summary of the known TLR agonists and existing or potential therapeutic agents

TLR	Ligand/agonist	Potential therapeutic agents
TLR1	Triacylated lipoproteins, lipoteichoic acid, peptidoglycans	PAM3CSK4 [93]
		BCG
TLR2	Endogenous DAMPs (HSP, HMGB1, uric acid, fibronectin, ECM proteins) [5]	PAM3CSK4 [94–96]
		BCG [97]
		HSP60 [98]
TLR3	Viral dsRNA, synthetic analogs of dsRNA	Poly-I:C [99]
		Ampligen (AMP-516) [100]
		IPH3102 [101]
		Poly-A:U [102]
TLR4	LPS, various DAMPs [5]	Eritoran [103]
		BCG [97]
TLR5	Bacterial flagellin	Flagellin, CBL502 [104]
TLR6	Diacylated lipopeptides and endogenous DAMPs (HSP, HMGB1, uric acid, fibronectin, ECM proteins)	MALP-2 [105, 106]
		CBLB613 [107]
TLR7	Viral ssRNA	Imiquimod [108]
		Imidazoquinoline 852A [109]
		IPH 3201 [110]
		SC-1, SC-2 [111]
TLR8	Viral ssRNA	Imiquimod [108]
		IPH 3201 [110]
		SC-2 [111]
TLR9	Unmethylated CpG DNA from bacteria and viruses [5, 93]	BCG
		CpG-ODNs [112]
		IMO-2055 [113]
		ISS1018 [114]
		PF-3512676 [115]

BCG bacillus Calmette-Guerin, *CPG ODN* CpG oligodeoxynucleotides, *DAMPs* damage-associated molecular patterns, *ECM* extracellular matrix, *HMGB1* high-mobility group box 1, *HSP* heat shock protein, *LPS* lipopolysaccharide, *MALP-2* macrophage-activating lipopeptide, *Poly-A:U* polyadenylic-polyuridylic acid, *Poly-I:C* polyinosinic-polycytidylic acid

forward and requires consideration of the downstream effects of TLR engagement on both specific tumor cells and immune cells within the tumor microenvironment.

TLR3 and TLR5 show the most promising results for eliciting direct antitumor effects, whereas TLR4, TLR7, TLR8, and TLR9 display primarily pro-tumor properties when directly stimulated on tumor cells [14]. Currently TLR agonists approved by the FDA for use in cancer treatment include bacillus Calmette-Guerin (BCG), monophosphoryl lipid A (MPL), and imiquimod. BCG is an attenuated strain of *Mycobacterium bovis*, used to successfully treat bladder cancer. BCG stimulates TLR2, TLR3, TLR4, and TLR9, leading to DC maturation in a TLR-dependent manner [15]. MPL is a component of lipopolysaccharide isolated from *Salmonella minnesota*, which acts as a TLR4 agonist with lower toxicity compared to LPS. MPL functions as a vaccine adjuvant and has been evaluated in several human clinical trials [16]. Imiquimod is a TLR7 agonist approved for the topical treatment of virally associated skin pathologies as well as superficial skin cancers. As a TLR7 agonist, imiquimod may be effective in a wide spectrum of tumor types and has demonstrated efficacy in Kaposi's sarcoma [17]. Table 17.1 summarizes the known TLR agonists as well as existing or potential therapeutic agents. While the broad activity of TLR manipulation in sarcoma has therapeutic potential, it is the identification of unique sarcoma antigens which serves as the basis for contemporary and focused immunotherapy approaches as discussed below.

17.3 Sarcoma Antigens as Targets for Immunotherapy

Like other cancers, sarcoma cells possess unique tumor antigens that may serve as targets for immunotherapy strategies. Many of the current immunogenic tumor markers have been discovered via serological analysis of recombinant cDNA expression libraries (SEREX). This assay is used to find antigens that have elicited an immune response in their respective hosts [18]. The tumor antigen family of "cancer-testis antigens" (CTAs) was originally discovered using this method and has several notable targets for immunotherapy. Well-known CTAs include NY-ESO-1 and the SSX and MAGE family of

proteins [19]. These proteins are grouped together into the subclass of *CT-X* genes as they are all located on the X chromosome and are preferentially expressed in the testis as opposed to other normal tissues. Importantly, these proteins have been found to be expressed in a variety of malignancies and have been associated with immune reactivity. These characteristics make this family of tumor antigens ideal targets of immune-based therapy, given its limited expression on normal tissues and degree of immune recognition.

17.3.1 NY-ESO-1

One of the best characterized CTAs is NY-ESO-1, a 180 amino acid intracellular protein that also belongs to the CT-X familial subgroup which is normally only expressed on testis tissues [20]. As a CTA it is expressed sporadically on soft tissue sarcomas as well as other malignancies but has a high incidence of expression on synovial sarcomas as well as myxoid/round cell sarcomas [21, 22]. NY-ESO-1 is most frequently expressed heterogeneously within tumors but is known to be highly immunogenic. As evidence of its immunogenicity, NY-ESO-1-specific antibodies are frequently found in patients with corresponding antigen-expressing tumors. In certain cancers it has been observed that as the stage of the disease increases, so does both the frequency and intensity of the immune response to NY-ESO-1. Despite the detection of antibodies, there has been no evidence that the presence of these antibodies confers a survival benefit. However, when antibodies to NY-ESO-1 are detected, a higher likelihood of response to ipilimumab (a CTLA-4-blocking antibody discussed in proceeding sections) has been observed [23]. Whether or not this is due to an enhanced immune response due to ipilimumab or if antibodies to NY-ESO-1 serve as a marker for a response has yet to be determined.

17.3.2 SSX

The pathognomonic characteristic of synovial sarcoma is a specific translocation between *SYT* (synovial sarcoma translocation) gene, currently also known as *SS18* (synovial sarcoma gene of chromosome 18), and *SSX* (synovial sarcoma X chromosome breakpoint) [24]. There are now over ten known members of the *SSX* gene family; however, only *SSX 1*, *SSX2*, and *SSX4* are known to occur in the setting of synovial sarcoma with translocations of *SSX 1* and *SSX2* representing the majority. This mutation occurs in greater than 95 % of all synovial sarcomas and as such this mutation can be utilized to help confirm the diagnosis [25]. Generally, the last 8 amino acids of SYT are replaced by the 78 amino acids from the C-terminus end of SSX to generate the fusion protein SYT-SSX [26]. While *SYT* overexpression alone does not lead to tumor formation, this specific combination with SSX does lead to activation and subsequent tumor formation [27]. Unfortunately, expression of *SSX* is seen intensely in only a subset of tumor cells with the majority of cells having little or no expression. Despite this, given the immunogenicity of SSX, it remains a potential target for immunotherapy.

17.3.3 ALK

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase (RTK) and was first described as an oncogene in 1994 in anaplastic large-cell lymphoma [28]. It has since been found to have a high occurrence rate in inflammatory myofibroblastic tumors (IMTs) as well as sporadic association with other sarcomas including multiple forms of lipomatous tumors/sarcomas, alveolar rhabdomyosarcoma, Ewing sarcoma/primitive neuroectodermal tumors, leiomyosarcomas, and extraskeletal myxoid chondrosarcomas [29]. The relevance of ALK expression in non-IMTs is unclear, but there is an approximately 50 % incidence of clonal rearrangement of the ALK gene in association with IMTs. Given the current enthusiasm for tyrosine kinase inhibitors (TKIs), ALK represents a promising target. The TKI crizotinib, which is a competitive inhibitor of both the ALK and MET tyrosine kinases, has been extensively evaluated in ALK-expressing

lung cancer but has also been reported as a therapy for IMT [30]. However, similar to other TKIs, crizotinib treatment resistance appears to be a limiting factor [31].

17.3.4 HHV8

The role of the immune system is clearly evidenced in Kaposi's sarcoma (KS), a low-grade vascular neoplasm that involves the epithelium. From an epidemiologic standpoint, KS can be grouped into four forms: classic KS in elderly men of European Jewish or Mediterranean descent, endemic KS in Central and Eastern Africa not associated with HIV, iatrogenic KS in the setting of immunosuppression for organ transplantation, and epidemic/AIDS-KS [32]. Human herpesvirus 8 (HHV8), also known as Kaposi's sarcoma herpesvirus (KSHV), is the causative agent for the development of KS; the rate of HHV8 infection directly correlates to the incidence of KS.

KS tumor cells arise from lymphatic endothelial cells, with HHV8 playing a critical role in the neoplastic transformation of these cells. Several factors are upregulated by HHV8 including vascular endothelial growth factor receptor 3, lymphatic vessel endothelial receptor 1, latency-associated nuclear antigen, and podoplanin [33]. Additionally, activation of the phosphatidylinositol 3-phosphate kinase/Akt/mammalian target of rapamycin pathway also plays a role in development of tumor formation [34]. Despite multiple upregulated genes, HHV8 infection does not usually lead to development of KS in immunocompetent individuals. KS tumor cells are unique from other tumors in that they do not generate tumors in nude mice, nor will they grow *in vitro* as they require cytokines for continued growth [32]. In addition to these factors, host immunosuppression is a key component to the development of clinical disease.

Initiation of highly active antiretroviral treatment (HAART) for HIV-associated KS or reduction of immunosuppression in organ-transplanted patients can lead to regression of KS, likely by increasing immune system activity against HHV8 but also possibly by reducing HIV-associated inflammation and associated cytokines. Initiation of HAART within the HIV population has been associated with a concurrent decrease in the incidence of KS. Prospective trials of HAART in patients with KS have shown decreases in both HIV and HHV8 viral loads and were subsequently associated with regression of the KS [35, 36]. Recently, a trial of rapamycin in combination with HAART showed some increased effectiveness in inducing a clinical response in KS tumors [34]. The use of antiviral agents, such as ganciclovir, may prevent the development of KS, but specific agents which take advantage of HHV8 once tumors are established are being explored [37].

17.4 Preclinical Models of Immunotherapy for Sarcoma

The ability to treat sarcomas by taking advantage of TLR agonists or immune-targeting of sarcoma-associated antigens has been enhanced by the use of preclinical animal models. Several models exist with distinct advantages and characteristics that allow for the testing of novel immunotherapy strategies. The simplest and classic animal model for sarcoma is using established *in vitro* sarcoma cell lines and immunocompromised mice. Cells cultivated *in vitro* could then be transplanted either subcutaneously or implanted as an orthotopic xenograft [38]. While important work has been done using this model, it has inherent flaws as the tumors develop without the selective pressures of an intact host immune system and various forms of immunotherapy are difficult or impossible to evaluate [39]. Additionally, serial passages of tumor cell lines in tissue culture can introduce mutations, favor cells that are capable of growing *in vitro*, and represent cells from a diverse patient population. Finally, there are a myriad of interactions between the mouse stroma and the human tumor cells that can affect aspects of tumor progression and experimental treatments.

17.4.1 Methylcholanthrene (MCA)

The classic model for spontaneous induction of sarcoma in a murine model is the injection of 3-methylcholanthrene (MCA), first described in 1943 by Gross [40]. Since that time, the complex mechanism of MCA-associated tumorigenesis has been elucidated. The process begins with the binding of MCA to cytochrome P450 and other enzymes [41]. The subsequent metabolites react with guanine, producing DNA adducts that lead to mutations in p53 [42]. MCA also becomes hydroxylated, ultimately forming a reactive carbonium ion that is highly mutagenic and carcinogenic, with these mutations ultimately leading to the development of a spindle cell sarcoma. MCA has been applied to various strains of mice to establish multiple *in vitro* sarcoma cell lines, thus allowing a homogenous population of tumor cells to be examined repeatedly *in vivo*. Since its first description, this model has been used extensively to further elucidate properties of soft tissue sarcomas. This model is characterized by the fact that the tumors which develop after administration of MCA are diverse and mimic clinical conditions but may make immunologic experimental design more complicated. For tumor grafts established by injection of an isolated, clonal cell line grown in culture, experimental design may favor immunologic therapy testing but have limited translation clinically.

17.4.2 p53 and Nf1

In an effort to further define the role of p53 in tumorigenesis, chimeric mice for wild-type and null p53 ($p53^{+/-}$) were generated. When these chimeric mice were crossed, it was found that the majority of mice that were homozygous for mutant p53 developed spontaneous tumors, namely, lymphomas and sarcomas, by 6 months of age [43]. In this model it was noted that common tumors in the original strain of mice (i.e., lymphomas and testicular tumors) developed spontaneously in the $p53^{-/-}$ mice at a much younger age, leading to the conclusion that loss of p53 function accelerates the development of predisposed tumors.

Similarly, a murine model of neurofibromatosis was attempted to be created; however, peripheral nerve sheath tumors were unable to be generated in the heterozygote model using the *Nf1* gene. As germ line homozygosity ($Nf1^{-/-}$) was found to be lethal at day 14 of gestation, a chimeric mouse model was generated that was partially composed of $Nf1^{-/-}$ [44]. These mice were then crossed with the p53 null mice to generate $p53^{-/+}$; $Nf^{1-/+}$. Mice that had a loss of heterozygosity (LOH) of only one of these genes survived for about 10 months. However, mice that lacked both functional copies of p53 and Nf1 on chromosome 11 survived for only 5 months and had a much higher predisposition for the development of soft tissue sarcomas. The tumors that develop in these mice are consistent with malignant peripheral nerve sheath tumors and serve as a useful platform for preclinical experimentation.

17.5 Undifferentiated Pleomorphic Sarcoma

Undifferentiated pleomorphic sarcoma (UPS), previously known as malignant fibrous histiocytoma (MFH), is a common soft tissue sarcoma with a high incidence of subsequent lung metastasis. No specific cell line or genetic mutation has been identified as the defining characteristic of UPS, and it is not known whether these tumors have a common cell line or if they represent a more diverse group of tumors arising from various mesenchymal cells [41]. As such, an animal model that adequately recapitulates this disease has been difficult to generate. Given the known role of both the tumor suppressor gene *p53* and the oncogene *Kras* in the oncogenesis of various sarcomas, a mouse model was made with conditional mutations in both of these genes.

This model is based on the site-specific recombinase Cre, which removes DNA bracketed by the loxP sequence. Transcription of a gene can be initiated by removing a stop sequence before the gene or the entire gene itself can be removed. In this instance, mice heterozygous for wild-type and mutant Kras (G12D) are used [45].

A loxP-stop-loxP sequence which normally prevents the transcription of the mutant *Kras* is removed by *Cre*, thus allowing the transcription of the oncogene. These mice are also homozygous for loxP-p53-loxP. With administration of Cre, both *p53* genes are removed. In this model *Cre* is delivered by injecting an adenovirus bearing *Cre* (Ad-Cre), such that the final result is that Kras is activated, while *p53* is suppressed.

The Kras/p53 model allows tumors to develop at specific anatomic sites in a defined amount of time as tumors develop in 2–3 months at the site of the Ad-Cre injection. Additionally, these tumors resemble human UPS in that there is an incidence of lung metastasis in approximately 50 % of mice. Compared to other animal models, tumors which arise most closely mimic human tumors and possess aggressive spontaneous metastatic potential.

17.6 Clinical Applications of Immunotherapy for Sarcoma

To take advantage of unique tumor antigens and the animal models described previously, a basic understanding of tumor immunology is necessary. To determine self from nonself, nucleated cells display type I major histocompatibility complexes (MHC I) and present a repertoire of cellular proteins. Immune surveillance by $CD8^+$ T cells can detect nonself proteins in the context of MHC I by interactions with unique T-cell antigen receptors (TCRs). TCRs confer specificity for a particular target antigen and allow clonal expansion of $CD8^+$ T cells [46]. Activated $CD8^+$ T cells differentiate into cytotoxic T-cell lymphocytes (CTL). Target cell destruction is then induced through the release of inflammatory cytokines such as tumor necrosis factor (TNF) and interferon gamma (IFNγ), the induction of TNF-related apoptosis-inducing ligand (TRAIL), and cytotoxic degranulation which leads to perforin-mediated lysis as an adaptive immunity to intracellular infections [47]. By the expression of specific co-stimulatory molecules, antigen-presenting cells (APCs), such as DCs, are capable of stimulating naive T cells to proliferate and differentiate in response to antigens and are powerful tools for manipulating the immune system [48]. As clinical evidence of the potency of DCs to influence immune responses, a set of ten pediatric patients with solid tumors underwent a DC vaccination protocol [49]. DCs were pulsed with tumor cell lysates and the immunogenic protein keyhole limpet hemocyanin, which generated significant regression of multiple metastatic sites in one patient and stable disease in five patients.

Unfortunately, tumor cells often lose MHC class I molecules from their cell surface, thereby escaping recognition by $CD8^+$ T cells [50–52]. While loss or downregulation of MHC I should lead to poorer clinical outcomes, results are mixed. When prognostic significance of MHC I expression was analyzed, patients with osteosarcoma and high expression of MHC class I showed significantly better overall and event-free survival, but no prognostic significance was found in patients with malignant fibrous histiocytoma [53]. In contrast, in patients with Ewing sarcoma family of tumors, downregulation or negative expression of MHC class I was associated with poorer survival [54].

17.6.1 Adoptive Cell Therapy

Adoptive cell therapy (ACT) harnesses a cancer patient's own antitumor T lymphocytes, which are expanded *ex vivo* into large numbers. These expanded cells are then reinfused back into the patient to achieve an antitumor effect. ACT allows several opportunities for immune modulations that can feasibly be applied to any tumor, including sarcoma, but have been most successfully applied to melanoma. Immune modulations include the selection of highly active, tumor-reactive lymphocyte cultures with optimal characteristics, rapid expansion of *ex vivo* lymphocyte cultures which circumvent immune regulatory mechanisms and the potentially suppressive tumor environment, and host systemic immunosuppression prior to cultured lymphocyte infusion to further enhance activity [55]. The discovery that normal human lymphocytes can be genetically

engineered to recognize cancer antigens and mediate cancer regression *in vivo* has opened opportunities for improving and extending the ACT approach to patients with a wide variety of cancer types [56]. The potential success of this approach for sarcomas has been evident for decades. In an early study, tumor-infiltrating lymphocytes (TILs) from transplantable mouse sarcomas were cultured in high levels of IL-2 where they showed specific lytic activity toward the cognate tumor cells *in vitro* and also mediated tumor regression when transferred into tumor-bearing, cyclophosphamide-conditioned mice [57].

17.6.1.1 Lymphokine-Activated Killers (LAKs)

In 1982, Grimm et al. demonstrated that IL-2 could activate peripheral blood lymphocytes to generate lymphokine-activated killer (LAK) cells capable of lysing human tumor cell lines [58]. The culture of peripheral blood lymphocytes (PBLs) with IL-2 generates NK cells, nonspecific T cells, and LAKs. While there has been moderate success with decreased size and number of pulmonary sarcoma metastases in a murine model, the limited *ex vivo* expansion and the cytolytic activity *in vivo* represent significant barriers [59–61]. The first clinical study combining LAK cells and IL-2 was initiated by Rosenberg and colleagues in 25 patients with advanced cancer [62]. By the end of the 1990s, all published phase II and phase III randomized trials using LAK therapy in cancer showed a clinical response rate of about 15–20 %, not superior to IL-2 monotherapy or IL-2 combined with IFN-α [63, 64].

17.6.1.2 Cytokine-Induced Killers (CIKs)

Cytokine-induced killer (CIK) cells are a heterogeneous population of effector $CD8^+$ T cells with diverse TCR specificities, possessing non-MHC-restricted cytolytic activities against tumor cells [65]. CIKs are generated by the addition of IFNγ and IL-1α to IL-2 in culture. Human CIK cells have been shown to have enhanced cytotoxicity and to proliferate more rapidly than lymphokine-activated killer (LAK) cells by both *in vitro* and *in vivo* studies. CIKs have been characterized as having a higher lytic activity when compared to LAKs, mainly due to the higher proliferation of $CD3^+CD56^+$ cells and to the increased cytotoxic activity of TCR-alpha/beta$^+$ cells in CIK cell cultures [66].

17.6.1.3 Natural Killers (NKs)

Natural killer (NK) cells are lymphocytes important to the innate immune responses, especially against bacteria and viruses. Unlike T or B cells, they do not require clonal expansion and differentiation and use cytokines to induce apoptosis. NKs are typically stimulated by distressed cells by the release of IL-12, IL-15, IL-18, and type I interferons, while MHC type I is often responsible for inhibition of NKs. To allow inhibition and activation of NKs, surface receptors such as Killer-cell immunoglobulin-like receptor (KIR) are used, as seen when MHC-expressing cells suppress NK [67, 68]. The loss of MHC antigen expression in a mouse lymphoma and the loss of MHC in human tumors were first recognized in the 1970s, where 25–75 % of tumor cells had MHC downregulation or losses and normal cells remained expressive [69]. Based on their "missing-self hypothesis," Ljunggren and Kärre hypothesized that NKs could target and lyse lymphoma cells due to the absence of or reduced MHC expression [70]. Therefore, it is conceivable that NK cells function not only as part of the innate immune system but also in adaptive immune responses against tumors that lack MHC expression.

In the development of a novel pediatric therapy, NKs collected from peripheral blood of healthy adult patients were sensitized with Ewing sarcoma, rhabdomyosarcoma, neuroblastoma, and osteosarcoma cell lines [71]. After *in vitro* studies, Ewing sarcoma and rhabdomyosarcoma cells appeared to be sensitive to the cytotoxicity of expanded and activated NK cells. Interestingly, as another example of NK cell activity in human sarcomas, it was observed that the tyrosine kinase inhibitor imatinib showed clinical benefit in gastrointestinal stromal tumors (GISTs) that lacked the mutant isoforms of KIT and PDGRF receptors. It was found that imatinib acted on DCs to promote NK cell activation *in vitro* and *in vivo*, where NKs generated an enhanced tumor response *in vivo* [72].

17.6.1.4 Engineered T Cells

Another approach to ACT involves genetically engineered T cells to express a specific tumor antigen-recognizing TCR. While several studies showed that transfer of TCR-engineered peripheral blood lymphocytes allowed recognition of tumor cells, these studies were mainly for the treatment of melanoma [73–75]. However, one unique study focused on sarcoma. NY-ESO-1, a cancer-testis antigen previously discussed, is expressed in 80 % of synovial cell sarcomas [76]. Using autologous T cells transduced against metastatic synovial cell sarcoma expressing NY-ESO-1, an objective clinical response was seen in four of six patients and a partial response lasting 18 months in one patient following adoptive transfer [77].

17.6.1.5 Chimeric Antigen Receptors (CARs)

The production of T cells expressing chimeric antigen receptors (CARs) is a novel treatment for osteosarcoma [78]. CARs were generated to enable T cells to overcome mechanisms by which tumors escape from immune surveillance including tumor cell MHC I downregulation or loss [79]. The structure of the CAR is comprised of an exodomain, typically derived from the antigen-binding portion of a monoclonal antibody linking the VH and VL domains to construct a single-chain fragment variable region. By doing so, CARs are highly targeted and allow antigen recognition even in tumors with MHC loss [78]. Using human epidermal growth factor receptor 2 (HER2)-specific CARs to overcome low expression of HER2 in a locoregional and metastatic mouse model, these genetically modified T cells demonstrated the ability to mediate regression of osteosarcoma tumors [80].

17.6.2 Sarcoma Immunotherapy of the Future: CTLA-4 and PD-1 Manipulation

17.6.2.1 CTLA-4

Found on the surface of activated and regulatory T cells and located on chromosome 2q33 is cytotoxic T-cell lymphocyte antigen-4 (CTLA-4), also known as cluster of differentiation 152 (CD152). While it is known that CD28 is an activator of T cells, CTLA-4 is a known inhibitory receptor. Both CTLA-4 and CD28 bind to CD80 and CD86, also called B7-1 and B7-2 respectively, on antigen-presenting cells. Early preclinical testing in colon carcinoma as well as fibrosarcoma suggested that blockade of CTLA-4 can lead to tumor cell recognition and elimination [81]. This led to successful clinical testing for metastatic melanoma where clinical responses were seen in 13–21 % of patients [82, 83]. Unfortunately, testing of anti-CTLA-4 monoclonal antibodies for various other cancers has yet to be as successful. In an *in vivo* mouse lymphoma model, CTLA-4 blockade enhanced the priming of T cells from vaccination, but did nothing to prevent the tolerance to tumor cells [84]. Similarly, a recent phase II trial of a CTLA-4-blocking antibody in patients with synovial sarcoma showed no clinical responses, nor was there a clinical benefit of antitumor antigen serological responses [85]. While data are mixed regarding the use of CTLA-4 blockade and antitumor activity, it is possible that blockade alone may not be adequate for continued tumor control.

Combination therapies that include CTLA-4 may have a greater clinical impact. In a mouse model of osteosarcoma, tumor lysate-pulsed DCs were used in combination with CTLA-4 blockade [86]. This combination resulted in a reduced number of regulatory T lymphocytes with increased $CD8^+$ T lymphocytes within metastatic deposits. These findings were associated with a reduction in metastases and improved survival.

Interestingly, CTLA-4 mutations have been related to increased risk of sarcoma development which further supports the critical role of the immune system. CTLA-4 +49G/A polymorphisms and GTAG haplotype are associated with increased risk of osteosarcoma when detected by polymerase chain reaction compared to controls in the Chinese population [87]. In addition, $CTLA\text{-}4^+$ 49G/A polymorphisms appear to be associated with an increased susceptibility to Ewing sarcoma [88] in the same population.

17.6.2.2 PD-1

Programmed death-1 (PD-1) is another immune checkpoint located on T and B cells, NKT cells, activated monocytes, and DCs. Its ligands PD-L1 and PD-L2, or B7–H1 and B7–H2, are expressed on APCs, tumor cells, and placental and non-hematopoietic cells that infiltrate tumors [89]. PD-L1 is the principal ligand of PD-1, and its expression has been found on a variety of tumors. IFNγ has been reported to upregulate the expression of PD-L1 in tumor cells *in vitro*. In preclinical *in vivo* experiments, it has been shown that blockade of the PD-1 checkpoints during cellular adoptive immunotherapy increases IFNγ produced by T cells as well as PD-L1 tumor expression, leading to an improved antitumor response [90]. Similarly, blockade of PD-L1 during DC vaccination has been shown to have a better therapeutic effect than DC vaccination alone by delaying tumor growth and prolonging survival times in a breast tumor-bearing hu-SCID model [91]. To determine synergy, dual blockade of PD-1 and CTLA-4 has resulted in the reversal of $CD8^+$ TIL dysfunction which led to tumor rejection in two-thirds of colon- and ovarian-bearing mice [92]. Currently, PD-1 blockade and its synergy with CTLA-4 blockade have yet to be extensively evaluated in preclinical or clinical studies for the treatment of sarcoma but have a strong rationale for application.

17.7 Concluding Remarks

Current understanding of the immune system combined with the identification of novel antigens expressed on sarcoma cells has created a renewed optimism for the immunotherapy of sarcoma. Active and passive immunotherapy approaches have been recently reported and serve as a foundation for further clinical study. In the near future, immunotherapy may become a standard treatment modality for the treatment of bone and connective tissue cancers.

Acknowledgment This manuscript was supported in part by Roswell Park Cancer Institute and National Cancer Institute (P30 CA016056).

References

1. McCarthy EF. The toxins of William B. Coley and the treatment of bone and soft-tissue sarcomas. Iowa Orthop J. 2006;26:154–8.
2. Coley II WB. Contribution to the knowledge of sarcoma. Ann Surg. 1891;14(3):199–220.
3. Nauts HC, McLaren JR. Coley toxins–the first century. Adv Exp Med Biol. 1990;267:483–500.
4. Kumar H, Kawai T, Akira S. Pathogen recognition by the innate immune system. Int Rev Immunol. 2011;30(1):16–34.
5. Rakoff-Nahoum S, Medzhitov R. Toll-like receptors and cancer. Nat Rev Cancer. 2009;9(1):57–63.
6. Adams S. Toll-like receptor agonists in cancer therapy. Immunotherapy. 2009;1(6):949–64.
7. Rossol M, Heine H, Meusch U, Quandt D, Klein C, Sweet MJ, et al. LPS-induced cytokine production in human monocytes and macrophages. Crit Rev Immunol. 2011;31(5):379–446.
8. Schreibelt G, Tel J, Sliepen KH, Benitez-Ribas D, Figdor CG, Adema GJ, et al. Toll-like receptor expression and function in human dendritic cell subsets: implications for dendritic cell-based anti-cancer immunotherapy. Cancer Immunol Immunother. 2010;59(10):1573–82.
9. Bjorkbacka H, Fitzgerald KA, Huet F, Li X, Gregory JA, Lee MA, et al. The induction of macrophage gene expression by LPS predominantly utilizes Myd88-independent signaling cascades. Physiol Genomics. 2004;19(3):319–30.
10. Kadowaki N, Ho S, Antonenko S, Malefyt RW, Kastelein RA, Bazan F, et al. Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. J Exp Med. 2001;194(6):863–9.
11. Steinman RM, Banchereau J. Taking dendritic cells into medicine. Nature. 2007;449(7161):419–26.
12. Oblak A, Jerala R. Toll-like receptor 4 activation in cancer progression and therapy. Clin Dev Immunol. 2011;2011:609579.
13. Sato Y, Goto Y, Narita N, Hoon DS. Cancer cells expressing toll-like receptors and the tumor microenvironment. Cancer Microenviron. 2009;2 Suppl 1:205–14.
14. Kaczanowska S, Joseph AM, Davila E. TLR agonists: our best frenemy in cancer immunotherapy. J Leukoc Biol. 2013;93(6):847–63.
15. Ridnour LA, Cheng RY, Switzer CH, Heinecke JL, Ambs S, Glynn S, et al. Molecular pathways: toll-like receptors in the tumor microenvironment–poor prognosis or new therapeutic opportunity. Clin Cancer Res. 2013;19(6):1340–6.
16. Cluff CW. Monophosphoryl lipid A (MPL) as an adjuvant for anti-cancer vaccines: clinical results. Adv Exp Med Biol. 2010;667:111–23.
17. Celestin Schartz NE, Chevret S, Paz C, Kerob D, Verola O, Morel P, et al. Imiquimod 5% cream for treatment of HIV-negative Kaposi's sarcoma skin

lesions: a phase I to II, open-label trial in 17 patients. J Am Acad Dermatol. 2008;58(4):585–91.
18. Chen YT, Gure AO, Scanlan MJ. Serological analysis of expression cDNA libraries (SEREX): an immunoscreening technique for identifying immunogenic tumor antigens. Meth Mol Med. 2005;103:207–16.
19. Caballero OL, Chen YT. Cancer/testis (CT) antigens: potential targets for immunotherapy. Cancer Sci. 2009;100(11):2014–21.
20. Cebon J, Knights A, Ebert L, Jackson H, Chen W. Evaluation of cellular immune responses in cancer vaccine recipients: lessons from NY-ESO-1. Expert Rev Vac. 2010;9(6):617–29.
21. Lai JP, Rosenberg AZ, Miettinen MM, Lee CC. NY-ESO-1 expression in sarcomas: a diagnostic marker and immunotherapy target. Oncoimmunology. 2012;1(8):1409–10.
22. Pollack SM, Jungbluth AA, Hoch BL, Farrar EA, Bleakley M, Schneider DJ, et al. NY-ESO-1 is a ubiquitous immunotherapeutic target antigen for patients with myxoid/round cell liposarcoma. Cancer. 2012;118(18):4564–70.
23. Yuan J, Gnjatic S, Li H, Powel S, Gallardo HF, Ritter E, et al. CTLA-4 blockade enhances polyfunctional NY-ESO-1 specific T cell responses in metastatic melanoma patients with clinical benefit. Proc Natl Acad Sci U S A. 2008;105(51):20410–5.
24. Smith HA, McNeel DG. The SSX family of cancer-testis antigens as target proteins for tumor therapy. Clin Dev Immunol. 2010;2010:150591.
25. Haldar M, Randall RL, Capecchi MR. Synovial sarcoma: from genetics to genetic-based animal modeling. Clin Orthop Relat Res. 2008;466(9):2156–67.
26. Crew AJ, Clark J, Fisher C, Gill S, Grimer R, Chand A, et al. Fusion of SYT to two genes, SSX1 and SSX2, encoding proteins with homology to the Kruppel-associated box in human synovial sarcoma. EMBO J. 1995;14(10):2333–40.
27. Nagai M, Tanaka S, Tsuda M, Endo S, Kato H, Sonobe H, et al. Analysis of transforming activity of human synovial sarcoma-associated chimeric protein SYT-SSX1 bound to chromatin remodeling factor hBRM/hSNF2 alpha. Proc Natl Acad Sci U S A. 2001;98(7):3843–8.
28. Morris SW, Naeve C, Mathew P, James PL, Kirstein MN, Cui X, et al. ALK, the chromosome 2 gene locus altered by the t(2;5) in non-Hodgkin lymphoma, encodes a novel neural receptor tyrosine kinase that is highly related to leukocyte tyrosine kinase (LTK). Oncogene. 1997;14(18):2175–88.
29. Li XQ, Hisaoka M, Shi DR, Zhu XZ, Hashimoto H. Expression of anaplastic lymphoma kinase in soft tissue tumors: an immunohistochemical and molecular study of 249 cases. Hum Pathol. 2004;35(6):711–21.
30. Butrynski JE, D'Adamo DR, Hornick JL, Dal Cin P, Antonescu CR, Jhanwar SC, et al. Crizotinib in ALK-rearranged inflammatory myofibroblastic tumor. N Engl J Med. 2010;363(18):1727–33.
31. Shaw AT, Engelman JA. ALK in lung cancer: past, present, and future. J Clin Oncol. 2013;31(8):1105–11.
32. Mesri EA, Cesarman E, Boshoff C. Kaposi's sarcoma and its associated herpesvirus. Nat Rev Cancer. 2010;10(10):707–19.
33. Radu O, Pantanowitz L. Kaposi sarcoma. Arch Pathol Lab Med. 2013;137(2):289–94.
34. Krown SE, Roy D, Lee JY, Dezube BJ, Reid EG, Venkataramanan R, et al. Rapamycin with antiretroviral therapy in AIDS-associated Kaposi sarcoma: an AIDS Malignancy Consortium study. J Acquir Immune Defic Syndr. 2012;59(5):447–54.
35. Gill J, Bourboulia D, Wilkinson J, Hayes P, Cope A, Marcelin AG, et al. Prospective study of the effects of antiretroviral therapy on Kaposi sarcoma–associated herpesvirus infection in patients with and without Kaposi sarcoma. J Acquir Immune Defic Syndr. 2002;31(4):384–90.
36. Wilkinson J, Cope A, Gill J, Bourboulia D, Hayes P, Imami N, et al. Identification of Kaposi's sarcoma-associated herpesvirus (KSHV)-specific cytotoxic T-lymphocyte epitopes and evaluation of reconstitution of KSHV-specific responses in human immunodeficiency virus type 1-Infected patients receiving highly active antiretroviral therapy. J Virol. 2002;76(6):2634–40.
37. Klass CM, Offermann MK. Targeting human herpesvirus-8 for treatment of Kaposi's sarcoma and primary effusion lymphoma. Curr Opin Oncol. 2005;17(5):447–55.
38. Sausville EA, Burger AM. Contributions of human tumor xenografts to anticancer drug development. Cancer Res. 2006;66(7):3351–4.
39. Dodd RD, Mito JK, Kirsch DG. Animal models of soft-tissue sarcoma. Dis Model Mech. 2010;3(9–10):557–66.
40. Gross L. Intradermal immunization of C3H mice against a sarcoma that originated in an animal of the same line. Cancer Res. 1943;3(5):326–33.
41. Malins DC, Anderson KM, Gilman NK, Green VM, Barker EA, Hellstrom KE. Development of a cancer DNA phenotype prior to tumor formation. Proc Natl Acad Sci U S A. 2004;101(29):10721–5.
42. Shimokado K, Watanabe H, Sumii M, Miyagawa K, Kamiya K, Dohi K, et al. p53 gene mutation and loss of heterozygosity of chromosome 11 in methylcholanthrene-induced mouse sarcomas. Jpn J Cancer Res. 1998;89(3):269–77.
43. Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery Jr CA, Butel JS, et al. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature. 1992;356(6366):215–21.
44. Cichowski K, Shih TS, Schmitt E, Santiago S, Reilly K, McLaughlin ME, et al. Mouse models of tumor development in neurofibromatosis type 1. Science. 1999;286(5447):2172–6.
45. Kirsch DG, Dinulescu DM, Miller JB, Grimm J, Santiago PM, Young NP, et al. A spatially and temporally restricted mouse model of soft tissue sarcoma. Nat Med. 2007;13(8):992–7.
46. Shastri N, Schwab S, Serwold T. Producing nature's gene-chips: the generation of peptides for display

by MHC class I molecules. Ann Rev Immunol. 2002;20:463–93.
47. Harty JT, Tvinnereim AR, White DW. CD8+ T cell effector mechanisms in resistance to infection. Ann Rev Immunol. 2000;18:275–308.
48. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature. 1998;392(6673):245–52.
49. Geiger J, Hutchinson R, Hohenkirk L, McKenna E, Chang A, Mule J. Treatment of solid tumours in children with tumour-lysate-pulsed dendritic cells. Lancet. 2000;356(9236):1163–5.
50. Marincola FM, Jaffee EM, Hicklin DJ, Ferrone S. Escape of human solid tumors from T-cell recognition: molecular mechanisms and functional significance. Adv Immunol. 2000;74:181–273.
51. Algarra I, Garcia-Lora A, Cabrera T, Ruiz-Cabello F, Garrido F. The selection of tumor variants with altered expression of classical and nonclassical MHC class I molecules: implications for tumor immune escape. Cancer Immunol Immunother. 2004;53(10):904–10.
52. Bubenik J. MHC class I down-regulation: tumour escape from immune surveillance? (review). Int J Oncol. 2004;25(2):487–91.
53. Tsukahara T, Kawaguchi S, Torigoe T, Asanuma H, Nakazawa E, Shimozawa K, et al. Prognostic significance of HLA class I expression in osteosarcoma defined by anti-pan HLA class I monoclonal antibody, EMR8-5. Cancer Sci. 2006;97(12):1374–80.
54. Yabe H, Tsukahara T, Kawaguchi S, Wada T, Torigoe T, Sato N, et al. Prognostic significance of HLA class I expression in Ewing's sarcoma family of tumors. J Surg Oncol. 2011;103(5):380–5.
55. Rosenberg SA, Restifo NP, Yang JC, Morgan RA, Dudley ME. Adoptive cell transfer: a clinical path to effective cancer immunotherapy. Nat Rev Cancer. 2008;8(4):299–308.
56. Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. Science. 2006;314(5796):126–9.
57. Rosenberg SA, Spiess P, Lafreniere R. A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. Science. 1986;233(4770):1318–21.
58. Grimm EA, Mazumder A, Zhang HZ, Rosenberg SA. Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. J Exp Med. 1982;155(6):1823–41.
59. Mule JJ, Shu S, Schwarz SL, Rosenberg SA. Adoptive immunotherapy of established pulmonary metastases with LAK cells and recombinant interleukin-2. Science. 1984;225(4669):1487–9.
60. Lotze MT, Grimm EA, Mazumder A, Strausser JL, Rosenberg SA. Lysis of fresh and cultured autologous tumor by human lymphocytes cultured in T-cell growth factor. Cancer Res. 1981;41(11 Pt 1):4420–5.
61. Parkhurst MR, Riley JP, Dudley ME, Rosenberg SA. Adoptive transfer of autologous natural killer cells leads to high levels of circulating natural killer cells but does not mediate tumor regression. Clin Cancer Res. 2011;17(19):6287–97.
62. Rosenberg SA, Lotze MT, Muul LM, Leitman S, Chang AE, Ettinghausen SE, et al. Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. N Engl J Med. 1985;313(23):1485–92.
63. Law TM, Motzer RJ, Mazumdar M, Sell KW, Walther PJ, O'Connell M, et al. Phase III randomized trial of interleukin-2 with or without lymphokine-activated killer cells in the treatment of patients with advanced renal cell carcinoma. Cancer. 1995;76(5):824–32.
64. Terme M, Ullrich E, Delahaye NF, Chaput N, Zitvogel L. Natural killer cell-directed therapies: moving from unexpected results to successful strategies. Nat Immunol. 2008;9(5):486–94.
65. Jiang J, Wu C, Lu B. Cytokine-induced killer cells promote antitumor immunity. J Transl Med. 2013;11:83.
66. Schmidt-Wolf IG, Lefterova P, Mehta BA, Fernandez LP, Huhn D, Blume KG, et al. Phenotypic characterization and identification of effector cells involved in tumor cell recognition of cytokine-induced killer cells. Exp Hematol. 1993;21(13):1673–9.
67. Moretta L, Bottino C, Pende D, Vitale M, Mingari MC, Moretta A. Different checkpoints in human NK-cell activation. Trends Immunol. 2004;25(12):670–6.
68. Bottino C, Moretta L, Pende D, Vitale M, Moretta A. Learning how to discriminate between friends and enemies, a lesson from natural killer cells. Mol Immunol. 2004;41(6–7):569–75.
69. Garrido F, Ruiz-Cabello F, Cabrera T, Perez-Villar JJ, Lopez-Botet M, Duggan-Keen M, et al. Implications for immunosurveillance of altered HLA class I phenotypes in human tumours. Immunol Today. 1997;18(2):89–95.
70. Ljunggren HG, Karre K. In search of the 'missing self': MHC molecules and NK cell recognition. Immunol Today. 1990;11(7):237–44.
71. Cho D, Shook DR, Shimasaki N, Chang YH, Fujisaki H, Campana D. Cytotoxicity of activated natural killer cells against pediatric solid tumors. Clin Cancer Res. 2010;16(15):3901–9.
72. Borg C, Terme M, Taieb J, Menard C, Flament C, Robert C, et al. Novel mode of action of c-kit tyrosine kinase inhibitors leading to NK cell-dependent antitumor effects. J Clin Invest. 2004;114(3):379–88.
73. Yee C, Thompson JA, Roche P, Byrd DR, Lee PP, Piepkorn M, et al. Melanocyte destruction after antigen-specific immunotherapy of melanoma: direct evidence of t cell-mediated vitiligo. J Exp Med. 2000;192(11):1637–44.
74. Clay TM, Custer MC, Sachs J, Hwu P, Rosenberg SA, Nishimura MI. Efficient transfer of a tumor antigen-reactive TCR to human peripheral

blood lymphocytes confers anti-tumor reactivity. J Immunol. 1999;163(1):507–13.
75. Hwu P, Yang JC, Cowherd R, Treisman J, Shafer GE, Eshhar Z, et al. In vivo antitumor activity of T cells redirected with chimeric antibody/T-cell receptor genes. Cancer Res. 1995;55(15):3369–73.
76. Jungbluth AA, Antonescu CR, Busam KJ, Iversen K, Kolb D, Coplan K, et al. Monophasic and biphasic synovial sarcomas abundantly express cancer/testis antigen NY-ESO-1 but not MAGE-A1 or CT7. Int J Cancer. 2001;94(2):252–6.
77. Robbins PF, Morgan RA, Feldman SA, Yang JC, Sherry RM, Dudley ME, et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. J Clin Oncol. 2011;29(7):917–24.
78. Weibo P, Zhaoming Y. Auto T cells expressing chimeric antigen receptor derived from auto antibody might be a new treatment for osteosarcoma. Med Hypotheses. 2012;78(5):616–8.
79. Eshhar Z, Waks T, Gross G, Schindler DG. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. Proc Natl Acad Sci U S A. 1993;90(2):720–4.
80. Ahmed N, Salsman VS, Yvon E, Louis CU, Perlaky L, Wels WS, et al. Immunotherapy for osteosarcoma: genetic modification of T cells overcomes low levels of tumor antigen expression. Mol Ther. 2009;17(10):1779–87.
81. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science. 1996;271(5256):1734–6.
82. Phan GQ, Yang JC, Sherry RM, Hwu P, Topalian SL, Schwartzentruber DJ, et al. Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. Proc Natl Acad Sci U S A. 2003;100(14):8372–7.
83. Attia P, Phan GQ, Maker AV, Robinson MR, Quezado MM, Yang JC, et al. Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4. J Clin Oncol. 2005;23(25):6043–53.
84. Sotomayor EM, Borrello I, Tubb E, Allison JP, Levitsky HI. In vivo blockade of CTLA-4 enhances the priming of responsive T cells but fails to prevent the induction of tumor antigen-specific tolerance. Proc Natl Acad Sci U S A. 1999;96(20):11476–81.
85. Maki RG, Jungbluth AA, Gnjatic S, Schwartz GK, D'Adamo DR, Keohan ML, et al. A pilot study of anti-CTLA4 antibody ipilimumab in patients with synovial sarcoma. Sarcoma. 2013;2013:168145.
86. Kawano M, Itonaga I, Iwasaki T, Tsumura H. Enhancement of antitumor immunity by combining anti-cytotoxic T lymphocyte antigen-4 antibodies and cryotreated tumor lysate-pulsed dendritic cells in murine osteosarcoma. Oncol Rep. 2013;29(3):1001–6.
87. Liu Y, He Z, Feng D, Shi G, Gao R, Wu X, et al. Cytotoxic T-lymphocyte antigen-4 polymorphisms and susceptibility to osteosarcoma. DNA Cell Biol. 2011;30(12):1051–5.
88. Feng D, Yang X, Li S, Liu T, Wu Z, Song Y, et al. Cytotoxic T-lymphocyte antigen-4 genetic variants and risk of Ewing's sarcoma. Gen Test Mol Biomark. 2013;17(6):458–63.
89. Weber J. Immune checkpoint proteins: a new therapeutic paradigm for cancer–preclinical background: CTLA-4 and PD-1 blockade. Semin Oncol. 2010;37(5):430–9.
90. Peng W, Lizee G, Hwu P. Blockade of the PD-1 pathway enhances the efficacy of adoptive cell therapy against cancer. Oncoimmunology. 2013;2(2):e22691.
91. Ge Y, Xi H, Ju S, Zhang X. Blockade of PD-1/PD-L1 immune checkpoint during DC vaccination induces potent protective immunity against breast cancer in hu-SCID mice. Cancer Lett. 2013;336(2):253–9.
92. Duraiswamy J, Kaluza KM, Freeman GJ, Coukos G. Dual blockade of PD-1 and CTLA-4 combined with tumor vaccine effectively restores T-cell rejection function in tumors. Cancer Res. 2013;73(12):3591–603.
93. So EY, Ouchi T. The application of toll like receptors for cancer therapy. Int J Biol Sci. 2010;6(7):675–81.
94. Salaun B, Zitvogel L, Asselin-Paturel C, Morel Y, Chemin K, Dubois C, et al. TLR3 as a biomarker for the therapeutic efficacy of double-stranded RNA in breast cancer. Cancer Res. 2011;71(5):1607–14.
95. Kanczkowski W, Morawietz H, Ziegler CG, Funk RH, Schmitz G, Zacharowski K, et al. Pam3CSK4 and LTA-TLRs ligands associated with microdomains induce IL8 production in human adrenocortical cancer cells. Horm Metab Res. 2007;39(6):457–60.
96. Lombardi V, Van Overtvelt L, Horiot S, Moussu H, Chabre H, Louise A, et al. Toll-like receptor 2 agonist Pam3CSK4 enhances the induction of antigen-specific tolerance via the sublingual route. Clin Exp Allergy. 2008;38(11):1819–29.
97. Yuk JM, Shin DM, Song KS, Lim K, Kim KH, Lee SH, et al. Bacillus calmette-guerin cell wall cytoskeleton enhances colon cancer radiosensitivity through autophagy. Autophagy. 2010;6(1):46–60.
98. Zanin-Zhorov A, Nussbaum G, Franitza S, Cohen IR, Lider O. T cells respond to heat shock protein 60 via TLR2: activation of adhesion and inhibition of chemokine receptors. FASEB J. 2003;17(11):1567–9.
99. Taura M, Fukuda R, Suico MA, Eguma A, Koga T, Shuto T, et al. TLR3 induction by anticancer drugs potentiates poly I:C-induced tumor cell apoptosis. Cancer Sci. 2010;101(7):1610–7.
100. Jasani B, Navabi H, Adams M. Ampligen: a potential toll-like 3 receptor adjuvant for immunotherapy of cancer. Vaccine. 2009;27(25–26):3401–4.
101. Hennessy EJ, Parker AE, O'Neill LA. Targeting toll-like receptors: emerging therapeutics? Nat Rev Drug Discov. 2010;9(4):293–307.
102. Geng D, Zheng L, Srivastava R, Velasco-Gonzalez C, Riker A, Markovic SN, et al. Amplifying

TLR-MyD88 signals within tumor-specific T cells enhances antitumor activity to suboptimal levels of weakly immunogenic tumor antigens. Cancer Res. 2010;70(19):7442–54.
103. Opal SM, Laterre PF, Francois B, LaRosa SP, Angus DC, Mira JP, et al. Effect of eritoran, an antagonist of MD2-TLR4, on mortality in patients with severe sepsis: the ACCESS randomized trial. JAMA. 2013;309(11):1154–62.
104. Yoon SI, Kurnasov O, Natarajan V, Hong M, Gudkov AV, Osterman AL, et al. Structural basis of TLR5-flagellin recognition and signaling. Science. 2012;335(6070):859–64.
105. Cataldi A, Yevsa T, Vilte DA, Schulze K, Castro-Parodi M, Larzabal M, et al. Efficient immune responses against Intimin and EspB of enterohaemorragic Escherichia coli after intranasal vaccination using the TLR2/6 agonist MALP-2 as adjuvant. Vaccine. 2008;26(44):5662–7.
106. Schill T, Schon MP, Pletz N, Emmert S, Schon M. Stimulation of pulmonary immune responses by the TLR2/6 agonist MALP-2 and effect on melanoma metastasis to the lung. Exp Dermatol. 2012;21(2):91–8.
107. Singh VK, Ducey EJ, Fatanmi OO, Singh PK, Brown DS, Purmal A, et al. CBLB613: a TLR 2/6 agonist, natural lipopeptide of Mycoplasma arginini, as a novel radiation countermeasure. Radiat Res. 2012;177(5):628–42.
108. Berman B, Poochareon VN, Villa AM. Novel dermatologic uses of the immune response modifier imiquimod 5% cream. Skin Ther Lett. 2002;7(9):1–6.
109. Dummer R, Hauschild A, Becker JC, Grob JJ, Schadendorf D, Tebbs V, et al. An exploratory study of systemic administration of the toll-like receptor-7 agonist 852A in patients with refractory metastatic melanoma. Clin Cancer Res. 2008;14(3):856–64.
110. Lu Z. Potential therapeutic interventions on toll like receptors for clinical applications. Res Pharm Biotechnol. 2010;2(1):7–13.
111. Hamm S, Rath S, Michel S, Baumgartner R. Cancer immunotherapeutic potential of novel small molecule TLR7 and TLR8 agonists. J Immunotoxicol. 2009;6(4):257–65.
112. Jurk M, Vollmer J. Therapeutic applications of synthetic CpG oligodeoxynucleotides as TLR9 agonists for immune modulation. BioDrugs. 2007;21(6):387–401.
113. Krieg AM. Toll-like receptor 9 (TLR9) agonists in the treatment of cancer. Oncogene. 2008;27(2):161–7.
114. Basith S, Manavalan B, Lee G, Kim SG, Choi S. Toll-like receptor modulators: a patent review (2006–2010). Exp Opin Ther Pat. 2011;21(6):927–44.
115. Tarhini AA, Kirkwood JM, Krieg AM. Early development of the toll-like receptor 9 agonist, PF-3512676, for the treatment of patients with advanced cancers. Expert Opin Drug Discov. 2009;4(5):587–603.

Immunopathology and Immunotherapy of Central Nervous System Cancer

18

Adam M. Sonabend, Christopher R. Showers, and Richard C.E. Anderson

Contents

A.M. Sonabend, MD (✉)
The Gabriele Bartoli Brain Tumor Research Laboratory, Department of Neurological Surgery, The Neurological Institute, Columbia University College of Physicians and Surgeons, New York, NY 10032, USA
e-mail: adam.sonabend@gmail.com

C.R. Showers, BS
The Gabriele Bartoli Brain Tumor Research Laboratory, Department of Neurological Surgery, The Neurological Institute, Columbia University College of Physicians and Surgeons, New York, NY 10032, USA

Department of Neurological Surgery, Columbia University Medical Center, New York, NY, USA

R.C.E. Anderson, MD
The Gabriele Bartoli Brain Tumor Research Laboratory, Department of Neurological Surgery, The Neurological Institute, Columbia University College of Physicians and Surgeons, New York, NY, 10032, USA

Department of Neurological Surgery, Morgan Stanley Children's Hospital of New York Presbyterian, New York, NY USA

18.1 Introduction

The immunopathology of central nervous system (CNS) cancers is unique; hence, they pose various challenges to designing effective immune-based therapeutic strategies. Considering the modest efficacy of current CNS cancer treatment, developments in preclinical and early clinical investigations of immune-based therapies appear promising. Cancers of the brain, spinal cord, and surrounding structures are diagnosed in approximately 9–11 per 100,000 people in the United States per year and effect an age-adjusted mortality rate of 4.3 per 100,000 per year [1], underscoring the general lethality of these tumors. Current treatments are rarely curative and often aimed primarily at reducing short-term mortality while minimizing neurological morbidity. Generally, these tumors are treated through a combination of cytoreductive surgery, chemotherapy, and radiation therapy, although exceptions exist for particular tumors. These treatments

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_18, © Springer-Verlag Berlin Heidelberg 2015

are largely nonspecific to cancer cells and therefore may be damaging to bystander neurological tissue while achieving only modest therapeutic benefits. For instance, in a recent series, treatment of glioblastoma multiforme (GBM) with surgery, radiation therapy, and chemotherapy with temozolomide led to an overall 2-year survival in only 27 % [2]. The vital functions and poor resiliency of neurologic tissue and the often diffusely infiltrating nature of CNS cancers pose a significant challenge to current therapies.

Antineoplastic properties of the immune system are well documented and known to be dysregulated in many human cancers, including those of the CNS [3]. Understanding the mechanisms by which immune cells may prevent CNS tumor development or by which these cells may contribute to tumor-mediated immune evasion is of key importance to combat cancer on a cell-specific level. Appreciation of the distinct features of immune activation and modulation within the CNS will be fundamental to the development of any immune-based therapy for brain tumors.

This chapter provides an overview of the intricacies of the immune system in the context of the CNS. The potential interactions between the immune system and a developing CNS tumor will be discussed. Additionally, some interesting immunotherapeutic approaches currently under development in the setting of CNS cancer will be discussed.

18.2 Antitumor Mechanisms of the Immune System

Generally, antitumor immune surveillance is thought to occur in three different circumstances. First, eradication of pathogens that cause chronic inflammation is believed to prevent the development of some cancers. The proposed explanation for this principle involves the inflammatory milieu, which contains free radicals and genotoxic agents that act as carcinogenic stimuli. Barricaded as a sterile space, immune mediators of the CNS are not routinely exposed to pathogens. Nevertheless, evidence from parenchymal infection or infarction and from autoimmune disease such as multiple sclerosis demonstrates the capacity to initiate classical inflammatory cascades within the CNS upon exposure to pathogens [4, 5]. Second, control of oncogenic viral infections through Cytotoxic T lymphocytes (CTLs) and natural killer (NK) cell immunity is key to prevention of viral induced transformation. Examples of viral induced cancers include some lymphomas caused by Epstein-Barr virus [6] and cervical carcinoma caused by papilloma virus [7]. Regarding CNS tumors, this principle may be relevant in the context of cytomegalovirus (CMV), which is hypothesized to underlie the development of gliomas. This highly controversial hypothesis is mainly supported by association studies, none of which have provided scientific evidence of causality. One of the most notable reports founding this hypothesis consists of a study in which CMV antigens were detected in glioma tissue specimen [8]. Lastly, CTLs and NK cells are capable of recognizing and eliminating tumor cells that overexpress developmental or tumor-specific antigens derived from cancer-related genetic alterations. Accumulating evidence suggests that immune mediators are capable of eliciting this mechanism for combating CNS cancers. Yet, additional evidence suggests that these effector immune cells are limited in doing so, that there is a scarcity of tumor antigens capable of being specifically recognized, and that these effector cells are overcome by tumor-derived immunosuppressive influences. A focus of ongoing CNS cancer immunotherapeutic research is the investigation of the limitations to antitumoral immunity and the design of strategies to enhance it.

18.3 Immune Compartment of the CNS

In the past, the CNS was perceived to possess little immunologic potential to resist tumor development [9].

This idea was based on a perceived lack of specialized antigen-presenting cells (APCs), restriction from circulating lymphocytes and

other immune mediators by the blood-brain barrier (BBB), and absence of lymphatic drainage in the CNS. However, evidence accumulated over the last 20 years has largely debunked this view of the CNS by demonstrating distinct immune activation cascades in response to cerebral ischemia, traumatic brain injury, and autoimmune diseases such as multiple sclerosis [5, 10]. In each of these pathological states, immune competence is contingent upon the activation of resident microglia and infiltrating macrophages capable of effective antigen presentation and lymphocyte activation, all permissible through inducible permeability of the BBB to leukocytes and immune mediators [4, 11]. Activated microglia have been shown to phenotypically resemble both macrophages and dendritic cells (DCs), capable of presenting antigens and activating T-cell lymphocytes [12]. Following activation, CNS APCs are capable of returning to the systemic circulation through drainage via perivascular Virchow-Robin spaces and the nasal mucosa as conduits to cervical lymph nodes [13–15]. Subsequently, both activated and naive T cells responding to chemotactic signals have been shown to traverse the BBB and engraft into sites of inflammation [16]. Activated T cells remain in the CNS, as demonstrated in tumor extracts from multiple CNS cancer types which display tumor antigen-specific CTLs and helper T cells capable of tumoricidal immune function *in vitro* [17–19]. Additionally, circulating CNS antigen-specific antibodies and CTLs have been isolated from the peripheral blood of patients with CNS cancer, further indicating the potential for competent tumor-specific responses within the CNS [20, 21].

On the other hand, opposing immunosuppressive phenomena have been described in the setting of CNS cancer. A series of reports have demonstrated anergy and apoptosis following TCR stimulation in CNS cancer-infiltrating T cells (reviewed in [18]), as well as an overwhelming presence of suppressive regulatory T cells (Tregs) within high-grade CNS tumors (reviewed in [22]). Furthermore, tumor-infiltrating macrophages have been shown to possess immunosuppressive and tumorigenic phenotypes in the setting of glioma [23, 24]. Understanding the forces driving lymphocyte activation *vs.* suppression following stimulation with tumor antigens within the CNS is imperative to the success of CNS cancer immune-based therapies.

18.4 CNS Tumor-Derived Immunosuppression

Suppression of both CNS immune surveillance and activated tumoricidal immune cells by tumor cells is a fundamental feature of tumor development. Unfolding evidence implicates many cellular participants in this process, including resident microglia, peripherally invading macrophages, and lymphocytes, most notably Tregs. Interactions among these players are believed to underlie the state of generalized immunosuppression observed in many patients with CNS cancers, likely extending systemically from the potently immunosuppressive local tumor microenvironment at the interface of tumor and immune cells. A brief overview of the main cellular players for CNS tumor-induced immunosuppression is provided here.

18.4.1 Tumor Cells

Transformed cells are clear targets for CNS immune sentinels responding to the expression of aberrant or mutated antigens, as well as to cellular stress antigens which are associated with cancer-induced cell proliferation and stromal remodeling. These antigens activate immune sentinel cells through stimulation of major histocompatibility complex (MHC) class I and II molecules, in coordination with co-stimulatory signals including B7 isoforms 1 and 2 (CD80/86) [25, 26]. As a principal means of evading tumoricidal immune activation, CNS tumor cells markedly downregulate expression of both MHC I and II proteins. In malignant glioma, the most extensively studied CNS cancer, an inverse correlation has been observed between the extent of MHC expression and tumor lymphocyte infiltration. MHC expression demonstrated an inverse corre-

lation with tumor grade [27], suggesting its downregulation as an immune-evasion mechanism for tumor cells. Additionally, through a potent cocktail of secreted mediators, glioma cells induce the downregulation of co-stimulatory molecules B7-1 and B7-2 on both tumor cells and surrounding APCs, most notably tumor-associated macrophages (TAM), removing a necessary signal for proper T-cell activation [28–30]. Furthermore, glioma cells can express immunosuppressive molecules such as the co-stimulatory molecule homologue B7-H1 [31], the expression of which is normally limited to Gemcitabine and carboplatin at the end of immune responses. B7-H1 expression has been demonstrated both on glioma cells themselves and on TAMs and functions to induce apoptosis in activated T cells [31, 32].

Upregulation of secreted molecules and cell surface proteins by glioma cells also contributes to potent immunosuppression and tumor propagation. Among the most extensively documented are transforming growth factor beta (TGF-β), prostaglandin E2 (PGE2), Fas ligand (FasL), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and the immunomodulatory cytokines IL-4, IL-6, and IL-10 (reviewed in [33]). TGF-β is also known to inhibit the development and activation of APCs, repress activation of NKs, and prevent the activation and differentiation of CTL [34]. PGE2 is associated with suppression of T-cell activation and proliferation and has been demonstrated to induce the production of Tregs [35]. Among the main pathways mediating programmed cell death in a variety of effector immune cell types is the cell surface protein FasL, which has been detected on the surface of tumor cells isolated from gliomas, as well as in multiple CNS cancer cell lines [36]. Both microglia and T cells express its receptor, Fas, and therefore may be susceptible to the death signal provided by FasL expressed on CNS tumor cells. Indeed, multiple studies have demonstrated that FasL was responsible for the death of T lymphocytes when cocultured with glioma cells *in vitro* and that the downregulation of FasL on tumor cells enhances tumor infiltration by T cells, reducing tumor growth *in vivo* [37]. Increased expression of the immunomodulatory cytokines IL-4, IL-6, and IL-10 has been demonstrated in high-grade gliomas, most notably GBM [38]; these cytokines limit inflammation, reduce immune activation, and drive the expression of immunosuppressive mediators such as TGF-β and PGE2 [39].

Recently, expression of indoleamine 2,3-dioxygenase (IDO) in gliomas has been implicated in the recruitment of immunosuppressive $CD4^+CD25^+FOX\text{-}P3$ Tregs and the subsequent ablation of antitumoral immunity. A series of *in vivo* experiments showed that IDO-derived Treg tumor infiltration led to a decrease of $CD8^+$ cytotoxic T-cell tumor infiltration and, in contrast, IDO silencing on tumor cells led to an increase in $CD8^+$ tumor infiltration and an increase in overall survival for mice bearing glioma xenografts. Interestingly, Wainwright et al. demonstrated that tumor cell-specific expression of IDO, rather than peripheral expression of this enzyme, is critical for maintaining this immunosuppressive state [40]. IDO might have a clinical and translational therapeutic potential, as its expression correlates with tumor grade and has a negative impact on overall survival for patients with gliomas [40, 41].

In addition to the mechanisms discussed above, cell-cell interactions might play a role in the complex local microenvironment involving tumor and immune cells, which are both potently immunosuppressive and tumorigenic [18].

18.4.2 Glioma Cancer Stem Cells

Cancer stem cells (CSCs) are a heterogeneous group of undifferentiated tumor cells which posses an enhanced capacity for self-renewal, multipotency, and tumorigenicity at low cell numbers and during isolation [42].

Some evidence suggests the implication of gCSC in tumor-mediated immunosuppression; gCSCs isolated from human glioma specimens and grown *in vitro* were shown to have reduced expression of MHC and co-stimulatory molecule expression but demonstrated high levels of expression of immune-inhibitory molecules [43]. Additionally, coculture experiments have shown

that gCSCs induced apoptosis of both naïve and activated T cells through secretion of galactin-3. In addition, gCSCs also inhibited phagocytosis and expression of tumor necrosis factor alpha (TNF-α) in macrophages through secretion of macrophage inhibitory cytokine 1 (MIC-1) [44]. Finally, gCSCs are believed to confer radiation and chemotherapeutic resistance [44, 45]. The near inevitability of glioma recurrence following standard treatments may result from recalcitrant gCSCs, which escape the therapeutic targeting and regenerate the parent tumor. Any therapeutic strategy designed to affect a lasting tumor remission should therefore target gCSCs.

18.4.3 Tumor-Associated Macrophages/Microglia

Tumor-associated macrophages/microglia (TAMs) are the predominant infiltrating immune cells in malignant glioma and can account for up to 40 % of the tumor cell mass [23]. Though phenotypically indistinguishable following activation, TAMs are derived both from resident CNS microglia and from bone marrow mononuclear cells that colonize the CNS under pathological conditions [36]. A series of studies have shown that in the case of gliomas, TAMs under the influence of tumor cells can acquire a phenotype that contributes to the immunosuppressive and tumor-promoting local tumor microenvironment [24].

Characterization of TAMs in glioma has led to delineation between classically activated inflammatory M1-type TAMs with tumoricidal potential and alternatively activated immunosuppressive M2-type TAMs, which are predominant in the CNS tumor microenvironment. Classically activated M1-type TAMs participate in the coordinated response to immunogenic antigens primarily through production of proinflammatory and tumoricidal mediators such as NO, TNF-α, IL-1B, and IL-12, upregulation of MHC and costimulatory molecules necessary for antigen presentation, and an overall enhanced ability to phagocytose pathogenic material (reviewed in [18]). Conversely, M2-type TAMs exert immune modulation through secretion of potent immunosuppressive mediators including IL-10, IL-6, and TGF-β. In addition to this cytokine cocktail, M2-type TAMs downregulate MHC and costimulatory molecules, show a decreased phagocytic capability, and upregulate the cell surface antigens FASL and B7-H1. The upregulation of these two molecules leads to the induction of anergy and apoptosis in effector T cells, which express Fas ligand. Thus, M2-type TAMs appear to play a role in the immunosuppressive environment seen on gliomas (Fig. 18.1).

Alternatively activated M2-type TAMs are the predominant immune cell type in malignant glioma, and their presence has been shown to correlate with histological grade [46]. A recent investigation revealed increased expression of the M2 markers CD163 and CD204 by TAMs in WHO grade IV gliomas, compared to WHO grades II and III gliomas [47]. The perverse polarization of TAM precursors, both resident microglia and peripheral derived monocytes, to the alternative M2 state is generally believed to occur as these cells encounter the myriad cytokines, growth factors, and surface antigens of the tumor microenvironment. Among the factors implicated in the active recruitment and altered polarization of monocytes by CNS tumor cells, monocyte chemoattractant proteins 1 (MCP-1/CCL-2) and monocyte colony-stimulating factor (M-CSF) are believed to drive local recruitment and proliferation of TAM precursors, while TGF-β, IL-4, IL-10, and IL-13 together orchestrate polarization to the alternative M2 phenotype [47, 48]. Importantly, this polarization toward a M2 TAM phenotype takes place in the absence of IFN-γ, a potent driver of the classical M1 phenotype [49].

The absence of IFN-γ is likely due to the suppression of its principle source, activated type 1 T-helper cells (discussed below).

18.4.4 Myeloid-Derived Suppressor Cells

Recent refinements of the M1/M2 TAM characterization scheme describe a more heterogeneous population of systemically distributed M2

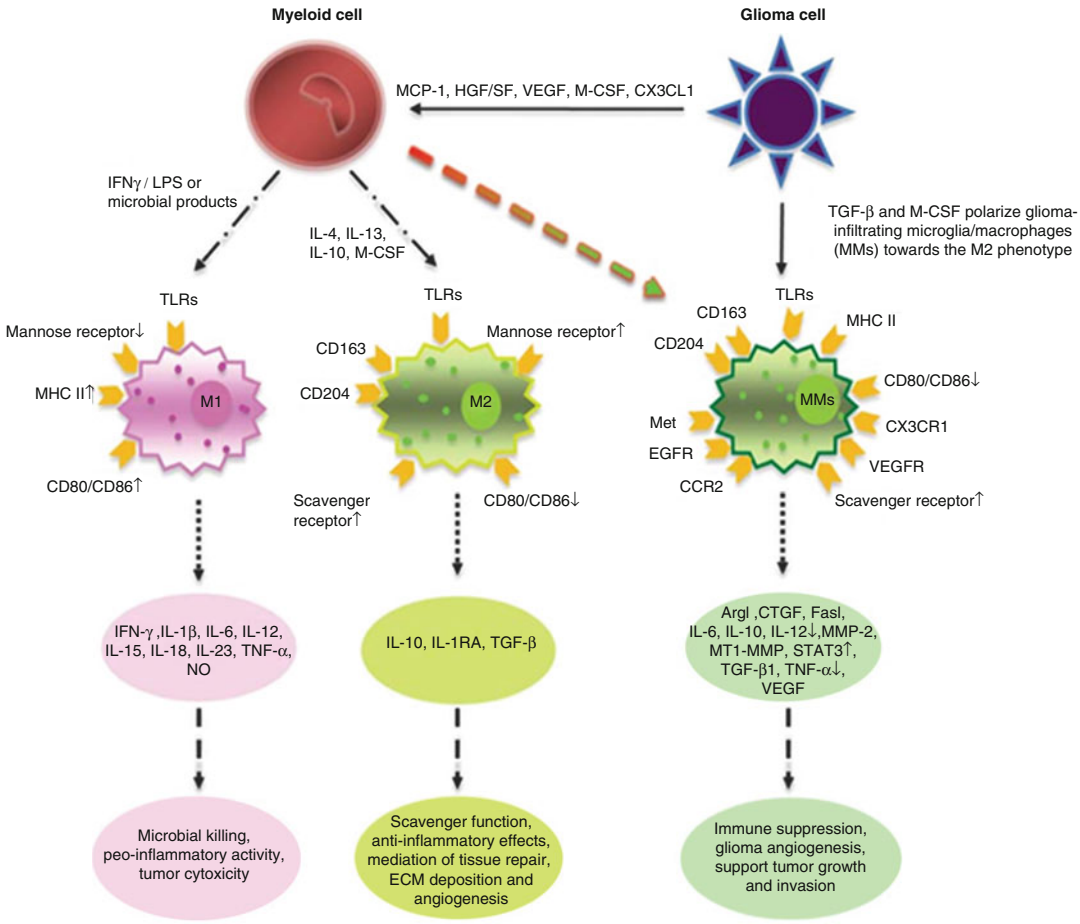


Fig. 18.1 Polarization of tumor-associated macrophages in glioma. Notice the distinct M1 and M2 phenotypes (Reprinted from Li and Graeber [18], with permission)

TAM-like myeloid-derived immunosuppressive cells at intermediate stages of maturation, which are able to suppress multiple phases of the immune response [50]. These myeloid-derived suppressor cells (MDSCs) have been shown both to perpetuate tumor-promoting microenvironments and to distribute peripherally to hinder lymphocyte activation in immune organs. MDSCs are therefore implicated in the general systemic immunosuppression observed in patients with malignant gliomas [48]. Recent evidence suggests that MDSC precursors must be exposed to the concentrated cocktail of immunomodulatory mediators and cell-cell interactions in the tumor microenvironment to become MDSCs [48]. These observations suggest that naïve monocyte traffic to the tumor microenvironment, mature into immunosuppressive MDSCs, and then redistribute systemically [48]. Systemically circulating MDSCs present a poorly understood hurdle to remediating CNS cancer immune suppression. Their heterogeneous expression profile and systemic distribution allow a potentially broad and widespread armament of immunosuppressive functions. If indeed these cells are generated by local tumor-derived factors of the microenvironment, as in the more clearly defined M2 TAM phenotype, then disabling the local "monocyte-educating" mechanisms of tumor cells may reduce the generation of MDSC.

18.4.5 Lymphocytes and Regulatory T cells

Lymphocyte effector cells are major players in antineoplastic immunity, yet lymphocytes which traffic to cancers of the CNS are disabled, reprogrammed to immunosuppressive phenotypes, and subsequently permitted to remain within tumor through failure of natural anergic cell deletion. As discussed above, the process of T-cell activation by APCs is severely hindered in CNS cancers by reductions in MHC and co-stimulatory molecules on both tumor cells and surrounding APCs and by the milieu of T-cell-deactivating mediators within the tumor microenvironment. NK cells are known to initiate deletion of T cells with reduced expression of MHC or co-stimulatory molecules, releasing TNF-α and IFN-γ (reviewed in [50]). This fail-safe mechanism is believed to be disabled by the immunosuppressive milieu of the local tumor microenvironment, most notably by IL-10, and by activation of the NK cell inhibitory receptor KIR2DL through the ligand HLA-G, which is expressed on Tregs [51]. Through these mechanisms, T lymphocytes that are polarized to immunosuppressive phenotypes are permitted to remain within CNS tumors.

Ongoing research implicates Tregs as a major lymphocyte player in CNS tumor immune biology. An increased systemic prevalence of Tregs among T cells has been observed in malignant glioma, consistent with their role in suppressing the immune rejection of neoplastic cells [52]. In addition, Treg infiltration of brain tumors has also been demonstrated, and in the case of gliomas, the fraction of Treg correlates with tumor grade [52, 53]. These observations reflect the significant role Tregs play as a negative immune modulator of lymphocytes both within the tumor and peripherally in lymphoid organs, leading to immune evasion by tumor cells.

Investigation of the origin, recruitment, expansion, and immunomodulatory effect of Tregs in malignant gliomas is an active effort within the tumor immunology field. Recent evidence shows that T cells may be converted to $CD4^+/Foxp3^+$-induced Tregs (iTregs) peripherally through exposure to APCs or suboptimal TCR stimulation in the presence of high levels of TGF-β, as is present in the tumor microenvironment [40].

Both iTregs and thymus-derived natural Tregs (nTregs) have been shown to infiltrate and proliferate within CNS tumors. These cells migrate in response to tumor-secreted MCP-1, which binds CCR4, a receptor highly expressed on Tregs and their precursors [51]. The mechanisms by which Tregs elicit immunosuppression involve Foxp3-mediated expression of the immunosuppressive cell surface ligands glucocorticoid-induced tumor necrosis factor receptor (GITR), cytotoxic T-lymphocyte antigen (CTLA-4), and human leukocyte antigen G (HLA-G), as well as through the contribution of immunosuppressive cytokines TGF-β and IL-10 into the microenvironment [54]. This inhibitory signal replaces the stimulatory interaction between T-cell protein CD28 and APC co-stimulatory molecules B7-1 and B7-2 to prevent activation. HLA-G on placental cells has been shown to contribute immune tolerance in pregnancy by binding the KIR2DL receptor of NK cells, blocking activation in the presence of cells lacking MHC or co-stimulatory molecules. By this mechanism, Tregs are hypothesized to disable NK cell surveillance.

18.5 STAT3 Pathway

As discussed, many soluble mediators and cell surface molecules expressed by tumor cells, TAMs, and Tregs participate to establish a potently immune-disabling microenvironment. Expression profiles across these various cellular players are similar, raising suspicion for unifying mediators of signal transduction or gene expression common to these shared phenotypes. Signal transducer and activator of transcription protein 3 (STAT3), a transcription factor active in both glioma cells and TAMs, has been shown to influence multiple immunosuppressive signaling pathways implicated in CNS tumor-induced immunosuppression [55].

Furthermore, considering the myriad targets of STAT3 modulation, activation of this intracellular mediator may also augment CNS tumor

angiogenesis and stromal remodeling [56]. STAT3 activation in glioma TAMs is induced downstream of many mediators known to constitute the local microenvironment such as IL-10, IL-6, EGF, and FGF [57]. In both tumor cells and TAMs, STAT3 decreases the expression of surface molecules necessary for antigen presentation such as MHC II, B7-1, and B7-2 and upregulates M2-specific immunomodulatory mediators including IL-10, EGF, VEGF, and various matrix metalloproteinases (MMPs) (reviewed in [18]). Experiments blocking the activation of STAT3 in gCSCs cocultured with allogeneic T-cell precursors demonstrate reduced Treg differentiation and reduced overall T-cell apoptosis [58]. Therefore, STAT3 may serve as a critical "molecular hub" linking multiple immunosuppressive pathways in CNS tumor cells and M2 TAMs. STAT3 target molecules such as IL-10 and IL-6 have been shown to subsequently trigger STAT3 activation [59], leading authors to propose a feedforward mechanism of reinforced STAT3 activation, which may account for its constitutive activation in both glioma cells and glioma-infiltrating TAMs.

18.6 Cytomegalovirus in Glioma

Accumulating evidence demonstrating an association between active human CMV infection and malignant glioma has inspired exciting innovations to current treatment strategies. A recent investigation reported the presence of CMV-associated nucleic acids and proteins in over 90 % of *ex vivo* GBM specimens analyzed. Neither HCMV-associated nucleic acids nor proteins were present in surrounding normal brain specimens, and over 80 % of recently diagnosed GBM patients also demonstrated CMV DNA in peripheral blood samples [60]. Though CMV is known to infect 50–80 % of the American population, effective immune control typically limits active disease to the immunosuppressed [61]. It remains unclear if the high prevalence of active CMV infection in glioma patients plays any role in tumor pathogenesis or if tumor growth simply provides an environment permissive of local reactivation and propagation of the virus. Regardless, the presence of CMV in these tumors may be important considering its known potential to modulate growth, invasiveness, and immunological recognition of infected cells (reviewed in [62]). Indeed, active CMV infection has been shown in astrocytes to reduce expression of molecules necessary for antigen presentation, increase the expression of TGF-β and IL-10, and limit the susceptibility of infected cells to apoptotic pathways [63, 64]. Elucidation of the impact CMV virus has on the immunosuppressive phenotypes of CNS tumor cells will require extensive investigation. The presence of viral antigens specifically in tumor cells may allow for tumor cell-specific targeting through the use of CMV antigens in CNS tumor vaccines. If in fact active CMV activation contributes to cellular transformation or malignant behavior, then vaccination strategies against its antigens could additionally provide a functionally disabling therapy toward preventing recurrence.

18.7 Immunoediting in CNS Cancer

As most human CNS tumor analysis is conducted on *ex vivo* specimens acquired from surgical excision following presentation of clinical deficits, the data and conclusions may not be representative of earlier stages of immune system and tumor interaction. Thus, whereas it is possible to study the immunosuppressive environment present in a malignant tumor, the sequence of events that leads to this state remains obscure. The theory of tumor immunoediting has emerged as a paradigm for understanding the dynamics of tumor progression and immunosuppression. The hypothesis proposes three distinct phases: an initial elimination, a period of equilibrium, and, finally, cancer cell immune escape [65] (tumor immunoediting is summarized in Fig. 18.2). Due to genetic instability and rapid proliferation, tumor cells are generated with different immunogenic antigens in a developing tumor. In the initial elimination phase, cytotoxic immune cells target and eliminate those cancer cells that are highly recognizable and lack immune-evasion

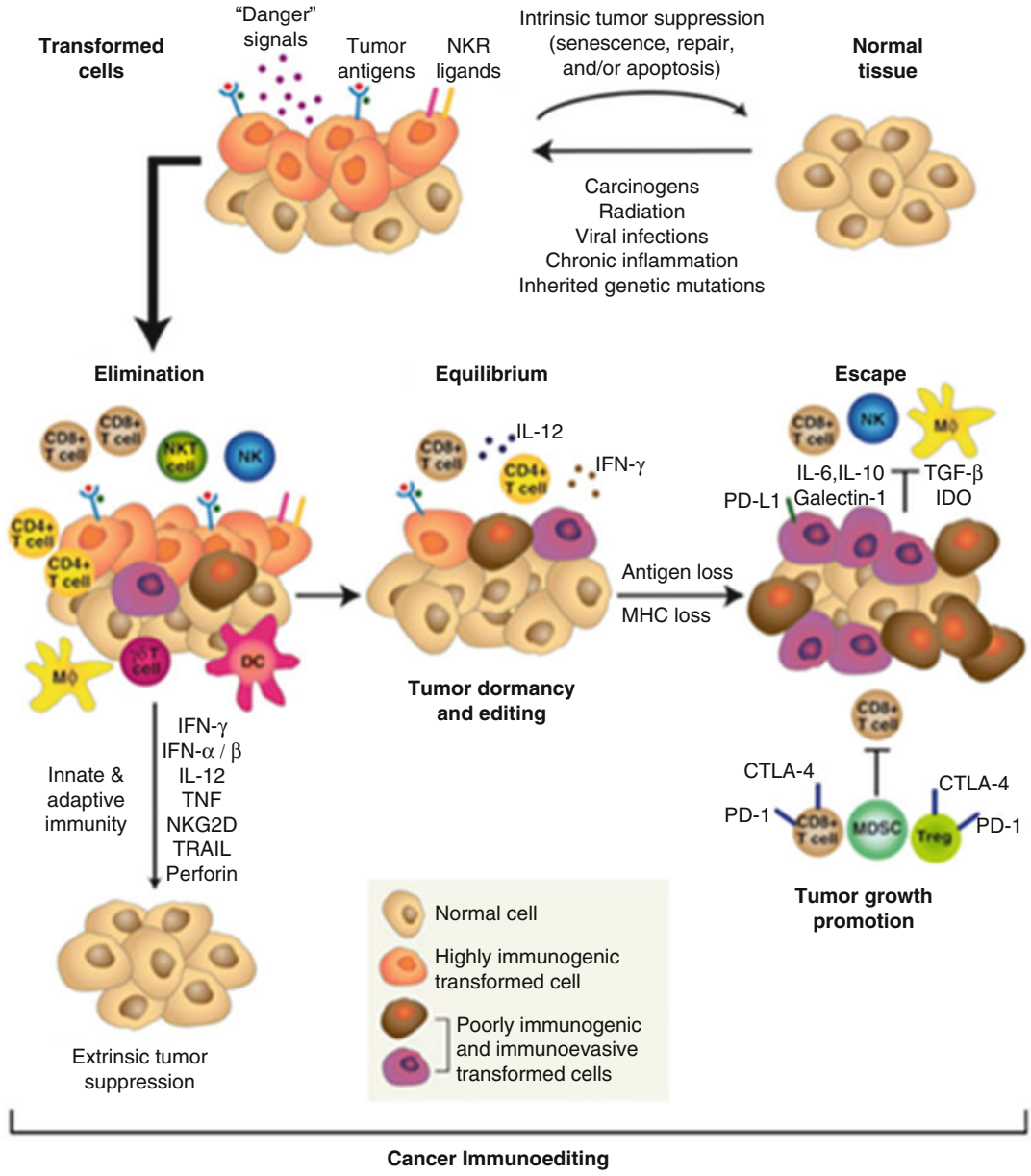


Fig. 18.2 Cancer immunoediting paradigm, highlighting the three proposed phases of immunoediting: elimination, equilibrium, and escape (Reprinted from Schreiber et al. [65], with permission of AAAS)

mechanisms, leading to the selection of poorly immunogenic and/or immunosuppressive tumor cells. Elimination is limited, and some tumor cells are not eradicated, either due to their antigenic or immunosuppressive-related gene expression profile, allowing these cells to survive the initial immune surveillance and enter an equilibrium phase. In this phase, there is a dynamic balance between the antitumoral immunity and tumor cell expansion. During this long phase, there is no clinical tumor burden. The prolonged latency period during equilibrium is thought to constitute an editing state in which neoplastic cells that are susceptible to the host immunity are

eradicated, and those that are not recognized are selected to survive. Finally, the escape phase occurs when those tumor cells that are not detectable or have developed mechanisms to avoid immune recognition are selected and grow into a symptomatic lesion (Fig. 18.2).

Considering the competence of immune surveillance and activation within the CNS, the principles of tumor immunoediting are believed to apply to CNS cancers. Support for the paradigm of immunoediting in CNS cancers comes from few transplant studies, citing the transmission of glioma tumors from liver and kidney organ donors to transplant recipients and from observations in ongoing immunotherapy trials. The first report of this phenomenon involved a 44-year-old woman with primary biliary cirrhosis who received an orthotopic liver transplant from a 14-year-old brain-dead donor with a glial tumor that had infiltrated the pons, pituitary, and spinal cord. Following 9 months of immunosuppression, the recipient developed several liver lesions that appeared histopathologically similar to that of the donor's glial tumor, suggesting immune escape of glioma cells maintained in quiescent immune equilibrium prior to transplantation [65].

A similar report documented two recipients who each received a kidney from a deceased donor with GBM. Both recipients developed renal masses after approximately 18 months, which upon organ removal were pathologically consistent with GBM [66]. Further evidence comes from current GBM vaccine trials (detailed below). Analysis of recurrent GBM specimens following use of a vaccine targeting the highly expressed variant EGFRvIII in GBM demonstrated a paucity of EGFRvIII expression, suggesting successful elimination of the EGFRvIII-expressing cells, followed by equilibrium and subsequent escape of cancer cell subpopulations which did not express EGFRvIII [67]. Ongoing investigation of the dynamic interactions between immune cells and tumor cells throughout the multiphasic progression of CNS tumors will test this theory of immunoediting in CNS cancers and potentially elucidate opportunities to enhance elimination and redirect the eventual failure of equilibrium.

18.8 Immunotherapy

In general terms, the CNS tumor immunotherapy strategies are focused on two goals: to direct the recognition of CNS cancer cells by immune effector cells necessary for a tumoricidal response and to counteract tumor-derived immunosuppression, thus leading to an effective antitumor activation state. A growing appreciation of the necessity for multimodal immune modulation in achieving durable control of CNS tumors through immune-based therapy has led to the combination of both strategies in preclinical and early clinical trials.

In efforts to enhance tumor detection by the immune system, antigen-specific vaccinations and primed dendritic cell-based infusions have both demonstrated promising results. With regard to efforts aimed at disabling immunosuppressive mechanisms, those targeting Tregs and immunomodulatory cytokines have shown preliminary success.

Some authors argue that surgery offers a means for disabling tumor-related immunosuppression by removing the bulk of immunosuppressive cells and mediators within the tumor [44]. Additionally, elimination of the mass effect and edema caused by a large tumor allows for discontinuation of steroids, which confer an iatrogenic immunosuppressive state to the patient. An example of the benefit of resection in the context of immunotherapy has been shown in post-resection GBM patients who, without a significant tumor mass and actively progressing disease, responded better to dendritic cell-based vaccines than did those who had received biopsy alone [68]. For this reason, many recent clinical trials of immune-based therapy in GBM patients are focused on patients who first receive a surgical resection of their tumor.

18.8.1 Adoptive Therapy

Considering the potent tumoricidal properties of activated lymphocyte effector cells, an obvious strategy toward overcoming the *in vivo* hindrances to adaptive immune activation utilizes

infusion of *in vitro* activated autologous lymphocytes back into patients. Lymphokine-activated killer (LAK) cells are populations of autologous peripheral lymphocytes that can be reinfused into tumor-bearing hosts either peripherally or intraoperatively into post-resection surgical cavities following *in vitro* culture in the presence of IL-2 [69]. Multiple phase I clinical trials have investigated LAKs in patients with high-grade gliomas and medulloblastomas (reviewed in [70, 71]. The most promising of these trials included 40 GBM patients treated with intratumoral LAKs and demonstrated a slight but significant increase in median survival in the absence of any toxicity [69]. Unfortunately, additional trials could not reproduce these effects and were further hindered by variant levels of toxicity as the reinfused LAKs demonstrated cytotoxic properties that were not specific to tumor cells. Lower cellular doses of intralesional LAK are under continued investigation as adjuvant treatment of GBM [69].

An extension of LAK strategies to direct more tumor-specific targeting involved the collection of lymphocytes from the lymph nodes or peripheral blood of patients with CNS tumors after peripheral injection of irradiated autologous tumor cells (ATCs) and granulocyte/macrophage colony-stimulating factor (GM-CSF). The harvested lymphocytes were then stimulated *in vitro* with IL-2 and subsequently reintroduced into the tumor-bearing host [72]. Variations in this scheme include additional *ex vivo* exposure to ATCs [72] or tumor-infiltrating lymphocytes isolated from resection specimens [19] during *in vitro* stimulation. Despite reduced toxicity and more objective tumor-specific targeting as compared to LAKs stimulated with IL-2 alone, effects on clinical outcome were minimal across these trials [19, 73].

18.8.2 Vaccination Strategies

Cancer vaccination strategies utilize tumor antigen-driven stimulation of host immune processes to target transformed cells. Cancer vaccines are designed to direct tumor-specific cellular immunity by stimulating the proliferation of high-avidity T cells capable of homing to and selectively attacking transformed cells within a tumor. Some of the major challenges to this strategy include failure of the delivered stimulus to adequately activate T cells, relative lack of tumor-specific antigens that are expressed by a large fraction of tumor cells, nonspecific targeting by stimulated T cells of healthy bystander cells resulting in toxic autoimmunity, and disabling of activated tumor cell-specific T cells by the local microenvironment. To overcome these issues, some vaccination strategies utilize reinfusion of autologous tumor material following *ex vivo* manipulation [74], as well as the use of non-antigen-specific tumor lysate preparations [75]. More recently, purified tumor antigen formulations have also been attempted as direct peptide infusions and as a priming stimulus to DCs prior to their infusion.

18.8.2.1 Autologous Tumor Material

ATCs may be harvested from *ex vivo* tumor resection specimens and used to generate direct CNS vaccination formulations. Subcutaneous or intradermal injection of autologous tumor material is believed to circumvent the immune-disabling tumor microenvironment by providing specific immune-stimulating material to peripheral DCs. Prior to their use in vaccination strategies, this tissue is processed to isolate whole tumor cells, parts of cells, or simply protein extracts and often inactivated by radiation or genetic modification. Eight trials have employed such strategies to treat GBM, including one phase I clinical trial [74], two case reports [76, 77], and five pilot vaccination studies (reviewed in [70]). In three of the pilot studies, processed cells were delivered concomitantly with adjuvant compounds, including IL-2 [76], IL-4 [77], and B7-2 plus GM-CSF infusions [78]; the amount of cells delivered varied across trials.

The induction of an immune response was demonstrated in over half the patients enrolled in each trial, with evidence both in peripheral blood [79] and at the tumor site [80]. Toxicity was minimal and no patient demonstrated severe adverse effects. Furthermore, clinical benefit was demonstrated with nearly 50 % overall survival

across five studies, which recorded three complete responses, four partial responses, two minor responses, and six cases of stable disease in 48 total GBM patients [74, 76–78]. The phase I clinical trial of ATC vaccination included a concomitant infusion of GM-CSF through a programmable pump and effected a significant increase in survival in three of the five patients who demonstrated a postvaccination immune response, out of a total of nine who were treated [74].

18.8.2.2 Dendritic Cell-Based Vaccination Strategies

As discussed, activation of T cells that specifically target brain tumor cells is limited by a reduction in the expression of molecules necessary for effective antigen presentation, including MHC class I/II and co-stimulatory molecules [28]. To overcome this limitation, DCs from patients with malignant brain tumors may be extracted, activated *in vitro* with tumor-derived antigens favoring APC maturation, and reintroduced as potent activators of tumor-specific T cells. This approach can lead to the generation of tumor-specific T-helper cells (Th) capable of altering the composition of the microenvironment through expression of immune-activating mediators and, subsequently, the activation of CTLs and NK cells capable of selectively eliminating tumor cells. Furthermore, the generation of memory T cells following introduction of tumor antigen-primed DCs presents the potential for lasting immunity to counter the recurrent proliferation of residual cancer cells. Indeed, coculture of glioma-associated antigen-primed DCs with undifferentiated lymphocytes has been shown to induce activation of T cells and subsequently T-cell cytotoxicity when autologous glioma cells were introduced [81, 82]. Furthermore, a robust cytotoxic (CTL) and memory T-cell lymphocyte infiltration into intracranial tumors was observed in murine models of glioma following vaccination and peripheral infusion of tumor antigen-primed DCs, favoring a Th1 lymphocyte activation state, capable of homing to and expanding within tumor tissue [83].

Though many investigative protocols for DC-based vaccination of malignant glioma differ with regard to protocol specifics, most involve extraction of DC precursors in the form of peripheral blood mononuclear cells (PBMCs); exposure to tumor-associated formulation in the presence of GM-CSF and IL-4, both known to direct APC maturation; and reintroduction though subcutaneous, intradermal, intranodal, or intratumoral injection.

The mechanism by which tumor-associated antigens are loaded *in vitro* into DCs is of critical importance. Multiple DC-loading strategies have been employed, including the use of autologous tumor lysates, formulations of apoptotic material following ATC irradiation, and purified or synthetic tumor-associated peptide antigens [84–86].

A potential advantage of loading strategies which do not isolate individual antigens, such as the use of autologous tumor lysates, is the induction of an immune response against multiple tumor epitopes, though likely at the expense of non-tumor-specific cross-reactivity and subsequent autoimmune toxicity. Those strategies using distinct and tumor-specific antigens, either purified or synthetic, limit the activation of cross-reactive lymphocytes, allowing for the escape of non-expressing clonal populations.

To date, 15 clinical trials including 316 total patients have evaluated the use of DC-based vaccination in the treatment of malignant gliomas including primary and recurrent GBM, anaplastic astrocytoma (AA), anaplastic oligoastrocytoma (AOA), and anaplastic oligodendroglioma (AO) (reviewed in [70]): eight phase I trials, six phase I/II trials, and one phase II trial. Table 18.1 summarizes the vaccination details and clinical results of these trials. Across all included in these trials, only one patient suffered grade IV neurotoxicity resulting from a large residual tumor and perilesional edema [90], highlighting the safety and feasibility of antigen-primed DC vaccinations for CNS cancers. Immune response was largely evaluated by delayed-type hypersensitivity (DTH); increased proportions of CTLs, NKs, and memory T cells both in peripheral blood and as infiltrating lymphocytes in subsequent tumor resections; increased tumor cell reactivity of postvaccination extracted PBMCs exposed to

Table 18.1 List of clinical trials utilizing dendritic cell vaccinations in patients with malignant gliomas

Patients	Phase	Route	Antigen format	Immune response	Clinical response	References
22 patients (13 recurrent GBM, 5 AA, 3 AO, 1 AOA	Phase I/II	Intranodal + intramuscular injections of poly-ICLC	Synthetic peptides for GAAs	Induced positive immune responses against at least one of the GAAs in PBMCs in 58 % of patients (after 4 vaccinations). Significant upregulation: interferon-alpha and CXCL10	4 recurrent GBMs are progression-free for at least 12 months; 1 CR (recurrent GBM). Median TTP, 4 months	Okada (2011)
23 patients (15 newly diagnosed GBM, 8 recurrent GBM)	Phase I	Intradermal + intramuscular injections of poly-ICLC	Autologous tumor lysate + imiquimod or poly-ICLC	No dose-limiting toxicity. Tumor samples with a mesenchymal gene expression signature had a higher number of CD3+ and CD8+ tumor-infiltrating lymphocytes	Newly diagnosed: median OS, 35.9 months, with a mean follow-up time of more than 4 years and 1-, 2-, and 3-year survival rates of 93, 77, and 58 %, respectively. Recurrent: median OS was 17.9 months from the time of initial glioblastoma diagnosis. OS was significantly longer for those who received DC vaccination at initial diagnosis compared with those who enrolled in this trial at the time or recurrence	Prins (2011)
8 patients (newly diagnosed GBM)	Phase I/II	Intradermal	Autologous tumor lysate	DTH (2/5) increased CD8+/CD25+ in PBL (6/7) ATR PBMC (5/8) (IFN-gamma ELISPOT)	Median OS, 24 months	Ardon (2010)
45 children (23 recurrent GBM, 5 AA, 1 AOA, 16 other HGG)	Phase I	Intradermal	Autologous tumor lysate + imiquimod	No data available	Median PFS for relapsed GBM, 4.3 months; median OS for relapsed GBM, 12.2 months	Ardon (2010)
12 patients (newly diagnosed GBM)	Phase I	Intradermal	EGFRvIII antigen + KLH	DTH EGFRvIII (5/9); DTH KLH (9/9); ATR PBMC (10/12) (EGFRvIII-induced proliferation)	Median OS, 22.8 months	Sampson (2009)

(continued)

Table 18.1 (continued)

Patients	Phase	Route	Antigen format	Immune response	Clinical response	References
56 patients (recurrent GBM)	Phase I/II	Intradermal	Autologous tumor lysate	DTH (9/21 at time of diagnosis, 9/17 after 2 vaccinations)	3-month PFS; OS, 9 months; 24-month OS, 14.8 %; total resection is a predictor for better PFS; younger age and total resection are predictors for better OS	De Vleeschouwer (2008)
34 patients (23 recurrent GBM, 11 newly diagnosed GBM)	Phase II	Subcutaneous	Autologous tumor lysate	Postvaccine antigen-directed IFN-gamma response in PBMCs (17/34); DTH test resulted in cutaneous GBM in 1 patient (DTH was subsequently discontinued)	Newly diagnosed: 8/17 (47 %) vaccine responders versus 3/15 (20 %) nonresponders. Recurrent: TTS, 621 ± 81 and 402 ± 49 days; TTP, 28 ±94 and 142 ± 22 days (8 responders and 13 nonresponders); TTP, 343 ± 116 and 136 ± 19 days (8 responders and 15 nonresponders)	Wheeler (2008)
24 patients (18 recurrent GBM, 6 grade III glioma)	Phase I/II	Intradermal or intradermal + intratumoral (Ommaya reservoir)	Autologous tumor lysate	DTH to tumor lysate (8/24); ATR PBMC (7/24) (IFN-gamma ELISPOT)	1 PR, 3 MR, 6 SD (GBM); 4 SD (grade III glioma); median OS, 16 months versus 13.3 months; longer survival if DC maturation or IC injection. One grade IV neurotoxicity event (stupor) observed	Yamanaka (2005)
12 patients (7 newly diagnosed GBM, 5 recurrent GBM)	Phase I	Intradermal	Acid-eluted tumor-associated peptides	CTL response (6/12); tumor infiltration CD8+ CD45RO+ cells (4/8)	Median TTP, 19.9 months, OS 18 to >58 months; median OS, 25.8 months. 1PR; median OS, 23.4 versus 18.3 months. No dose-limiting toxicity observed	Liau (2005)
14 patients (1 newly diagnosed GBM, 9 recurrent GBM, 4 AA)	Phase I	Subcutaneous	Autologous tumor lysate	Increased IFN-gamma RNA in PBMC (6.10) ATR T cells (4/9) (HER-2, GP100, MAGE-1 tetramers); CD8+, CD45RO+ cell infiltration (3.6)	Median survival, 33.3 versus 7.5 months (8/9 recurrent GBM)	Yu (2004)

15 patients (6 recurrent GBM, 7 AA, 2 AOA)	Phase I	Intradermal	DC fusion with autologous glioma cells	DTH to tumor lysate (15/15); increased cytotoxic activity (2/15); increased intracellular IFN-gamma in CD8+ T cells (1/15)	1 SD (GBM); 3 PR, 1 MR (AA); 1 PR, 1 SD (AOA)	Kikuchi (2004)
7 patients (2 recurrent GBM, 1 AA, 4 other HGG)	Phase I	Intradermal	Autologous tumor RNA	No antitumor responses (0/3) (IFN-gamma ELISA)	1 PR (1XA); 4 SD (1AA, 3 other HGG)	Caruso (2004)
25 patients (newly diagnosed GBM: 13 plus chemotherapy, 12 without chemotherapy)	Phase I/II	Intradermal	Autologous tumor lysates or peptide elutions	Vaccine alone: ATR PBMC (4/11) vaccine + chemotherapy: ATR PBMC (4/13) (lytic activity and IFN-gamma PCR)	Vaccine or chemotherapy alone: 24-month survival 8 %; vaccine + chemotherapy, 3 PR; 24-month survival 42 %	Wheeler (2004)
10 patients (7 recurrent GBM after radiotherapy, 3 recurrent grade III glioma)	Phase I/II	Intradermal and/or intratumoral (Ommaya)	Autologous tumor lysate	Increase in NK cells in PBMCs (5/10); DTH to tumor lysate (3/10); increased T-cell-mediated antitumor activity (2/10)	2 MR, 2SD (GBM), 2SD (grade III glioma); OS >50 months	Yamanaka (2003)
9 patients (7 newly diagnosed GBM, 2 AA after radiotherapy)	Phase I	Subcutaneous	Tumor-specific MHC I-associated peptides	Systemic CTL cytotoxicity against tumor (4/9) (lytic activity); tumor infiltration: CD4+, CD8+, CD45RO+ cells (2/4)	Prolonged median survival compared to control group: 15.2 versus 8.6 months (GBM)	Yu (2001)

AA anaplastic astrocytoma, *AO* anaplastic oligodendroglioma, *AOA* anaplastic oligoastrocytoma, *ATR* antitumor responses, *CR* complete response, *DTH* delayed-type hypersensitivity, *GAA* glioblastoma-associated antigen, *GBM* glioblastoma multiforme, *HGG* high-grade glioma, *KLH* keyhole limpet hemocyanin, *MR* minor response, *OS* overall survival, *PBMC* peripheral blood mononuclear cells, *PFS* progression-free survival, *PR* partial response, *PXA* pleomorphic xanthoastrocytoma, *SD* stable disease, *TMZ* temozolomide, *TTP* time to tumor progression, *TTS* time to tumor survival, *XA* xanthoastrocytoma

ATC *in vitro*; and increased presence of IFN-γ both peripherally and within the tumor-infiltrating lymphocytes. Over half of the patients enrolled in these trials demonstrated some evidence of an immune response following vaccination, and all 15 studies reported a survival benefit following vaccination (Table 18.1). Moreover, two of these trials focusing on patients with GBM demonstrated an improved response to chemotherapy delivered in a second phase following DC-based vaccination, suggesting an exciting potential for synergy with these treatments [85, 96].

Despite the variability across these trials, salient insights include the safety of DC-based approaches to CNS tumor vaccination and the feasibility of these immune strategies as some features of an elicited immune response were demonstrated in over half of all patients enrolled. Other interesting results include the improved success of matured DC vaccinations generated by combining antigen priming with maturation factors such as TNF-α, Toll-like receptor (TLR) ligands, or IFN-γ and the potentially synergistic effect of DC-based vaccination and chemotherapy in treating brain tumors. Details regarding precise protocols for loading of DCs, amount and site of injection, and composition of accompanying adjuvants remain to be optimized.

An additional trial evaluated the use of DC-based immunotherapy in 45 pediatric patients with high-grade glioma, medulloblastoma, primitive neuroectodermal tumors (PNETs), ependymoma, and atypical teratoid-rhabdoid tumors (ATRTs) [97]. The authors utilized autologous tumor lysates to load PBMC-derived DCs and delivered these by intradermal injections followed by two subsequent boost vaccinations of tumor lysate. No severe adverse effects occurred in those patients with high-grade gliomas and ATRTs, and additionally, overall survival was increased compared to historical controls in those two tumor types. In those patients with PNET and medulloblastoma tumors, vaccinations were discontinued due to adverse effects. These findings show the potential of the DC-based immunotherapy to pediatric brain tumors, but data regarding efficacy remains preliminary and poorly controlled.

The optimization of tumor-associated antigen-loading strategies is under active exploration. A recent study compared specific antigenic peptide-loaded *vs.* autologous tumor lysate-loaded DC vaccines for treating malignant glioma [98].

Twenty-eight patients were treated with autologous tumor lysate-pulsed DC vaccines, whereas six patients were treated with glioma-associated antigen peptide-pulsed DCs, utilizing a synthetic formulation of four epitopes known to be expressed on malignant gliomas. These antigens included survivin, HER-2/neu, gp100, and TRP-2, which are present in approximately 60, 80, 60, and 50 % of malignant gliomas specimens, respectively [27]. No adverse events were reported in either study group. The median survival of patients on the autologous tumor lysate-DC trial was 34.4 months, whereas that of patients on the synthetic glioma-associated antigen-DC group was significantly different with a median survival of 14.5 months [27]. Though limited to small cohorts under individual protocols, these results support the use of autologous tumor lysate preparation in priming DCs for vaccination in CNS cancer. The authors also noted a significant correlation between decreased Treg ratios (pre- *vs.* postvaccination) and overall survival, evident in both study groups.

18.8.2.3 Antigen-Specific Peptide Strategies

In contrast to the DC-based techniques discussed above, direct peptide vaccines rely on the ability of host APCs in the periphery to process, migrate, and present the introduced antigens. Extensive preclinical analysis has demonstrated the ability of peripheral APCs to activate T cells within lymph nodes regional to the site of injection in animal models of brain tumors [70, 99]. Refinements to direct antigen vaccination strategies have demonstrated the utility of adjuvant compounds such as keyhole limpet hemocyanin (KHL) as an immunogenic peptide carrier protein [100] and GM-CSF as a mitogenic stimulus for APCs [101], both of which ultimately augment antigen presentation.

The selection of tumor-associated peptides to enable selective tumor cell targeting with minimal secondary autoimmunity is critical to the

success of any vaccination utilizing target peptide sequences, both in direct peptide injection and in specific antigen-primed DC infusion. Considerable effort has been expended in identifying antigens differentially or exclusively expressed in CNS tumors, including genes only normally expressed during embryological development, differently spliced or mutated genes, and genes giving rise to fusion proteins, which result from the general genetic instability of transformed cells, as well as housekeeping or metabolic pathway antigens which may be exclusive to tumor cells [90]. Nevertheless, an increasing appreciation of intratumor clonal heterogeneity [102] complicates effective targeting of a clinically significant proportion of tumor cells through a specific antigen vaccination strategy.

The National Cancer Institute (NCI) recently performed an in-depth review of 75 general tumor-associated antigens to evaluate their potential as targets for immunotherapy [103]. The potential of tumor antigens to serve as targets for immunotherapy was graded according to the following criteria: therapeutic function, immunogenicity, oncogenic function, specificity, expression level in tumors, in cancer stem cells, percentage of tumors that express it and cellular localization of the protein. Based on this, the antigens that showed the most potential for immunotherapy where. The highest-ranked antigens included WT-1, MUC1, LMP2, HPV E6/E7, HER2/neu, EGFRvIII, melanoma antigen-encoding (MAGE)-A3, and NY-ESO-1. While expression of some of these antigens in CNS cancer is well established, such as the expression of EGFRvIII in GBM [104], the presence or absence of others in CNS cancers warrants future investigation. Additional insight into CNS cancer-specific antigen targets for immune-based therapy has come from tumor antigen investigations in melanoma [105]. The genes MAGE-1 [106], MAGE-E1 [107], MAGE-3, and glycoprotein-240 (a cell surface glycoprotein of 240,000 molecular weight present in most melanomas) [108] were expressed in many different glioma subtypes but never in normal brain tissue and therefore present as potential targets for CNS tumor-specific immunotherapy. Many additional CNS cancer-associated antigens have been described as potential tumor-selective targets for immunotherapy in a variety of CNS tumor types; these include but are not limited to tenascin, homo sapiens testis (HOM-TES)-14 (also known as stromal cell-derived protein (SCP)-1), HOM-TES-85, synovial sarcoma X chromosome breakpoint (SSX)-1, SSX-2, GAGE-1, SRY-related high-mobility group (HMG)-box-containing gene (SOX)-5, cancer testis antigen 6, IL-13 receptor a2, ephrin (Eph) A2, antigen isolated from immunoselected melanoma (AIM)-2, squamous cell carcinoma antigen recognized by T cells (SART)1, SART3, and kinesin superfamily protein (KIF)1C and KIF3C [70]. See Table 18.2 for a list of GBM-associated antigens under preclinical or clinical investigation in tumor vaccines. Ongoing effort to characterize these many relevant antigens in various CNS cancer subtypes will hopefully yield firm footing of which to launch future antigen-specific immune-based therapies.

Following promising preclinical results, a number of clinical trials utilizing direct vaccination with some of the aforementioned peptides are currently underway for cancers of the CNS. Among those under most active vaccine developments are the IL-13 receptor a2 (IL-13Ra) [73, 113] and EphA2 [109, 142] though disproportionate attention and success have come from vaccination strategies against EGFRvIII. A potent mitogenic signaling motif, stimulation of the EGF receptor, is believed to play a significant role in the development of malignant glioma; approximately 50–60 % of glial tumors overexpress EGFR, and 24–67 % express the most commonly mutated form, EGFRvIII [143]. The functional relevance of EGFRvIII for malignant gliomas is also suggested by the fact that presence of EGFRvIII is associated with reduced survival on multivariate analysis [144] and also may confer malignant cells with resistance to radiation and chemotherapy [145]. The amino acid sequence obtained from the fusion of the remaining exons 1 and 8 includes the addition of a glycine residue at the junctional site (LEEKKGNYVVTDH), rendering a totally novel peptide [143]. Consequently, the resultant protein is unique to glioma cells and therefore may allow for the generation of an immune response which does not cross-react with wild-

Table 18.2 List of glioma-associated antigens (GAAs) which may serve as immunotherapeutic targets

GAA	Characteristic/potential function	References
AIM2: absent in melanoma 2	AIM2 could be used as a tumor antigen target for monitoring vaccine trials or for developing antigen-specific active immunotherapy for glioma patients	Okada (2009), Liu (2004)
BMI1: BMI1 polycomb ring finger oncogene	Expressed in human GBM tumors and highly enriched in CD133+ GSC cells	Abdough (2009)
COX-2: cyclooxygenase-2	Overexpressed in many tumors including CD133+ GSC cells; COX-2 inhibitor celecoxib will become a nice weapon for GBM therapy	Shono (2001)
TRP-2: tyrosinase-related protein 2	Highly expressed in GSCs	Saikali (2007), Driggers (2010)
GP100: glycoprotein 100	Melanocyte lineage-specific antigen, expressed in GSCs as well	Saikali (2007), Driggers (2010)
EGFRvIII: epidermal growth factor receptor variant III	EGFRvIII is the most prevalent of several EGFR mutations found in human gliomas and is expressed in 20–25 % of GBM. GSC-associated antigen	Saikali (2007), Driggers (2010)
EZH2: enhancer of zeste homologue 2	Upregulated in malignant gliomas in GSC cells	Orzan (2011)
LICAM: human L1 cell adhesion molecule	Highly expressed in GSCs. Invasion-associated proteins	Cheng (2011)
Livin and Livin β	Livin β was more related with the high survival rate. It is a cancer-associated member of the inhibitor of apoptosis protein (IAP)	Jin (2010)
MRP-3: multidrug-resistance protein 3	GBMs overexpress MRP-3 at both mRNA and protein levels. Multidrug-resistance protein 3 has potential correlation with survival. Highly expressed in GSC cells as well	Driggers (2010), Kuan (2010)
Nestin	Nestin plays important roles in cell growth, migration, invasion, and adhesion to extracellular matrices in glioma cells. Overexpressed in GSCs	Ishiwata (2011)
OLIG2: oligodendrocyte transcription factor 2	GSC marker, OLIG2 is highly expressed in all diffuse gliomas. Immunohistochemistry and microarray analyses demonstrated higher OLIG2 in anaplastic oligodendrogliomas versus glioblastomas, which are heterogeneous with respect to OLIG2 levels	Ligon (2004)
SOX2: SRY-related HMG-box 2	SOX2 expression and amplification in gliomas and GSC cell lines	Xu (2009)
ART1: antigen recognized by T cells 1	Pediatric GBM express ART1, ART4, SART1, SART2, and SART3; they were identified within GBM cell lines as well	Zhang (2008), Driggers (2010)
ART4: antigen recognized by T cells 4		
SART1: squamous cell carcinoma antigen recognized by T cells 1		
SART2: squamous cell carcinoma antigen recognized by T cells 2		
SART3: squamous cell carcinoma antigen recognized by T cells 3		
β-Catenin	β-Catenin and Gli 1 are prognostic markers in GBM	Rossi (2011)
Gli 1: glioma-associated oncogene homologue 1	Gli 1 is correlated with glioma recurrence after chemotherapy; Gli 1 plays a dominant role in chemoresistance of glioma cells. Located nuclear, might be fluctuating between the cytoplasm and the nucleus	Rossi (2011), Cui (2010)
Cav-1: caveolin-1	Expressed in most HGG, correlated with proliferation and invasive potential of tumor	Senetta (2011)

Table 18.2 (continued)

GAA	Characteristic/potential function	References
Cathepsin B	Overexpression of cathepsin B during the progression of human gliomas	Sivaprvathi (1995)
CD74: cluster of differentiation 74	Contribute to TMZ resistance. Also known as HLA class II histocompatibility antigen gamma chain	Kitange (2010)
E-cadherin: epithelial calcium-dependent adhesion	Expression in gliomas correlated with an unfavorable clinic outcome	Lewis-Tuffin (2010)
EphA2/Eck: EPH receptor A2/ epithelial cell kinase	Overexpressed in both pediatric and adult GBM. Used as a novel target for glioma vaccines	Okada (2009), Driggers (2010)
Fra-1/Fosl 1: Fos-related antigen 1	Plays an important role in maintenance/progression of various cancers, including GBM. Highly expressed in pediatric GBM	Driggers (2010), Wykosky (2008)
GAGE-1: G antigen 1	A potential target for specific immunotherapy and diagnostic markers in high-grade brain tumors	Scarcella (1999)
Ganglioside/GD2	Expression in astrocytic tumors	Mennel (2005)
GnT-V, β 1, 6-N: acetylglucosaminyltransferase-V	Plays an important role in regulating invasivity of human glioma	Driggers (2010), Yamamoto (2000)
Her2/neu: human epidermal growth factor receptor 2	A tumor-associated antigen that is expressed by up to 80 % of GBMs but not by normal postnatal neurons or glia	Driggers (2010), Xu (2009)
Ki67: nuclear proliferation-associated antigen of antibody Ki67	Prognostic marker for glioma, especially for the lower grades	Kogiku (2008)
Ku70/80: human Ku heterodimer protein subunits (molecular weight: 70 kDa/80 kDa)	A therapeutic potential target antigen. Highly expressed in GBM	Persson (2010)
IL-13Ralpha2: interleukin-13 receptor subunit alpha-2	Overexpressed in GBM but diminished in several GSC cell lines	Saikali (2007), Driggers (2010)
MAGE-A: melanoma-associated antigen 1	MAGE-A1, MAGE-A3, and NY-ESO-1 can be upregulated in neuroblastoma cells to facilitate cytotoxic MAGE-A3: melanoma-associated antigen 3 T-lymphocyte-mediated tumor cell killing	Bao (2011)
MAGE-A3: melanoma-associated antigen 3		
NY-ESO-1: New York esophageal squamous cell carcinoma 1		
MART-1: melanoma antigen recognized by T cells	Melanoma antigen also associated with glioma	Jian (2007)
PROX1: prospero homeobox protein 1	Strongly expressed in GBM, frequently coexpress early neuronal proteins MAP2 and β III-tubulin but not the mature neuronal marker NeuN	Elsir (2010)
PSCA: prostate stem cell antigen	GPI-anchored cell surface protein, represented as a novel GAA	Geiger (2011)
SOX10: SRY-related HMG-box 10	The SOX10 expression was restricted to gliomas and melanomas. All glioma types expressed SOX10, and tumors of low-grade glioma had a much broader distribution of SOX10 compared with high-grade gliomas	Ferletta (2007)
SOX11: SRY-related HMG-box 11	The transcription factor SOX11 is with highly specific overexpression in human malignant gliomas	Schmitz (2007), Driggers (2010)
Survivin	Quantitatively determined survivin expression levels are of prognostic value in human gliomas	Kogiku (2008), Driggers (2010)
UPAR: urokinase-type plasminogen activator receptor	UPAR and cathepsin B, known to be overexpressed in high-grade gliomas and strongly correlated with invasive cancer phenotypes	Gondi (2004)
WT-1: Wilms' tumor protein 1	A transcription factor overexpressed in glioma	Ueda (2007)

GBM glioblastoma, *TMZ* temozolomide, *GSC* glioma stem cells

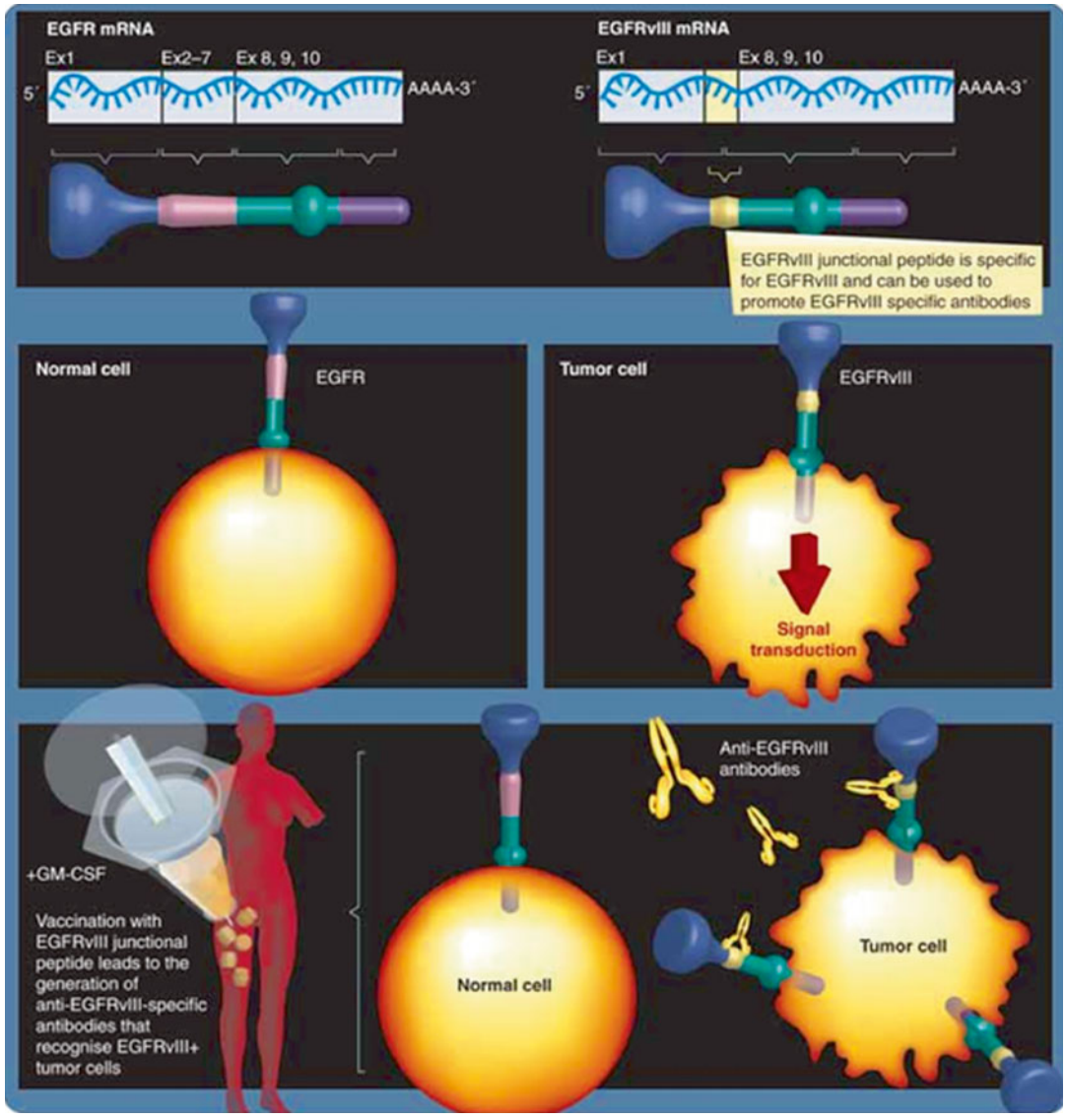


Fig. 18.3 Schematic of EGFRvIII-targeted vaccination. Notice the formation of EGFRvIII-specific antibodies, which selectively target tumor cells (Reprinted from Sonabend et al. [54], with permission)

type EGFR protein. See Fig. 18.3 for a synopsis of a vaccination strategy developed to target EGFRvIII.

An early phase I trial to evaluate the safety of GBM vaccination against EGFRvIII was performed in which treatment with an intradermal KLH-conjugated EGFRvIII-based peptide (PEPvIII) was utilized. No serious adverse effects were reported, and immunological responses were detected *ex vivo* [12]. Subsequently, a multicenter phase II trial, entitled "A Complimentary Trial of an Immunotherapy Against Tumor Specific EGFRvIII" (ACTIVATE), was performed, in which 19 patients with GBM were treated with PEPvIII and adjuvant GM-CSF following tumor resection and standard radiation plus chemotherapy [146]. Importantly, EGFR amplification and EGFRvIII expression were not criteria for enrollment. No patient experienced adverse effects aside from local injection site

reactions, and both humoral and delayed-type hypersensitivity immune responses specific to EGFRvIII were observed in the majority [147]. Furthermore, in the patients who demonstrated an immune response to vaccination, median time to tumor progression (TTP) and overall survival were significantly increased when compared to historical controls (TTP 12 months *vs.* 7.1 months, $p<0.05$, with a median survival of over 32 months *vs.* 14 months in historical controls $p<0.01$). Interestingly, histological analysis of recurrent tumor specimens revealed complete absence of EGFRvIII expression in all patients demonstrating an immune response. Though pretreatment EGFRvIII expression was not published, this finding may suggest successful targeting of antigen-bearing tumor cells.

The promising results of this trial were further extended in a subsequent phase II trial which enrolled 21 GBM patients in a similar KLH-conjugated PEPvIII plus GM-CSF vaccination schedule, concurrent with two different temozolomide (TMZ) chemotherapeutic dosing schedules [148].

Although grade II TMZ-associated lymphopenia was observed in nearly all treated patients, immune responses specific to EGFRvIII were documented in the majority of patients. Unexpectedly, antigen-specific immune responses were observed to be either sustained or enhanced with successive TMZ treatments. Follow-up investigations, including an ongoing randomized phase III clinical trial, hope to validate this observation and to further elucidate optimal vaccination regimens. The possibility of synergy between immunotherapy and chemotherapy in CNS cancer is promising. Together, these trials suggest that vaccination with a peptide containing an EGFRvIII tumor epitope safely elicits a specific immune response against EGFRvIII and that this approach might be effective against cancers bearing the variant antigen.

18.8.2.4 Heat Shock Protein Peptide Complex 96

An exciting new frontier of immune-based therapy for CNS cancers involves the use of heat shock protein peptide complexes (HSPPCs). HSPs are known to lead to "chaperone" protein folding and protein-protein interactions and are unregulated in states of cellular stress [149]. Certain HSPs have been shown to play instrumental roles in the delivery and intracellular processing of antigens in APCs and are therefore an attractive target for exploitation in immunotherapy [150]. HSPPC-96 is composed of HSP gp-96 and a wide array of bound chaperoned proteins, including antigenic peptides. This protein complex can be easily purified from solid tumor specimens of patients with a variety of solid tumor types [151]. Immunization strategies with HSPPC-96 work by interacting with APCs via specific receptors, including CD91 [152]. After binding to CD91, the HSPPC-96 complex is internalized, and the chaperoned peptides are presented by class I and class II MHC molecules. The highly specific nature of the interaction between HSPPC-96 and APCs may present an advantage over the aforementioned vaccine approaches and has been shown to facilitate robust T-helper cell and CTL immune responses [153].

Vaccination with HSPPC-96 was recently extended to patients with CNS tumors: 12 patients with recurrent high-grade gliomas were treated with autologous HSPPC-96 vaccines derived from resected tumor tissue [154]. No toxicity attributable to HSPPC-96 was observed in any of the 12 patients treated. In 11 of the 12 patients, a significant immune response was demonstrated, as indicated by robust activation of peripheral blood leukocytes isolated postvaccination, when exposed to antigenic peptides carried on the HSPPC-96 complex (gp-96). Vaccination led to a significant increase in IFN-γ expression as compared to peripheral blood leukocytes isolated prior to vaccination. Furthermore, an increase in IFN-γ-positive T-helper cells, CTL, and NK cells accompanied a decrease in Tregs in biopsy specimens from all 11 patients who responded, whereas these findings were not observed in the one patient who did not respond. The 11 responders had an overall survival of 47 weeks compared with 16 weeks in the one non-responder. Collectively, these results suggest the safety, feasibility, and potential therapeutic benefit of autologous HSPPC-96 vaccination in patients with high-grade gliomas.

18.8.3 Immunotherapy Targeting CNS Cancer-Induced Immunosuppression

In addition to directed activation of immune mediators against tumor-specific antigens, parallel efforts to counteract CNS cancer-induced immunosuppression have gained attention. A comprehensive inventory of ongoing efforts is beyond the scope of this chapter, but cytokine therapy and antibody-mediated neutralization of Tregs will be discussed as two notable examples of this therapeutic principle.

As key transmitters of cellular communication, cytokines are known to play predominant roles both in proper immune cell activation schemes and in the irregular immunosuppressive milieu of the CNS tumor microenvironment. Cytokines direct the phenotypic fate of stimulated monocytes and lymphocytes and are involved in signaling exchanges upon encountering pathogenic or neoplastic stimuli. Thought to precipitate escape from immune equilibrium, the aberrant cytokine expression profile of transformed CNS cells eventually acts to alter the cytokine expression profiles of resident (microglia) and infiltrating (monocytes) myeloid cells, and subsequently lymphocytes, which thereafter collude to construct potent local immunosuppression. The therapeutic introduction or inhibition of immune-modulating cytokines is hypothesized to reorient M2 TAMs back to tumoricidal effector phenotypes [24]. Among the cytokines identified for such efforts, TGF-β, IL-2, IL-4 [41, 155], IL-12 [156, 157], and IFN-γ [158, 159] have received the most attention, both as principal treatment and as adjuvants in combination with the antigen-based strategies discussed earlier.

As discussed above, TGF-β plays a prominent role in the multiple pathways implicated in CNS tumor-induced immunosuppression, proliferation, angiogenesis, and invasion-permitting stromal remodeling. TGF-β expression is observed to increase following radiation treatment in both in vitro [160] and in vivo [161], raising the possibility of a therapeutic benefit derived from TGF-β modulation in conjunction with radiation treatments in patients with GBM. Trabedersen (AP12009) is an antisense molecule consisting of 18 DNA oligonucleotides which specifically targets TGF-β2 mRNA, inhibiting its protein synthesis [162]. Trabedersen's utility in AA was recently investigated in a phase IIb clinical trial which reported significantly improved 14-month tumor control rates evaluated by the presence of recurrent tumor on MRI ($p<0.05$) when compared to standard chemotherapy [152]. Overall, patients with GBM in this trial did not demonstrate the same tumor control benefit, though a subgroup analysis of young GBM patients with good performance status did suggest a trend toward improved 2- and 3-year survival ($p=0.08$). Trabedersen is currently in phase III clinical trials for treatment of AA [155].

IL-2 is known to be an essential stimulus for the proliferation and differentiation of both Th type 1 cells and CTL following TCR antigen recognition, and it has been shown to abrogate the immunosuppressive effects of TGF-β [163]. Commonly used to stimulate the expansion and maturation of PBMCs in the development of LAK, multiple investigators have attempted to use IL-2 as an immunotherapeutic agent in CNS cancer. Early clinical trials with high-dose IL-2 delivered intratumorally or intraventricularly were discontinued on account of significant adverse effects resulting from local edema [164]. An *IL-2* transgene was delivered into the tumors of 12 patients with recurrent GBM, followed by systemic treatment with acyclovir. In this trial, a retroviral vector was used as a vehicle for IL-2 and herpes simplex virus thymidine kinase (HSV-tk), which helped the selective elimination of infected cells with acyclovir [164]. None of these patients demonstrated adverse effects to treatment, and although no complete response was recorded, two experienced a partial response, four a minor response, and four stable disease. Additionally, expression analysis on posttreatment biopsies in three of the patients with a partial or minor response demonstrated increased expression of TNF-α, IFN-γ, IL-2, IL-1B, and IL-10, suggesting the induction of a local Th1 immune response. Together, these findings suggest that local IL-2 transgene delivery may be a safe and at least a modestly effective therapeutic

strategy for further development in CNS cancer. Efforts to integrate IL-2 into combination strategies for the treatment of CNS cancers are ongoing [165].

The immunotherapeutic potential of IL-2 manipulation extends further through its impact on the potently immunosuppressive Tregs. Defined by a set of constitutively expressed antigens which include the high-affinity IL-2 receptor alpha chain (IL-2Rα/CD25), Tregs may be selectively targeted and functionally impaired by interventions specific for this component of the IL-2 receptor complex. Indeed, blockage of the IL-2Rα in murine models of glioma was observed to deactivate Treg-induced suppression through functional inhibition as well as depletion [165, 166]. Still, as discussed above, IL-2-mediated stimulation of the IL-2 receptor (heterotrimer of α, β, γ chains) is necessary for the proliferation and differentiation of Th1 cells and CTLs, particularly following the administration of therapeutic vaccines designed to stimulate a tumor antigen-specific lymphocyte response. Thus, blockage of IL-2 signaling, though it may hinder the expansion of immunosuppressive Treg, may also limit the production of tumoricidal immune effector cells and therefore be of limited benefit.

Initial investigations into the use of the humanized anti-IL-2Rα monoclonal antibody (mAb) daclizumab for the treatment of malignant melanoma demonstrated this suspicion. Though tumor infiltration and peripheral Treg populations were effectively depleted, the functionality of vaccine-induced tumor-specific T cells was impaired, and the formation of vaccine-induced humoral immunity was minimal [167]. Still, attempts to use daclizumab in the treatment of malignant cancers were not abandoned, largely as a result of the observation of different IL-2 signaling responses in Tregs as compared to mature effector T cells during times of lymphopenia, such as is induced by chemotherapy. A preclinical investigation attempting to exploit this discrepancy delivered daclizumab during TMZ-induced lymphopenia in a murine model of glioma and demonstrated an effective depletion of Treg populations while sparing tumor-specific vaccine-activated effector cells [60]. The authors additionally reported an increased reduction in tumor growth in the vaccinated mice given daclizumab after TMZ as compared to those treated with TMZ followed by vaccination alone. These encouraging findings were extended in a pilot clinical trial of six patients with recently diagnosed EGFRvIII-expressing GBM, undergoing standard TMZ treatment followed by a single-dose infusion of daclizumab concurrent with a course of PEPvIII peptide EGFRvIII-targeting vaccination [168]. No adverse events were reported beyond minor irritation at the vaccine injection site. Peripheral lymphocyte analysis demonstrated a significant reduction in circulating Tregs in the group treated with daclizumab without a corresponding depletion of overall $CD4^+$ or $CD8^+$ T cells, suggesting a Treg-specific inhibition of proliferation under lymphopenic circumstances. Furthermore, increases in vaccination-induced anti-PEPvIII antibodies directly correlated with reductions in Tregs and with increases in effector cell to Treg ratios. Together, these results suggest that mAb blockade of IL-2Rα in TMZ-treated malignant glioma may create an environment conducive to further immunotherapeutic intervention. Moreover, these encouraging findings underscore the potential benefit of multimodal combinations of immune-modulating therapies in treating CNS cancers.

18.9 Concluding Remarks

A comprehensive understanding of the dynamic balance between tumoricidal immunity and tumor-derived immunosuppression that take place during CNS cancer development is essential for successful immunotherapy for this disease. As this chapter highlights, ever-unfolding insight into CNS-distinct immune mechanisms and their derailment by transformed tumor cells has already allowed for innovative, safe, and therapeutically promising techniques. The feasibility of individual immune-altering therapies is leading to a combination of strategies for achieving the ultimate goal of synergistic tumoricidal immunity. Such efforts might rely on use of DC- or peptide-based vaccines with

either chemotherapy or biological therapy. The final goal of these interventions is augmenting lymphocyte and NK cell activation or disabling tumor-derived immunosuppressive barriers. Additionally, recent insight into the clonal heterogeneity of CNS tumors, the presence of recalcitrant gCSCs, and the expression of CMV antigens in a majority of transformed cells in some CNS cancers have spurred new and innovative strategies that are being evaluated to further enhance antitumoral immunity.

References

1. Davis FG, Freels S, Grutsch J, Barlas S, Brem S. Survival rates in patients with primary malignant brain tumors stratified by patient age and tumor histological type: an analysis based on Surveillance, Epidemiology, and End Results (SEER) data, 1973–1991. J Neurosurg. 1998;88:1–10.
2. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol. 2009;10:459–66.
3. Parney I. Basic concepts in glioma immunology. Adv Exp Med Biol. 2012;746:42–52.
4. Harling-Berg C, Hallett J, Park J, Knopf P. Hierarchy of immune responses to antigen in the normal brain. In: Protective and pathological immune responses in the CNS. Berlin/New York: Springer; 2002. p. 1–22.
5. Gehrmann J, Banati R, Wiessnert C, Hossmann KA, Kreutzberg G. Reactive microglia in cerebral ischaemia: an early mediator of tissue damage? Neuropathol Appl Neurobiol. 1995;21:277–89.
6. Flavell K, Murray P. Hodgkin's disease and the Epstein-Barr virus. Mol Pathol. 2000;53:262–9.
7. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999;189:12–9.
8. Cobbs CS, Harkins L, Samanta M, Gillespie GY, Bharara S, King PH, et al. Human cytomegalovirus infection and expression in human malignant glioma. Cancer Res. 2002;62:3347–50.
9. Fabry Z, Raine CS, Hart MN. Nervous tissue as an immune compartment: the dialect of the immune response in the CNS. Immunol Tod. 1994;15:218–24.
10. Beschorner R, Nguyen TD, Gözalan F, Pedal I, Mattern R, Schluesener HJ, et al. CD14 expression by activated parenchymal microglia/macrophages and infiltrating monocytes following human traumatic brain injury. Acta Neuropathol. 2002;103:541–9.
11. Owens T, Renno T, Taupin V, Krakowski M. Inflammatory cytokines in the brain: does the CNS shape immune responses? Immunol Tod. 1994;15:566–71.
12. Ling EA, Wong WC. The origin and nature of ramified and amoeboid microglia: a historical review and current concepts. Glia. 1993;7:9–18.
13. Goldmann J, Kwidzinski E, Brandt C, Mahlo J, Richter D, Bechmann I. T cells traffic from brain to cervical lymph nodes via the cribroid plate and the nasal mucosa. J Leukoc Biol. 2006;80:797–801.
14. Cserr HF, Knopf PM. Cervical lymphatics, the blood-brain barrier and the immunoreactivity of the brain: a new view. Immunol Tod. 1992;13:507–12.
15. Weller RO, Engelhardt B, Phillips MJ. Lymphocyte targeting of the central nervous system: a review of afferent and efferent CNS-immune pathways. Brain Pathol. 1996;6:275–88.
16. Brabb T, von Dassow P, Ordonez N, Schnabel B, Duke B, Goverman J. In situ tolerance within the central nervous system as a mechanism for preventing autoimmunity. J Exp Med. 2000;192:871–80.
17. Heimberger AB, Sampson JH. Immunotherapy coming of age: what will it take to make it standard of care for glioblastoma? Neuro Oncol. 2011;13:3–13.
18. Li W, Graeber MB. The molecular profile of microglia under the influence of glioma. Neuro Oncol. 2012;8:958–78.
19. Quattrocchi KB, Miller CH, Cush S, Bernard SA, Dull ST, Smith M, et al. Pilot study of local autologous tumor infiltrating lymphocytes for the treatment of recurrent malignant gliomas. J Neuro Oncol. 1999;45:141–57.
20. Gigliotti F, Lee D, Insel RA, Scheld WM. IgG penetration into the cerebrospinal fluid in a rabbit model of meningitis. J Infect Dis. 1987;156:394–8.
21. Stemmler H-J, Schmitt M, Willems A, Bernhard H, Harbeck N, Heinemann V. Ratio of trastuzumab levels in serum and cerebrospinal fluid is altered in HER2-positive breast cancer patients with brain metastases and impairment of blood-brain barrier. Anticancer Drugs. 2007;18:23–8.
22. Sonabend AM, Rolle CE, Lesniak MS. The role of regulatory T cells in malignant glioma. Anticancer Res. 2008;28:1143–50.
23. Kostianovsky AM, Maier LM, Anderson RC, Bruce JN, Anderson DE. Astrocytic regulation of human monocytic/microglial activation. J Immunol. 2008;181:5425–32.
24. Kennedy BC, Showers CR, Anderson DE, Anderson L, Canoll P, Bruce JN, et al. Tumor-associated macrophages in glioma: friend or foe? J Oncol. 2013;2013:486912.
25. Conti A, Gulì C, La Torre D, Tomasello C, Angileri FF, Aguennouz MH. Role of inflammation and oxidative stress mediators in gliomas. Cancer. 2010;2:693–712.
26. Dunwiddie TV. The physiological role of adenosine in the central nervous system. Int Rev Neurobiol. 1985;27:63.

27. Dunn GP, Fecci PE, Curry WT. Cancer immunoediting in malignant glioma. Neurosurgery. 2012;71:201–23.
28. Anderson RC, Anderson DE, Elder JB, Brown MD, Mandigo CE, Parsa AT, et al. Lack of B7 expression, not human leukocyte antigen expression, facilitates immune evasion by human malignant gliomas. Neurosurgery. 2007;60:1129–36.
29. Badie B, Bartley B, Schartner J. Differential expression of MHC class II and B7 costimulatory molecules by microglia in rodent gliomas. J Neuroimmunol. 2002;133:39–45.
30. Schartner JM, Hagar AR, Van Handel M, Zhang L, Nadkarni N, Badie B. Impaired capacity for upregulation of MHC class II in tumor-associated microglia. Glia. 2005;51:279–85.
31. Wintterle S, Schreiner B, Mitsdoerffer M, Schneider D, Chen L, Meyermann R, et al. Expression of the B7-related molecule B7-H1 by glioma cells a potential mechanism of immune paralysis. Cancer Res. 2003;63:7462–7.
32. Kjellman C, Olofsson SP, Hansson O, Von Schantz T, Lindvall M, Nilsson I, et al. Expression of TGF-β isoforms, TGF-β receptors, and SMAD molecules at different stages of human glioma. Int J Cancer. 2000;89:251–8.
33. Li W, Graeber MB. The molecular profile of microglia under the influence of glioma. Neuro Oncol. 2012;14:958–78.
34. Merzak A, McCrea S, Koocheckpour S, Pilkington G. Control of human glioma cell growth, migration and invasion in vitro by transforming growth factor β1. Br J Cancer. 1994;70:199–203.
35. Payner T, Leaver HA, Knapp B, Whittle IR, Trifan OC, Miller S, et al. Microsomal prostaglandin E synthase-1 regulates human glioma cell growth via prostaglandin E2–dependent activation of type II protein kinase A. Mol Cancer Ther. 2006;5:1817–26.
36. Badie B, Schartner J. Role of microglia in glioma biology. Microsc Res Tech. 2001;54:106–13.
37. Jansen T, Tyler B, Mankowski JL, Recinos VR, Pradilla G, Legnani F, et al. FasL gene knock-down therapy enhances the antiglioma immune response. Neuro Oncol. 2010;12:482–9.
38. Wagner S, Czub S, Greif M, Vince GH, Süss N, Kerkau S, et al. Microglial/macrophage expression of interleukin 10 in human glioblastomas. Int J Cancer. 1999;82:12–6.
39. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer. 2004;4:71–8.
40. Wainwright DA, Sengupta S, Han Y, Lesniak MS. Thymus-derived rather than tumor-induced regulatory T cells predominate in brain tumors. Neuro Oncol. 2011;13:1308–23.
41. Mitsuka K, Kawataki T, Satoh E, Asahara T, Horikoshi T, Kinouchi H. Expression of indoleamine 2, 3-dioxygenase and correlation with pathological malignancy in gliomas. Neurosurgery. 2013;72:1031–9.
42. Galli R, Binda E, Orfanelli U, Cipelletti B, Gritti A, De Vitis S, et al. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res. 2004;64:7011–21.
43. Wu A, Oh S, Gharagozlou S, Vedi RN, Ericson K, Low WC, et al. In vivo vaccination with tumor cell lysate plus CpG oligodeoxynucleotides eradicates murine glioblastoma. J Immunother. 2007;30:789–97.
44. Hatiboglu MA, Wei J, Wu ASG, Heimberger AB. Immune therapeutic targeting of glioma cancer stem cells. Target Oncol. 2010;5:217–27.
45. Ji B, Chen Q, Liu B, Wu L, Tian D, Guo Z, et al. Glioma stem cell-targeted dendritic cells as a tumor vaccine against malignant glioma. Yonsei Med J. 2013;54:92–100.
46. Badie B, Schartner JM. Flow cytometric characterization of tumor-associated macrophages in experimental gliomas. Neurosurgery. 2000;46:957–62.
47. Komohara Y, Ohnishi K, Kuratsu J, Takeya M. Possible involvement of the M2 anti-inflammatory macrophage phenotype in growth of human gliomas. J Pathol. 2008;216:15–24.
48. Rodrigues JC, Gonzalez GC, Zhang L, Ibrahim G, Kelly JJ, Gustafson MP, et al. Normal human monocytes exposed to glioma cells acquire myeloid-derived suppressor cell-like properties. Neuro Oncol. 2010;12:351–65.
49. Boehm UKT, Groot M, Howard JC. Cellular responses to interferon-gamma. Annu Rev Immunol. 1997;15:749–95.
50. Filipazzi P, Huber V, Rivoltini L. Phenotype, function and clinical implications of myeloid-derived suppressor cells in cancer patients. Cancer Immunol Immunother. 2012;61:255–63.
51. Lowther DE, Hafler DA. Regulatory T cells in the central nervous system. Immunol Rev. 2012;248:156–69.
52. El Andaloussi A, Lesniak MS. An increase in CD4+ CD25+ FOXP3+ regulatory T cells in tumor-infiltrating lymphocytes of human glioblastoma multiforme. Neuro Oncol. 2006;8:234–43.
53. Heimberger AB, Abou-Ghazal M, Reina-Ortiz C, Yang DS, Sun W, Qiao W, et al. Incidence and prognostic impact of FoxP3+ regulatory T cells in human gliomas. Clin Cancer Res. 2008;14:5166–72.
54. Sonabend AM, Dana K, Lesniak MS. Targeting epidermal growth factor receptor variant III: a novel strategy for the therapy of malignant glioma. Expert Rev Anticancer Ther. 2007;12:S45–50.
55. Zhang L, Alizadeh D, Van Handel M, Kortylewski M, Yu H, Badie B. Stat3 inhibition activates tumor macrophages and abrogates glioma growth in mice. Glia. 2009;57:1458–67.
56. Schaefer LK, Menter DG, Schaefer TS. Activation of stat3 and stat1 DNA binding and transcriptional activity in human brain tumour cell lines by gp130 cytokines. Cell Signal. 2000;12:143–51.

57. Wang T, Niu G, Kortylewski M, Burdelya L, Shain K, Zhang S, et al. Regulation of the innate and adaptive immune responses by Stat-3 signaling in tumor cells. Nat Med. 2004;10:48–54.
58. Sherry MM, Reeves A, Wu JK, Cochran BH. STAT3 is required for proliferation and maintenance of multipotency in glioblastoma stem cells. Stem Cells. 2009;27:2383–92.
59. Brantley EC, Benveniste EN. Signal transducer and activator of transcription-3: a molecular hub for signaling pathways in gliomas. Mol Cancer Res. 2008;6:675–84.
60. Mitchell DA, Cui X, Schmittling RJ, Sanchez-Perez L, Snyder DJ, Congdon KL, et al. Monoclonal antibody blockade of IL-2 receptor α during lymphopenia selectively depletes regulatory T cells in mice and humans. Blood. 2011;118:3003–12.
61. Sissons J, Carmichael A. Clinical aspects and management of cytomegalovirus infection. J Infect. 2002;44:78–83.
62. Cinatl J, Vogel JU, Kotchetkov R, Wilhelm DH. Oncomodulatory signals by regulatory proteins encoded by human cytomegalovirus: a novel role for viral infection in tumor progression. FEMS Microbiol Rev. 2004;28:59–77.
63. Kossmann T, Morganti-Kossmann MC, Orenstein JM, Britt WJ, Wahl SM, Smith PD. Cytomegalovirus production by infected astrocytes correlates with transforming growth factor-β release. J Infect Dis. 2003;187:534–41.
64. Reddehase MJ. The immunogenicity of human and murine cytomegaloviruses. Curr Opin Immunol. 2000;12:390–6.
65. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science. 2011;331:1565–70.
66. Ruiz J, Cotorruelo J, Tudela V, Ullate P, Val-Bernal F, de Francisco A, et al. Transmission of glioblastoma multiforme to two kidney transplant recipients from the same donor in the absence of ventricular shunt. Transplantation. 1993;55:682–3.
67. Pellegatta S, Cuppini L, Finocchiaro G. Brain cancer immunoediting: novel examples provided by immunotherapy of malignant gliomas. Expert Rev Anticancer Ther. 2011;11:1759–74.
68. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. Nat Med. 2004;10:909–15.
69. Dillman RO, Duma CM, Schiltz PM, DePriest C, Ellis RA, Okamoto K, et al. Intracavitary placement of autologous lymphokine-activated killer (LAK) cells after resection of recurrent glioblastoma. J Immunother. 2004;27:398–404.
70. Xu X, Stockhammer F, Schmitt M. Cellular-based immunotherapies for patients with glioblastoma multiforme. J Immunol Res. 2012;2012:764213.
71. Sonabend AM, Ogden AT, Maier LM, Anderson DE, Canoll P, Bruce JN, et al. Medulloblastoma: challenges for effective immunotherapy. J Neurooncol. 2012;108:1–10.
72. Sloan AE, Dansey R, Zamorano L, Barger G, Hamm C, Diaz F, et al. Adoptive immunotherapy in patients with recurrent malignant glioma: preliminary results of using autologous whole-tumor vaccine plus granulocyte-macrophage colony-stimulating factor and adoptive transfer of anti-CD3-activated lymphocytes. Neurosurg Focus. 2000;9:1–8.
73. Wykosky J, Gibo DM, Stanton C, Debinski W. Interleukin-13 receptor α2, EphA2, and Fos-related antigen 1 as molecular denominators of high-grade astrocytomas and specific targets for combinatorial therapy. Clin Cancer Res. 2008;14(1):199–208.
74. Clavreul A, Piard N, Tanguy J-Y, Gamelin E, Rousselet M-C, Leynia P, et al. Autologous tumor cell vaccination plus infusion of GM-CSF by a programmable pump in the treatment of recurrent malignant gliomas. J Clin Neurosci. 2010;17(7):842–8.
75. Wu AWS, Xiao J, et al. Expression of MHC I and NK ligands on human CD1331 glioma cells: possible targets of immunotherapy. J Neurooncol. 2007;82(2):121–31.
76. Sobol R, Fakhrai H, Shawler D, Gjerset R, Dorigo O, Carson C, et al. Interleukin-2 gene therapy in a patient with glioblastoma. Gene Ther. 1995;2(2):164–7.
77. Okada H, Lieberman FS, Edington HD, Witham TF, Wargo MJ, Cai Q, et al. Autologous glioma cell vaccine admixed with interleukin-4 gene transfected fibroblasts in the treatment of recurrent glioblastoma: preliminary observations in a patient with a favorable response to therapy. J Neurooncol. 2003;64(1):13–20.
78. Parney IF, Chang L-J, Farr-Jones MA, Hao C, Smylie M, Petruk KC. Technical hurdles in a pilot clinical trial of combined B7-2 and GM-CSF immunogene therapy for glioblastomas and melanomas. J Neurooncol. 2006;78:71–80.
79. Andrews DW, Resnicoff M, Flanders AE, Kenyon L, Curtis M, Merli G, et al. Results of a pilot study involving the use of an antisense oligodeoxynucleotide directed against the insulin-like growth factor type I receptor in malignant astrocytomas. J Clin Oncol. 2001;19:2189–200.
80. Steiner HH, Bonsanto MM, Beckhove P, Brysch M, Geletneky K, Ahmadi R, et al. Antitumor vaccination of patients with glioblastoma multiforme: a pilot study to assess feasibility, safety, and clinical benefit. J Clin Oncol. 2004;22:4272–81.
81. Yu JS, Wheeler CJ, Zeltzer PM, Ying H, Finger DN, Lee PK, et al. Vaccination of malignant glioma patients with peptide-pulsed dendritic cells elicits systemic cytotoxicity and intracranial T-cell infiltration. Cancer Res. 2001;61(3):842–7.
82. Steinman RM, Banchereau J. Taking dendritic cells into medicine. Nature. 2007;449(7161):419–26.
83. Yu JS, Liu G, Ying H, Yong WH, Black KL, Wheeler CJ. Vaccination with tumor lysate-pulsed dendritic cells elicits antigen-specific, cytotoxic T-cells in patients with malignant glioma. Cancer Res. 2004;64(14):4973–9.

84. Prins RM, Soto H, Konkankit V, Odesa SK, Eskin A, Yong WH, et al. Gene expression profile correlates with T-cell infiltration and relative survival in glioblastoma patients vaccinated with dendritic cell immunotherapy. Clin Cancer Res. 2011;17(6): 1603–15.
85. Wheeler CJ, Black KL, Liu G, Mazer M, Zhang X-X, Pepkowitz S, et al. Vaccination elicits correlated immune and clinical responses in glioblastoma multiforme patients. Cancer Res. 2008;68(14): 5955–64.
86. Okada H, Kalinski P, Ueda R, Hoji A, Kohanbash G, Donegan TE, et al. Induction of CD8+ T-cell responses against novel glioma-associated antigen peptides and clinical activity by vaccinations with α-type 1 polarized dendritic cells and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in patients with recurrent malignant glioma. J Clin Oncol. 2011;29(3):330–6.
87. Ardon H, Gool SV, Lopes IS, et al. Integration of autologous dendritic cell-based immunotherapy in the primary treatment for patients with newly diagnosed glioblastoma multiforme: a pilot study. J Neurooncol. 2010;99(2):261–72.
88. Sampson JH, Archer GE, Mitchell DA, et al. An epidermal growth factor receptor variant III-targeted vaccine is safe and immunogenic in patients with glioblastoma multiforme. Mol Cancer Ther. 2009;8(10):2773–9.
89. De Vleeschouwer S, Fieuws S, Rutkowski S, Van Calenbergh F, Van Loon J, Goffin J, et al. Postoperative adjuvant dendritic cell-based immunotherapy in patients with relapsed glioblastoma multiforme. Clin Cancer Res. 2008;14(10): 3098–104.
90. Yamanaka R, Homma J, Yajima N, Tsuchiya N, Sano M, Kobayashi T, et al. Clinical evaluation of dendritic cell vaccination for patients with recurrent glioma: results of a clinical phase I/II trial. Clin Cancer Res. 2005;11(11):4160–7.
91. Liau LM, Prins RM, Kiertscher SM, et al. Dendritic cell vaccination in glioblastoma patients induces systemic and intracranial T-cell responses modulated by the local central nervous system tumor microenvironment. Clin Cancer Res. 2005;11(15): 5515–25.
92. Kikuchi T, Akasaki Y, Abe T, et al. Vaccination of glioma patients with fusions of dendritic and glioma cells and recombinant human interleukin 12. J Immunother. 2004;27(6):452–9.
93. Caruso LM, Orme AM, Neale AM, et al. Results of a phase 1 study utilizing monocyte-derived dendritic cells pulsed with tumor RNA in children and young adults with brain cancer. Neuro Oncol. 2004;6(3): 236–46.
94. Wheeler CJ, Das A, Liu G, Yu JS, Black KL. Clinical responsiveness of glioblastoma multiforme to chemotherapy after vaccination. Clin Cancer Res. 2004;10(16):5316–26.
95. Yamanaka R, Abe T, Yajima N, et al. Vaccination of recurrent glioma patients with tumour lysate-pulsed dendritic cells elicits immune responses: results of a clinical phase I/II trial. Br J Cancer. 2003;89(7): 1172–9.
96. Fadul CE, Fisher JL, Hampton TH, Lallana EC, Li Z, Gui J, et al. Immune response in patients with newly diagnosed glioblastoma multiforme treated with intranodal autologous tumor lysate-dendritic cell vaccination after radiation chemotherapy. J Immunother. 2011;34:382.
97. Ardon H, De Vleeschouwer S, Van Calenbergh F, Claes L, Kramm CM, Rutkowski S, et al. Adjuvant dendritic cell-based tumour vaccination for children with malignant brain tumours. Pediatr Blood Cancer. 2010;54:519–25.
98. Prins RM, Wang X, Soto H, Young E, Lisiero DN, Fong B, et al. Comparison of glioma-associated antigen peptide-loaded versus autologous tumor lysate-loaded dendritic cell vaccination in malignant glioma patients. J Immunother. 2013;36(2):152–7.
99. Moscatello DK, Ramirez G, Wong AJ. A naturally occurring mutant human epidermal growth factor receptor as a target for peptide vaccine immunotherapy of tumors. Cancer Res. 1997;57(8):1419–24.
100. Lateef SS, Gupta S, Jayathilaka LP, Krishnanchettiar S, Huang J-S, Lee B-S. An improved protocol for coupling synthetic peptides to carrier proteins for antibody production using DMF to solubilize peptides. J Biomol Tech. 2007;18:173.
101. Chang DZ, Lomazow W, Joy Somberg C, Stan R, Perales M-A. Granulocyte-macrophage colony stimulating factor: an adjuvant for cancer vaccines. Hematology. 2004;9(3):207–15.
102. Aparicio S, Caldas C. The implications of clonal genome evolution for cancer medicine. N Engl J Med. 2013;368:842–51.
103. Cheever MA, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT, et al. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. Clin Cancer Res. 2009;15:5323–37.
104. Ekstrand AJ, James CD, Cavenee WK, Seliger B, Pettersson RF, Collins VP. Genes for epidermal growth factor receptor, transforming growth factor α, and epidermal growth factor and their expression in human gliomas in vivo. Cancer Res. 1991;51:2164–72.
105. Lallier TE. Cell lineage and cell migration in the neural crest. Ann N Y Acad Sci. 1991;615:158–71.
106. Kuramoto T. Detection of MAGE-1 tumor antigen in brain tumor. Kurume Med J. 1996;44:43–51.
107. Sasaki M, Nakahira K, Kawano Y, Katakura H, Yoshimine T, Shimizu K, et al. MAGE-E1, a new member of the melanoma-associated antigen gene family and its expression in human glioma. Cancer Res. 2001;61:4809–14.
108. Kurpad SN, Wikstrand CJ, Batra SK, McLendon RE, Bigner DD. Tumor antigens in astrocytic gliomas. Glia. 1995;15:244–56.
109. Okada H, Kohanbash G, Zhu X, Kastenhuber ER, Hoji A, Ueda R, et al. Immunotherapeutic approaches for glioma. Crit Rev Immunol. 2009;29(1):1–42.

110. Liu G, Yu JS, Zeng G, et al. AIM-2: a novel tumor antigen is expressed and presented by human glioma cells. J Immunother. 2004;27(3):220–6.
111. Abdouh M, Facchino S, Chatoo W, Balasingam V, Ferreira J, Bernier G. BMI1 sustains human glioblastoma multiforme stem cell renewal. J Neurosci. 2009;29(28):8884–96.
112. Shono T, Tofilon PJ, Bruner JM, Owolabi O, Lang FF. Cyclooxygenase-2 expression in human gliomas: prognostic significance and molecular correlations. Cancer Res. 2001;61(11):4375–81.
113. Saikali S, Avril T, Collet B, Hamlat A, Bansard J-Y, Drenou B, et al. Expression of nine tumour antigens in a series of human glioblastoma multiforme: interest of EGFRvIII, IL-13Rα2, gp100 and TRP-2 for immunotherapy. J Neurooncol. 2007;81(2):139–48.
114. Driggers L, Zhang JG, Newcomb EW, Ge L, Hoa N, Jadus MR. Immunotherapy of pediatric brain tumor patients should include an immunoprevention strategy: a medical hypothesis paper. J Neurooncol. 2010;97(2):159–69.
115. Orzan F, Pellegatta S, Poliani PL, et al. Enhancer of Zeste 2 (EZH2) is up-regulated in malignant gliomas and in glioma stem-like cells. Neuropathol Appl Neurobiol. 2011;37(4):381–94.
116. Cheng L, Wu Q, Guryanova OA, et al. Elevated invasive potential of glioblastoma stem cells. Biochem Biophys Res Commun. 2011;406(4):643–8.
117. Jin F, Zhao L, Guo YJ, et al. Influence of Etoposide on anti-apoptotic and multidrug resistance-associated protein genes in CD133 positive U251 glioblastoma stem-like cells. Brain Res. 2010;1336:103–11.
118. Kuan CT, Wakiya K, Herndon JE, et al. MRP3: a molecular target for human glioblastoma multiforme immunotherapy. BMC Cancer. 2010;10:468.
119. Ishiwata T, Teduka K, Yamamoto T, Kawahara K, Matsuda Y, Naito Z. Neuroepithelial stem cell marker nestin regulates the migration, invasion and growth of human gliomas. Oncol Rep. 2011; 26:91–9.
120. Ligon KL, Alberta JA, Kho AT, et al. The oligodendroglial lineage marker OLIG2 is universally expressed in diffuse gliomas. J Neuropathol Exp Neurol. 2004;63(5):499–509.
121. Xu G, et al. Adenoviral-mediated interleukin-18 expression in mesenchymal stem cells effectively suppresses the growth of glioma in rats. Cell Biol Int. 2009;33(4):466–74.
122. Zhang JG, Kruse CA, Driggers L, et al. Tumor antigen precursor protein profiles of adult and pediatric brain tumors identify potential targets for immunotherapy. J Neurooncol. 2008;88(1):65–76.
123. Rossi M, Magnoni L, Miracco C, et al. β-catenin and Glil are prognostic markers in glioblastoma. Cancer Biol Ther. 2011;11(8):753–61.
124. Cui D, Xu Q, Wang K, Che X. Gli1 is a potential target for alleviating multidrug resistance of gliomas. J Neurol Sci. 2010;288(1–2):156–66.
125. Senetta R, Miracco C, Lanzafame S, et al. Epidermal growth factor receptor and caveolin-1 coexpression identifies adult supratentorial ependymomas with rapid unfavorable outcomes. Neuro Oncol. 2011;13(2): 176–83.
126. Sivaparvathi M, Sawaya R, Wang SW, et al. Overexpression and localization of cathepsin B during the progression of human gliomas. Clin Exp Metastasis. 1995;13(1):49–56.
127. Kitange GJ, Carlson BL, Schroeder MA, et al. Expression of CD74 in high-grade gliomas: a potential role in temozolomide resistance. J Neurooncol. 2010;100(2):177–86.
128. Lewis-Tuffin LJ, Rodriguez F, Giannini C, et al. Misregulated E-cadherin expression associated with an aggressive brain tumor phenotype. PLoS One. 2010;5(10):e13665.
129. Scarcella DL, Chow CW, Gonzales MF, Economou C, Brasseur F, Ashley DM. Expression of MAGE and GAGE in high-grade brain tumors: a potential target for specific immunotherapy and diagnostic markers. Clin Cancer Res. 1999;5(2):335–41.
130. Mennel HD, Lell B. Ganglioside (GD2) expression and intermediary filaments in astrocytic tumors. Clin Neuropathol. 2005;24(1):13–8.
131. Yamamoto H, Swoger J, Greene S, et al. β1,6-N-acethylglucosamine-bearing N-glycans in human gliomas: implications for a role in regulating invasivity. Cancer Res. 2000;60(1):134–42.
132. Kogiku M, Ohsawa I, Matsumoto K, et al. Prognosis of glioma patients by combined immunostaining for survivin, Ki-67 and epidermal growth factor receptor. J Clin Neurosci. 2008;15(11):1198–203.
133. Persson O, Salford LG, Fransson J, Widegren B, Borrebaeck CAK, Holmqvist B. Distribution, cellular localization, and therapeutic potential of the tumor-associated antigen Ku70/80 in glioblastoma multiforme. J Neurooncol. 2010;97(2):207–15.
134. Bao L, Dunham K, Lucas K. MAGE-A1, MAGEA3, and NY-ESO-1 can be upregulated on neuroblastoma cells to facilitate cytotoxic T lymphocyte-mediated tumor cell killing. Cancer Immunol Immunother. 2011;60(9):1299–307.
135. Jian JG, Eguchi J, Kruse CA, et al. Antigenic profiling of glioma cells to generate allogeneic vaccines or dendritic cellbased therapeutics. Clin Cancer Res. 2007;13(2):566–75.
136. Elsir T, Eriksson A, Orrego A, Lindström MS, Nisté M. Expression of PROX1 is a common feature of high grade malignant astrocytic gliomas. J Neuropathol Exp Neurol. 2010;69(2):129–38.
137. Geiger KD, Hendruschk S, Rieber EP, et al. The prostate stem cell antigen represents a novel glioma-associated antigen. Oncol Rep. 2011;26(1): 13–21.
138. Ferletta M, Uhrbom L, Olofsson T, Pontén F, Westermark B. Sox10 has a broad expression pattern in gliomas and enhances platelet-derived growth factor-B—induced gliomagenesis. Mol Cancer Res. 2007;5(9):891–7.
139. Schmitz M, Wehner R, Stevanovic S, et al. Identification of a naturally processed T cell epitope

derived from the glioma associated protein SOX11. Cancer Lett. 2007;245(1–2):331–6.
140. Gondi CS, Lakka SS, Dinh DH, Olivero WC, Gujrati M, Rao JS. RNAi-mediated inhibition of cathepsin B and uPAR leads to decreased cell invasion, angiogenesis and tumor growth in gliomas. Oncogene. 2004;23(52):8486–96.
141. Ueda R, Low KL, Zhu X, et al. Spontaneous immune responses against glioma-associated antigens in a long term survivor with malignant glioma. J Transl Med. 2007;5:68.
142. Wykosky J, Gibo DM, Stanton C, Debinski W. EphA2 as a novel molecular marker and target in glioblastoma multiforme. Mol Cancer Res. 2005;3:541–51.
143. Ekstrand AJ, Longo N, Hamid ML, Olson JJ, Liu L, Collins VP, et al. Functional characterization of an EGF receptor with a truncated extracellular domain expressed in glioblastomas with EGFR gene amplification. Oncogene. 1994;9:2313–20.
144. Heimberger AB, Hlatky R, Suki D, Yang D, Weinberg J, Gilbert M, et al. Prognostic effect of epidermal growth factor receptor and EGFRvIII in glioblastoma multiforme patients. Clin Cancer Res. 2005;11(4):1462–6.
145. Choi KSC, Sampson JH. Tumors of the central nervous system. In: Hayat MA, editor. Tumors of the central nervous system, vol. 1. Dordrecht: Springer Netherlands; 2011.
146. Heimberger AB, Hussain S, Aldape K, Sawaya R, Archer G, Friedman H, et al. Tumor-specific peptide vaccination in newly-diagnosed patients with GBM. J Clin Oncol. 2006;24 Suppl 18:2529.
147. Schmittling RJ, Archer GE, Mitchell DA, Heimberger A, Pegram C, Herndon II JE, et al. Detection of humoral response in patients with glioblastoma receiving EGFRvIII-KLH vaccines. J Immunol Methods. 2008;339:74–81.
148. Sampson J, Archer G, Bigner D, Davis T, Friedman H, Keler T, et al. Effect of EGFRvIII-targeted vaccine (CDX-110) on immune response and TTP when given with simultaneous standard and continuous temozolomide in patients with GBM. J Clin Oncol. 2008;26 Suppl 15:2011.
149. De Maio A. Heat shock proteins: facts, thoughts, and dreams. Shock. 1999;11:1–12.
150. Nishikawa M, Takemoto S, Takakura Y. Heat shock protein derivatives for delivery of antigens to antigen presenting cells. Int J Pharm. 2008;354:23–7.
151. Belli F, Testori A, Rivoltini L, Maio M, Andreola G, Sertoli MR, et al. Vaccination of metastatic melanoma patients with autologous tumor-derived heat shock protein gp96-peptide complexes: clinical and immunologic findings. J Clin Oncol. 2002;20:4169–80.
152. Bogdahn PH, Stockhammer G, et al. Targeted therapy for high-grade glioma with the TGF-βeta2 inhibitor trabedersen: results of a randomized and controlled phase IIb study. Neuro Oncol. 2011;13(1):132–42.
153. Yang I, Fang S, Parsa AT. Heat shock proteins in glioblastomas. Neurosurg Clin N Am. 2010;21:111–23.
154. Crane CA, Han SJ, Ahn B, Oehlke J, Kivett V, Fedoroff A, et al. Individual patient-specific immunity against high-grade glioma after vaccination with autologous tumor derived peptides bound to the 96 KD chaperone protein. Clin Cancer Res. 2013;19(1):205–14.
155. Jaschinski TR, Jachimczak P, Seitz C, Schneider A, Schlingensiepen KH. The antisense oligonucleotide trabedersen (AP 12009) for the targeted inhibition of TGF-βeta2. Curr Pharm Biotechnol. 2011;12:2203–13.
156. Morantz RA, Wood GW, Foster M, Clark M, Gollahon K. Macrophages in experimental and human brain tumors: part 2: studies of the macrophage content of human brain tumors. J Neurosurg. 1979;50:305–11.
157. Morse JH, Turcotte JG, Merion RM, Campbell Jr DA, Burtch GD, Lucey MR. Development of a malignant tumor in a liver transplant graft procured from a donor with a cerebral neoplasm. Transplantation. 1990;50:875–6.
158. Buckner JC, Schomberg PJ, McGinnis WL, Cascino TL, Scheithauer BW, O'Fallon JR, et al. A phase III study of radiation therapy plus carmustine with or without recombinant interferon-α in the treatment of patients with newly diagnosed high-grade glioma. Cancer. 2001;92(2):420–33.
159. Olson JJ, McKenzie E, Skurski-Martin M, Zhang Z, Brat D, Phuphanich S. Phase I analysis of BCNU-impregnated biodegradable polymer wafers followed by systemic interferon alfa-2b in adults with recurrent glioblastoma multiforme. J Neurooncol. 2008;90(3):293–9.
160. Tabatabai G, Frank B, Möhle R, Weller M, Wick W. Irradiation and hypoxia promote homing of haematopoietic progenitor cells towards gliomas by TGF-β-dependent HIF-1α-mediated induction of CXCL12. Brain. 2006;129:2426–35.
161. Ehrhart PS, Tsang MLS, Carroll AG, Barcellos-Hoff MH. Latent transforming growth factor β1 activation in situ: quantitative and functional evidence after low-dose γ-irradiation. FASEB J. 1997;11:991–1002.
162. Schlingensiepen KH, Jaschinski F, Lang SA, Moser C, Geissler EK, Schlitt HJ, et al. Transforming growth factor-beta 2 gene silencing with trabedersen (AP 12009) in pancreatic cancer. Cancer Sci. 2011;102(6):1193–200.
163. Gansbacher B, Zier K, Daniels B, Cronin K, Bannerji R, Gilboa E. Interleukin 2 gene transfer into tumor cells abrogates tumorigenicity and induces protective immunity. J Exp Med. 1990;172:1217–24.
164. Colombo F, Barzon L, Franchin E, Pacenti M, Pinna V, Danieli D, et al. Combined HSV-TK/IL-2 gene therapy in patients with recurrent glioblastoma mul-

tiforme: biological and clinical results. Cancer Gene Ther. 2005;12:835–48.
165. Fecci PE, Sweeney AE, Grossi PM, Nair SK, Learn CA, Mitchell DA, et al. Systemic anti-CD25 monoclonal antibody administration safely enhances immunity in murine glioma without eliminating regulatory T cells. Clin Cancer Res. 2006;12(14):4294–305.
166. Kohm AP, McMahon JS, Podojil JR, Begolka WS, DeGutes M, Kasprowicz DJ, et al. Cutting edge: anti-CD25 monoclonal antibody injection results in the functional inactivation, not depletion, of CD4+ CD25+ T regulatory cells. J Immunol. 2006;176:3301–5.
167. Jacobs JF, Punt CJ, Lesterhuis WJ, Sutmuller RP, Brouwer HM-H, Scharenborg NM, et al. Dendritic cell vaccination in combination with anti-CD25 monoclonal antibody treatment: a phase I/II study in metastatic melanoma patients. Clin Cancer Res. 2010;16:5067–78.
168. Sampson JH, Schmittling RJ, Archer GE, Congdon KL, Nair SK, Reap EA, et al. A pilot study of IL-2Rα blockade during lymphopenia depletes regulatory T-cells and correlates with enhanced immunity in patients with glioblastoma. PLoS One. 2012; 7:e31046.

Immunotherapy of Lung Tumors

19

Shahe Boghossian

Contents

S. Boghossian, MD, PhD (✉)
Department of Surgery, University Hospital of Worcester, Worcester, UK

Department of Surgery and Vascular Surgery, University Hospital of North Staffordshire, West Midlands Deanery, Stoke on Trent, West Midlands, UK
e-mail: shaboghru@gmail.com

19.1 Introduction

Lung tumors are one of the most common tumors worldwide in both incidence and mortality. The estimated worldwide incidence was 1.7 million cases in 2011. Lung cancer creates a difficult financial burden even for developed countries; however, for the developing countries, it is particularly different because of relatively costly adjuvant chemotherapy or even surgery. The main treatment method of lung tumors is still surgery, but chemotherapy and immunotherapy have become quite robust in the last decade. Throughout this chapter, of particular observation that we have to make are the shift and emphasis on non-small cell lung tumors which have become more common in the last 25 years with the introduction of low-tar cigarettes and the lower incidence of small cell lung carcinomas (SCLCs) as compared to non-small cell lung carcinomas (NSCLCs). This has been particularly significant because SCLCs are classified as central tumors which present quite late and are not amenable to extensive intervention, whereas peripheral tumors are at least amenable to surgical resection if detected early and hence are more prone to be investigated further with immunotherapy. A brief description of lung demographics is displayed in Table 19.1 followed by key features about lung cancer in Tables 19.2 and 19.3. Finally, Table 19.4 gives a comparative analysis between SCLCs and NSCLCs.

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_19, © Springer-Verlag Berlin Heidelberg 2015

Table 19.1 Brief overview of lung cancer demography as described by WHO in 2009

Lung cancer demography
85 % of lung tumors are NSCLCs
15 % of lung tumors are SCLCs
45 % of NSCLC cases are adenocarcinoma
45 % of NSCLC are squamous cell carcinoma
10 % of NSCLC are large cell

Table 19.2 Displays the key facts about lung tumors

Key facts about lung tumors
Peak incidence between 40 and 70
Four main types: squamous, small cell anaplastic, adenocarcinoma, and large cell anaplastic
Clinical division into small cell (SCLC) and non-small cell (NSCLC)
TNM staging used for NSCLC while either limited or extensive staging for SCLC stages
Small proportion are operable due to late and advanced presentation
Tumors can be central or peripheral (more shift lately toward peripheral)
Overall survival 5–30 % at 5 years depending on histology and stage

Table 19.3 Describes features of lung tumors that are amenable for surgery

Key features of lung tumors that make them amenable to surgery
Tumors <3 cm
Tumors at least 2 cm distal to the carina
No contralateral nodal involvement (T3)
No effusion
No distant metastasis
No obstruction of bronchi or pulmonary atelectasis
No involvement of trachea, great vessels, pericardium, vertebrae, esophagus, diaphragm, or pericardium

Table 19.4 Comparing the key features of SCLC and NSCLC

SCLC (small cell lung cancer)	NSCLC (non-small cell lung cancer)
Previously called oat cell	Arise from alveolar cells
Arise from neuroendocrine cells (Kulchitsky)	Include adenocarcinomas, squamous and large cell tumors
Secrete peptides	Nonsecretory
Can cause SIADH (syndrome of inappropriate secretion of ADH)	Adenocarcinomas arise from basal bronchial cells and type II pneumocytes

19.2 Why Immunotherapy?

Chemotherapy regimens have yielded roughly the same results no matter what the combination albeit the fact that the introduction of tyrosine kinase inhibitors has extended the overall survival of EGFR mutant tumors, as described in Fig. 19.1 [1], hence grew the need for immunotherapy, which started with dendritic cell (DC) therapies. Dendritic cells or antigen-presenting cells (APC) are well known for their plasticity, but the use of dendritic cell immunotherapy has at best yielded only 15 % success rate. This has been due to several factors mainly maturity, timing, antigenic dose, anergy, and several other receptor-ligand interactions. Of particular note has been the recent paradigm shift with the introduction of ipilimumab (Yervoy®) which has boosted the success rates of immunotherapy to well above 20 %, whereby combined with surgery, tissue specific chemotherapy, and immunotherapy, patients have a higher chance of being in remission or possibly cure. Our discussion will henceforth focus on humoral as well as targeted cellular modalities which have been successfully implemented for the last decade.

19.3 Antiangiogenic Agents and Monoclonals

19.3.1 Ziv-Aflibercept (Zaltrap®)

Ziv-Aflibercept (*Zaltrap*®) is a recombinant fusion protein that binds (scavenges) to VEGF-A, VEGF-B, and placental growth factor and hence does not allow the binding of VEGF to its receptors as shown in Fig. 19.2. This relatively new agent has been randomized in one trial for NSCLC. The VITAL study is a randomized multinational double-blind trial that enrolled 913 patients with *non-squamous* NSCLC (adenocarcinoma making 80 % of the NSCLC) who have already failed one platinum doublet therapy. The randomization was 1:1 with one subgroup receiving docetaxel (75 mg/m^2) and aflibercept (6 mg/kg) every

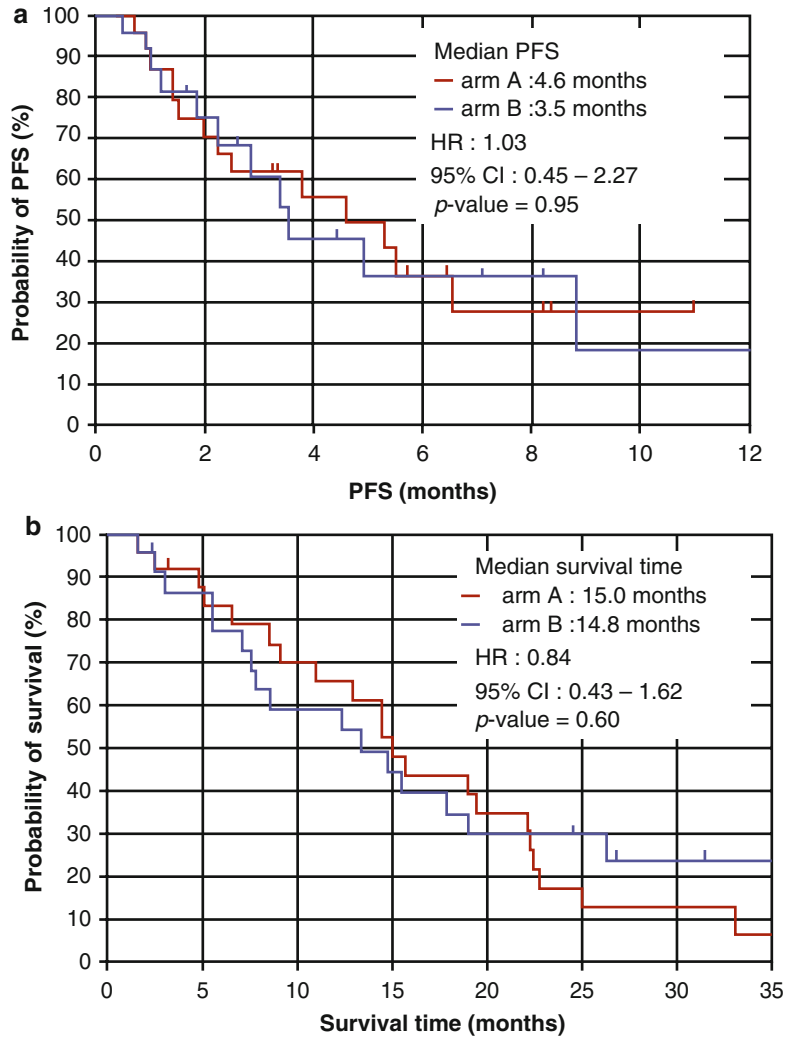


Fig. 19.1 (**a**, **b**) Showing a Kaplan-Meier curve showing the probability of progression-free survival (*PFS*) and overall survival (*OS*) in patients with carboplatin and paclitaxel (arm **a**) *vs*. carboplatin and gemcitabine (arm **b**). The survival clearly demonstrates that the overall benefits are roughly the same with no statistically significant difference (Image courtesy of BMC Res Notes, Permission Free Open Access Journal [1])

3 weeks, while the control subgroup received docetaxel only. The primary endpoint was overall survival (OS) and the secondary endpoints were progression-free survival (PFS) and overall response rate (ORR). A total of 12.3 % of the patients had already received prior bevacizumab therapy. The OS in the treatment subgroup was 10.1 months, while it was 10.4 months in the placebo group. PFS and ORR were 5.2 % versus 4.1 % and 23.3 % versus 8.9 % in the treatment and placebo subgroup, respectively [2]. This pioneering study did not show any advantage of scavenger fusion proteins in the treatment of NSCLC.

19.3.2 Bevacizumab

Bevacizumab or Bev is a humanized monoclonal antibody aimed against VEGF-A, developed by Genentech/Roche, and marketed as Avastin®. VEGF-A is one of the key components in promoting de novo angiogenesis. Bev has been recommended in renal cell carcinoma, carcinoma of the breast (in some countries), glioblastoma multiforme, colonic cancer, and advanced stages of NSCLC. Recent evidence in using Bev has been counterbalanced by the unpredictable nature of the response. This is due to the neutralizing effect of another isoform of VEGF-165b which is an antiangiogenic form of VEGF-A

and neutralizes the effect of Bev [3]. This has prompted the development of scavenger agents: ziv-aflibercept and VEGF-receptor inhibitors. So far, none of the VEGFR inhibitors have undergone any clinical trials in relation to pulmonary tumors.

One the first clinical trials employing Bev was E4599 (Eastern Cooperative Oncology Group) which randomized 878 patients with recurrent or advanced non-squamous NSCLC (IIIB or IV) to receive either paclitaxel or carboplatin ($N=444$) versus the same therapy plus bevacizumab ($N=434$). Chemotherapy was given for 3 weeks for six cycles, while Bev was given every 3 weeks until disease progression or increased toxicity. The primary endpoint was overall survival. The overall difference between the two groups was 2 months in favor of the chemotherapy+Bev group ($p=0.003$). Progression-free survival was 6.2 months in the chemotherapy+Bev versus 4.5 months in chemotherapy alone [4]. This trial subsequently led to the European AVAiL trial which confirmed the positive results of adding bevacizumab (7.5 or 15 mg/kg) to gemcitabine and cisplatin in treating non-squamous NSCLC tumors. The results showed a significant benefit in progression-free survival (PFS) (6.7 months in the low-dose group *vs*. 6.1 for the placebo group and 6.5 months for the high-dose group *vs*. 6.1 months for the placebo).

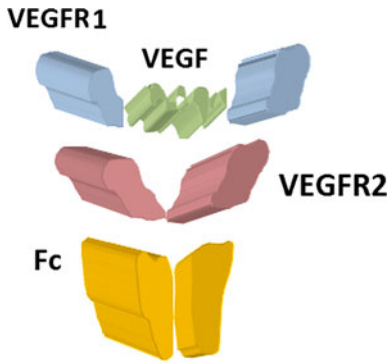


Fig. 19.2 VEGF-Trap/aflibercept is a fully human fusion protein made from a human IgG1 Fc fragment (*orange*) and domain 3 of VEGFR-2 (Ig) (*dark red*) as well as an apical VEGFR-1 portion (*blue*) capable of trapping VEGF (*green*) and PIGF

Two additional complimentary trials with Bev have emerged as maintenance therapies after chemotherapy. These are AVAPERL and POINTBREAK trials. AVAPERL was a European phase III trial that randomized 362 patients previously treated with pemetrexed, cisplatin, and bevacizumab. The patients were further randomized to receive either maintenance Bev or Bev + pemetrexed every 3 weeks until progression. The trial demonstrated the superiority of maintenance therapy with combined medications (PFS of 10.2 months *vs*. 6.6 months) but contrasted the futility of having bevacizumab alone in any treatment modality. Finally, POINTBREAK as referred in Fig. 19.3 is a very recent North American trial which was primarily powered for

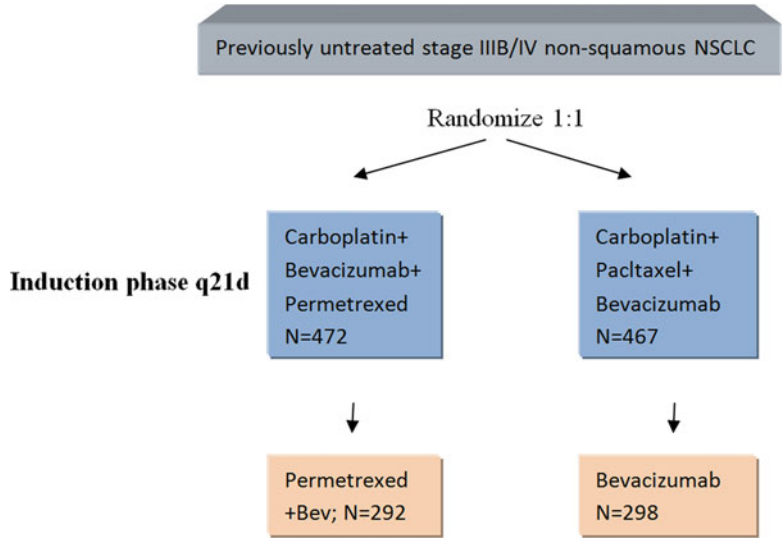


Fig. 19.3 Maintenance phase q21d. Demonstrating the randomization protocol of POINTBREAK trial, both subgroups were given chemotherapy as described in the induction phase for four cycles and then subsequently were maintained on Bev+permetrexed or Bev alone. The maintenance phase was administered every 21 days until disease progression

overall survival and PFS as well as RR as secondary endpoints (see below). It proved to be a negative study with absolutely no difference in overall survival between the two arms of the study (13 months) and very similar progression-free survival, but it did show that maintenance therapy was tolerable with significantly higher level of thrombocytopenia in the combined arm.

19.3.3 PD-1 Monoclonal

The birth of the monoclonal antibody against programmed death and its ligand PD1/PD1-L was heralded as a great leap in immunotherapy. PD-1 is a member of the CD28 family. It is expressed on T cells, memory cells, and regulatory T cells. The binding of PD-1 to its corresponding ligand PD-1L downregulates the activity of the T cell and inhibits it. The expression of PD-1L heralds poor tumor prognosis. PD1/PD-1L is regarded as a secondary signal after the initial interaction of T-cell receptor with antigen and MHC—Fig. 19.4. This secondary signaling pathway acts as an inhibitor of death. Henceforth, an antibody blocking the PD1 motif will cause cell death. BMS-936558 is an IgG4 monoclonal that blocks the docking of PD-1L and PD-2L onto PD-1. The current literature demonstrates good safety and efficacy in a preliminary phase I study by Brahmer and colleagues, whereby 39 patients with advanced solid tumors were given dose-escalating infusions of 0.3, 1, 3, and 10 mg/kg. The patient pool contained melanoma, renal cell carcinoma (RCC), colorectal carcinoma, NSCLC, and castration-resistant prostatic carcinoma (CRPC). The treatment was well tolerated; however, in nine patients observed, the success of the treatment was correlated with PD-1L expression [5]. Yet, in another study, SL Topalian and colleagues recruited 296 patients with advanced solid tumors who were given anti-PD-1 antibody at doses from 0.1 mg/kg up to 10 mg/kg every 2 weeks. Response was assessed after 8 cycles, but patients were given up to 12 cycles of therapy until complete response or disease progression. Of the 236 assessed, objective responses were more common in patients with NSCLC, melanoma, and RCC; this was further reflected with the response rate of PD-1L-expressing tumors which was 36 % (9 out

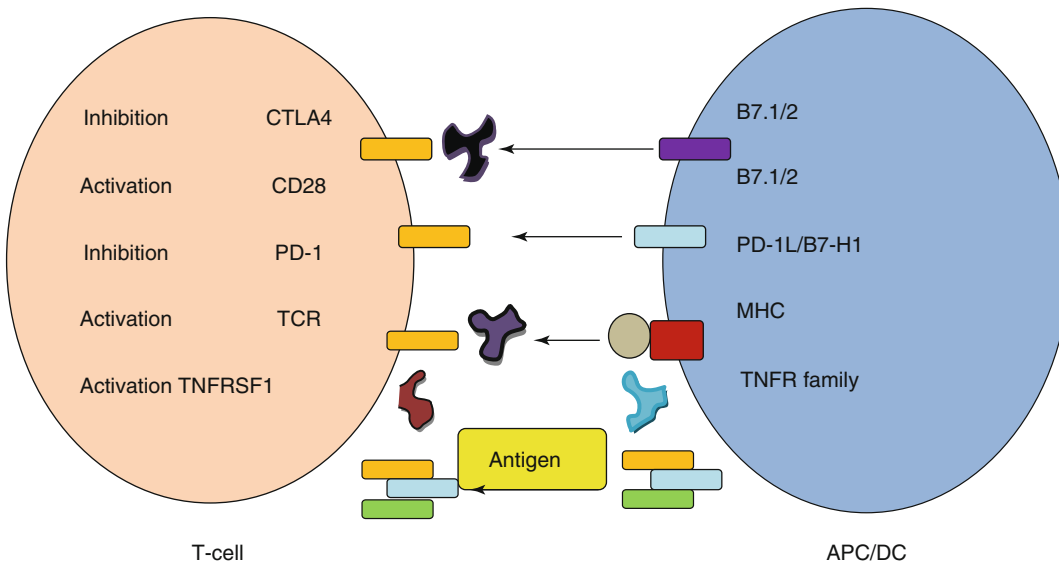


Fig. 19.4 Demonstrates the interaction of T cell with antigen-presenting cell. The antigen (*yellow*) is sandwiched between the MHC and the T-cell receptor. Secondary activating signals between CD28 and B7.1 are important in consolidating an immune response. Inhibitory responses mainly between PD-1 and PD-1L as well as CTLA4 and B7.1 are important in the abrogation of the immune response. In this representation, BMS936558 (*purple*) and ipilimumab (*black*) inhibit these inhibitory interactions and thus cause an inhibition of inhibitory stimuli, therefore priming the T cell

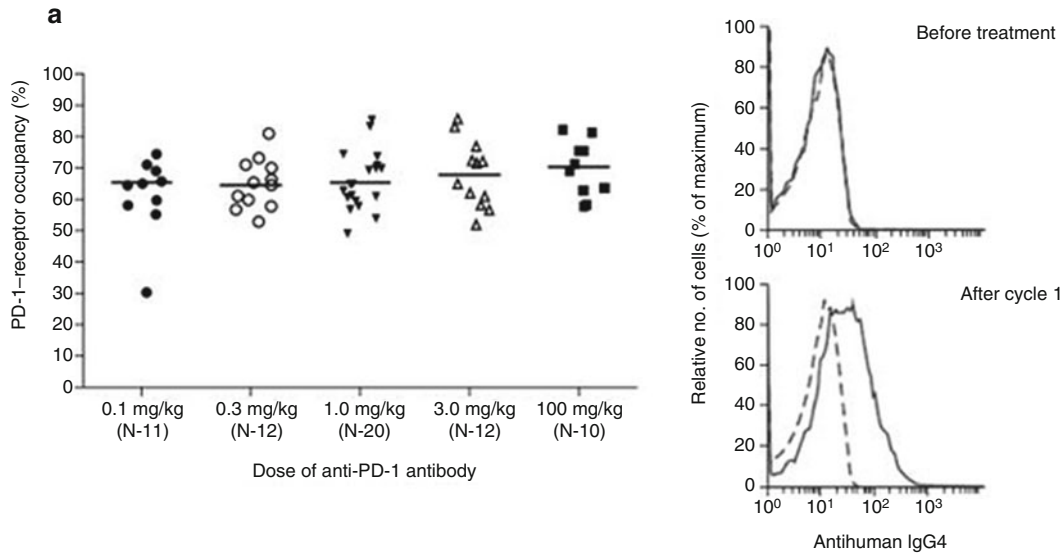
of 25) and totally negative in the tumors not expressing PD-1L (Fig. 19.5). Overall response rate in the NSCLC subgroup was 18 % (14 out of 76), 28 % in the melanoma subgroup, and 27 % in the RCC subgroup [6]. Although this was not a randomized trial, it did prove the principle that in tumors expressing PD-1L, it is well worth examining the option of using anti-PD1 antibodies. Another phase II multicenter trial (CA 209-063) with BMS 936558 will be looking into patients with advanced squamous cell NSCLC who have received at least two prior chemotherapy regimens. The study is still recruiting patients and should be finished by 2014. Thus, although we do not have a large well-powered randomized trial with BMS 936558, this relatively new monoclonal antibody could hold much promise in the future.

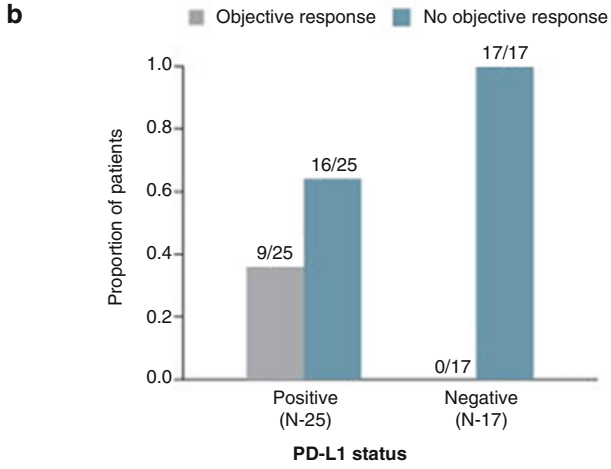
19.3.4 Cetuximab

Cetuximab is a monoclonal antibody that targets epidermal growth factor receptor (EGFR), which is an extracellular receptor with complex downstream signaling. Cetuximab is an IgG1 chimeric antibody that prevents the dimerization of its receptor with five- to tenfold affinity compared to its native ligand, thus preventing further receptor action. It also mediates antibody-dependent cell-mediated cytotoxicity (ADCC) and downregulation of the extracellular receptor. The dogma of using cetuximab in NSCLC is based upon the fact that 80 % of the diagnosed patients have high expression of EGFR extracellularly on immunohistochemical analysis. However, further analysis in colorectal cancer (CRC) and squamous cell carcinoma of the head and neck (SCCHN), whereby cetuximab and another antibody, panitumumab, are used to treat advanced stage disease, revealed that mutational variations in K-ras, a further downstream GTPase, have a significantly different outcome. Colorectal cancer and squamous cell carcinoma of the head and neck patients treated with EGFR monoclonal antibodies against wild-type K-ras have a significantly better outcome than those harboring mutations as demonstrated from the phase III CRYSTAL and EXTREME trials, respectively. In diametric contrast, analysis of K-ras mutations in NSCLC, whether wild type or mutated, this correlation has not been demonstrated, thus suggesting that further biomarkers are required in the determination of prognosis. Crucial to the use of cetuximab in NSCLC has been the FLEX trial which has shown that the addition of cetuximab to first-line chemotherapy in advanced NSCLC significantly improved survival in patients as compared to chemotherapy alone. In 1,121 patients with positive immunohistochemistry for EGFR staining that were further subdivided into

Fig. 19.5 (**a**) Shows PD-1-receptor occupancy by anti-PD-1 antibody. The graph at the left shows PD-1-receptor occupancy on circulating T cells in 65 patients with melanoma after one cycle (8 weeks) of treatment at a dose of 0.1–10.0 mg per kilogram every 2 weeks. *Bars* indicate median values. The graphs at the *right* show the flow cytometric analysis of PD-1-receptor occupancy on CD3-gated peripheral blood mononuclear cells from a patient with melanoma who received 0.1 mg per kilogram, before treatment (*top*) and after one treatment cycle (*bottom*). *Dashed lines* indicate isotype staining controls, and *solid lines* antihuman IgG4. Panel (**b**) shows the correlation of pretreatment tumor cell-surface expression of PD-1 ligand (PD-L1), as determined with immunohistochemical analysis of formalin-fixed, paraffin-embedded specimens, with an objective response to PD-1 blockade in 42 patients with advanced cancers: 18 with melanoma, 10 with non-small cell lung cancer, 7 with colorectal cancer, 5 with renal cell cancer, and 2 with castration-resistant prostate cancer. Tumor cell-surface expression of PD-L1 was significantly correlated with an objective clinical response (graph at the *left*). No patients with PD-L1-negative tumors had an objective response. Of the 25 patients with PD-L1-positive tumors, two who were categorized as not having had a response at the time of data analysis are still under evaluation. Shown at the right are immunohistochemical analysis with the anti-PD-L1 monoclonal antibody 5H1 in a specimen of a lymph node metastasis from a patient with melanoma (*top*), a nephrectomy specimen from a patient with renal cell cancer (RCC) (*middle*), and a specimen of a brain metastasis from a patient with lung adenocarcinoma (*bottom*). The *arrow* in each specimen indicates one of many tumor cells with surface membrane staining for PD-L1. The *asterisk* indicates a normal glomerulus in the nephrectomy specimen, which was negative for PD-L1 staining (Images and data reproduced from Topalian et al. [6], with permission from the Massachusetts Medical Society)

high (345) and low (776), the overall survival in the high EGFR-expressing chemotherapy plus cetuximab group versus the chemotherapy alone group was significantly higher (12.0 months *vs.* 9.6 months), whereas the low EGFR-expressing subgroup showed no overall benefit (9.8 cetux +chemo. *vs.* 10.3 months for chemotherapy alone) [7]. However, there has been some reluctance in using cetuximab in some communities due to high cost and availability of oral tyrosine kinase inhibitors that act further downstream from EGFR receptor.

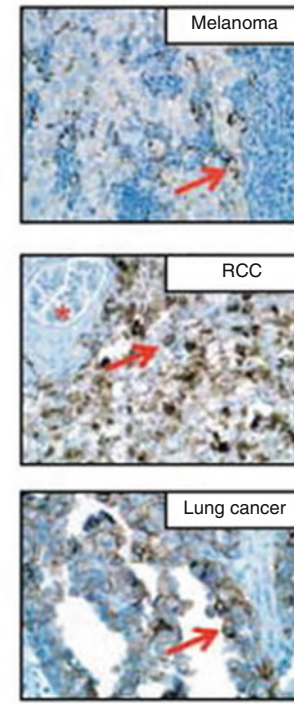




Association between pretreatment tumor PD-L1 expression and clinical response

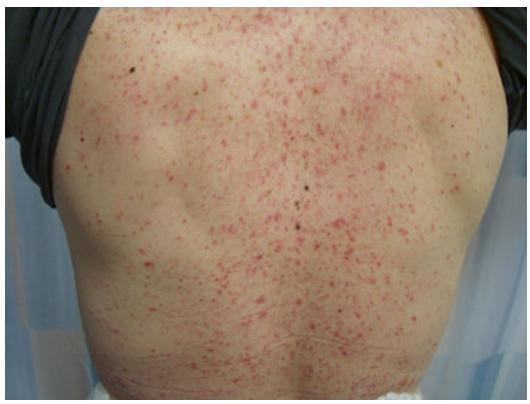
Response status	PD-L1–positive	PD-L1–negative	Total
		number (percent)	
Objective response	9 (36)	0	9 (21)
No objective response	16 (64)	17 (100)	33 (79)
All	25	17	42

P - 0.006 for association by Fisher's exact test



19.3.4.1 The EGFR Inhibitor Rash

A well-known dermatitic rash develops with use of EGFR inhibitors as demonstrated in Fig. 19.6. The rash develops in almost two thirds of patients using EGFR inhibitors. The association of the rash with anticancer activity was observed by Saltz and colleagues with increasingly apparent association between cutaneous toxicity and favorable EGFR activity. Almost all clinical trials report a positive association; nonetheless, there is a significant reporter bias. The underlying mechanism of action is difficult to interpret as clinicians have aimed to relate tumor EGFR with skin EGFR. However, unlike other tumor predictors of EGFR (mutant/amplification), the same cannot be said about the skin. Additionally, the tumor mutations are somatic mutations and do not involve the skin. Alternatively, a much pragmatic explanation would be that the rash is a clear indication of adequate drug exposure whether it is EGFR inhibitors or tyrosine kinase inhibitor (TKI) like gefitinib. To simplify matters, two mechanisms have been postulated: one is the inhibition of the skin itself and the second one is the result of systemic immunologic reaction. The last finding is based upon the fact that in some patients, the rash is self-limiting in a small number of patients and improves over time while being managed with steroids. While the inhibitor-associated rash is clinically related to anticancer activity, the treatment of this rash will permit more patients to benefit from inhibitor treatment [8].



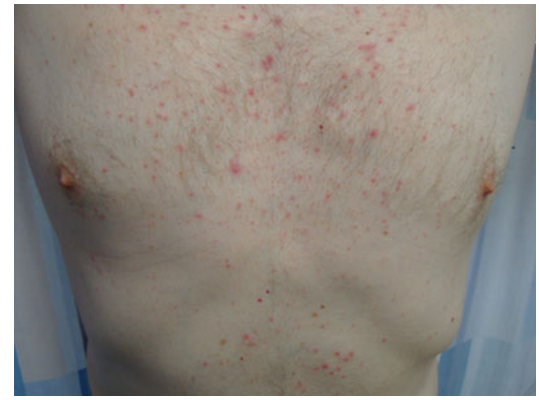


Fig. 19.6 Showing a patient receiving with rash after receiving four cycles of cetuximab (The permission was granted by the patient. Copyright of the chapter author)

19.3.4.2 The Measurement of Immune Response in Monoclonals

Before undertaking the discussion of the next monoclonal antibody, I would like to take the opportunity to explain the major differences between standardized response rates as classified by the World Health Organization and the immune-related criteria (IRC). Traditional dogma dictates that the response to chemotherapy should be evident in a few weeks. However, immunotherapy does not obey those rules; it requires the activation of T cells and their proliferation, but for this to achieve clinically measurable antitumor effect, it takes a few months, and finally, the ultimate outcome on survival is possibly looking at a few years.

Wolchok and colleagues elegantly described the patterns of response in ipilimumab which is clearly shown in Fig. 19.7 and Table 19.5. This describes the patterns of response and warrants clinicians and immunologists that albeit the fact that tumors might eventually decrease in overall size, there can be an initial increase; this is termed pseudoprogression which might be evident for up to 6 months in some cases [9]. It is important to appreciate the clinical significance of pseudoprogression as modern-day biologicals are likely to have a delayed onset of action and possibly a longer duration

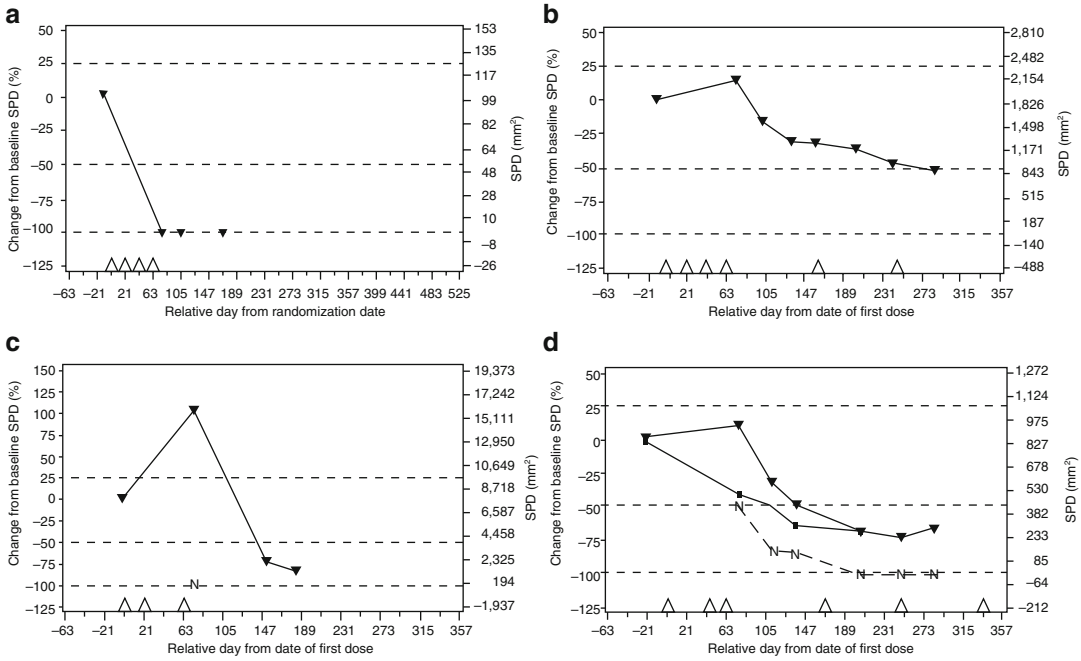


Fig. 19.7 Showing the patterns or response to ipilimumab which were observed in advanced melanoma. Four patterns of response have been observed in advanced melanoma patients treated with ipilimumab at 10 mg/kg in the CA184-008 and CA184-022 trials. (**a**) Immediate response; (**b**) "stable disease" with slow, steady decline in total tumor volume; (**c**) response after initial progression; (**d**) initial mixed response. *SPD* sum of the product of perpendicular diameters, *N* tumor burden of new lesions (**c**, **d**). Panel (**d**) *top line*, total tumor burden; *middle line*, tumor burden of baseline lesions; *bottom line*, tumor burden of new lesions. *Triangles* on the x-axis: ipilimumab dosing time points; *dashed lines*, thresholds for response or progressive disease/immune-related progressive disease [9] (Reprinted by permission from the American Association for Cancer Research: Wolchok et al. [9])

Table 19.5 Displaying the differences between WHO and immune-related response criteria (irRC)

	CR	**PR**	**SD**	**PD**
WHO criteria	All lesions gone	SPD of index lesions decreases ≥50 %	SPD of index lesion of neither CR, PR, nor PD	SPD of index lesion increases by 25 % and/or new lesions develop
		New lesions not allowed		
	irCR	**irPR**	**irSD**	**irPD**
Immune-related response criteria (irRC)	All lesions gone	SPD of index and any new lesion decreases by ≥50 %	SPD of index and any new lesions but neither irCR, irPR, nor irPD	SPD of index lesion and any new lesions increases by 25 %
		New lesions are allowed		

With clear differences in the partial response (irPR/PR) and stable disease (irSD/SD)
CR complete response, *PD* progressive disease

of action. Additionally, these new agents might prompt clinicians to terminate therapy early, although it might be that the immune response is just starting to recruit the necessary reactive cells.

19.3.5 Ipilimumab

Ipilimumab is another monoclonal antibody targeted against CTLA-4. Ipilimumab (Ipb) has been recently used against prostatic carcinoma,

Table 19.6 CA 184-41, ipilimumab in combination with carboplatin and paclitaxel as first-line treatment in stage III/IV NSCLC showing the randomization and treatment protocol of CA 184-041

CA 184-041 Trial		Treatment phase ($n = 203$) Dosed every 3 weeks	Maintenance phase Dosed every 12 weeks
Randomise 203 patients into 3 subgroups (1:1:1)	Subgroup A Chemo+Ipb Concurrent	C C C C C C → Ipb Ipb Ipb Ipb p p	Ipb Ipb →
	Subgroup B Chemo+Ipb Phased	C C C C C C → p p Ipb Ipb Ipb Ipb	Ipb Ipb →
	Subgroup C Chemo only Placebo	C C C C C C → p p p p p p	P p →

Three subgroups are randomized into concurrent (Ipb plus chemotherapy), followed by maintenance doses of ipilimumab, phased, chemotherapy first, and then chemotherapy plus ipilimumab. The placebo group was given chemotherapy only

Ipb ipilimumab, *C* chemotherapy, *P* placebo

melanoma, as well as NSCLC. Ipilimumab is a fully human IgG1 isotype, whereas its sister antibody tremelimumab is an IgG2 isotype. The key to understanding of the mechanism of action of this antibody is the *inhibition* of inhibitory signals from the dendritic cells (DCs) or antigen-presenting cell (APC) to the T cell or the cytotoxic T lymphocyte (CTL) upon antigen presentation or tumor peptide presentation. This inhibition will naturally create a state of anergy and subsequently lead to the apoptosis of activated T cells. Thus, blocking this inhibition allows CTLs to destroy tumor cells.

Initial trials with Ipb were focused against melanoma; however, it soon was evident that it was effective against other solid tumors including castration-resistant prostate cancer and NSCLC. Ipb has been approved for use by the FDA and the UK for use in advanced melanoma. The current literature holds one crucial clinical trial CA 184-041 described in Table 19.6. The objective of the study was to evaluate the safety and efficacy of ipilimumab in combination with chemotherapy (carboplatin, AUC = 6, and paclitaxel 175 mg/m^2) with primary endpoint being immune-related progression-free survival (irPFS). The study was also intended to additionally evaluate two different schedules: concurrent (started at the same time of chemotherapy) and phased (give chemotherapy for two cycles). To be included in the trial, the patients were meant to be chemotherapy naive with Eastern Cooperative Oncology Group 0–1 performance status without any brain metastasis or autoimmune disease. The results of this phase II study were quite valuable and positive. With respect to immune-related progression-free survival, the results were 5.5 months ($p = 0.094$) for the concurrent group versus 5.7 (0.026) months for the phased group and 4.6 months for the placebo group. As for overall survival, the results were 11 months ($p = 0.429$) versus 11.6 months ($p = 0.104$) for the concurrent and phased groups, respectively. The overall survival for the placebo group was 10 months. Thus, looking back at these results, although they do not show a dramatic difference, they will definitely herald newer trials with Ipb and NSCLC [10].

19.3.6 Talactoferrin

Talactoferrin is a new development by Agennix® which is based on lactoferrin, a breast milk pro-

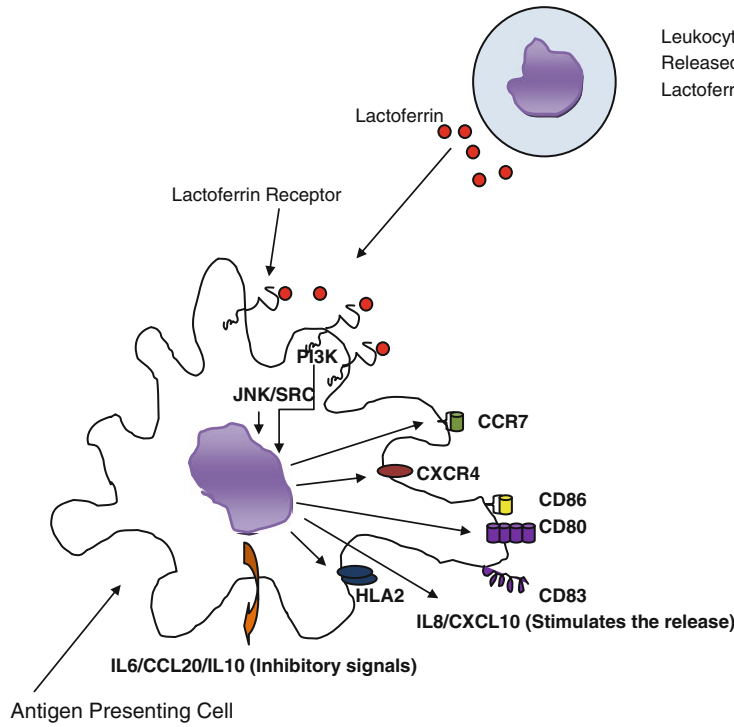


Fig. 19.8 The immunobiology of talactoferrin and its immunostimulatory and inhibitory mechanisms of action

tein secreted in cycles during puerperium. The orally available talactoferrin has been genetically modified to bypass the gastric mucosa and is given twice a day for 2 weeks and then 2 weeks off. It is an 80 kDa protein synthesized in *Aspergillus niger* strains. The exact mechanism of action is poorly understood, but it is postulated to stimulate the dendritic cells in the gut (GALT) and prime the T cells against tumors or immunopotentiate them as shown in Fig. 19.8. The crucial trial that upholds the credibility of talactoferrin is the FORTIS-M trial which has just been completed. In an effort aimed at overall survival (OS) after successful phase II trials [11], FORTIS-M was a randomized double-blind multicenter trial that recruited 742 previously treated, stage IIIb/IV NSCLC patients to receive either talactoferrin or placebo. Unfortunately, the results were negative with OS being 7.5 months versus 7.7 months for talactoferrin and placebo, respectively (data from Agennix). However, committing this agent to the shelf is very early, and the possibility of combining this agent early on in chemotherapy might be another useful alternative.

19.4 Peptide-Based Vaccines

19.4.1 MAGE-3

Known as melanoma antigen-3, MAGE is also defined as a cancer-testis antigen protein (cancer-testis database). Its exact function in normal somatic cells is unknown; neither is their embryonal role. The *MAGE* groups of genes are clustered on the Xq28. The MAGE proteins bind to E3 ubiquitin ligases and inhibit the interaction of p53 with their cognate receptors on chromatin. MAGE is highly expressed in NSCLC, melanomas, and myelomas by well over 50 %, making them ideal targets for immunotherapy. The recent vaccine used in clinical trials for immunotherapy is MAGE-A3 fused with *Haemophilus influenzae* protein D along with a proprietary adjuvant. Expression of MAGE-A3 and other cancer-testis antigens are more common in

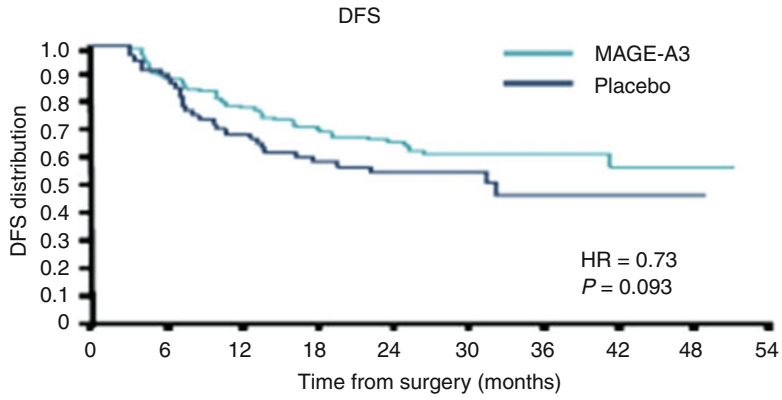


Fig. 19.9 Disease-free survival after 42 months of follow-up demonstrating a hazard ratio of 0.73 in favor of MAGE vaccine with a 27 % risk reduction of cancer recurrence (Adapted from Vansteenkiste et al. [33], with permission of ASCO)

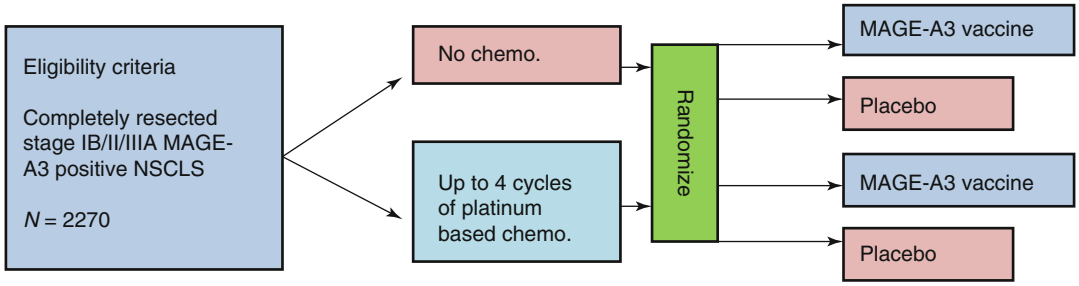


Fig. 19.10 Demonstrating the randomization and recruitment criteria for MAGRIT, with a primary endpoint being disease-free survival while the secondary endpoints are lung cancer-specific survival; OS; 2-, 3-, 4-, and 5-year DFS; anti-MAGE and anti-protein D seropositivity; as well as safety profile and serious adverse events

squamous cell subtypes and are associated with embryologically earlier stages of maturity making them less amenable to standard chemotherapy or to host immune responses [12].

The use of MAGE-A3 as an immunotherapeutic agent was based on solid evidence from phase II trials and high expression in more than 50 % of cases of NSCLC as demonstrated in Figs. 19.9 and 19.10. Additionally, being a cancer-testis antigen, autoimmunity would not have been a problem. In the phase II trial for NSCLC, 182 patients with MAGE-A3-positive stage Ib and II tumors were randomized (2:1) to receive either the vaccine or placebo after curative surgery (122 receiving MAGE *vs*. 60 receiving placebo). As described earlier, it consisted of full length MAGE-A3 with *H. influenzae* protein D mixed with saponin and monophosphoryl lipid A. The vaccine (300 μg) was administered intramuscularly every 3 weeks for five doses and then once every 3 months for a total period of 2 years. The primary endpoint was disease-free interval (DFI) with secondary endpoints being disease-free survival and overall survival safety. The vaccine proved to be effective and heralded a new phase III trial—MAGRIT. The MAGRIT trial is based on the successes of its predecessor; in addition to using MAGE whole protein, the adjuvant activity was potentiated with the addition of TLR9 agonist, CpG7909, which is known to be effective in hepatitis B vaccines. MAGRIT started recruiting in 2007 and intends to recruit almost 2,300 patients from 150 centers worldwide. Unfortunately, the results of the trial did not meet the primary endpoint of disease-free survival as well as the secondary endpoint which was gene signatures that allow response to MAGE-A3.

19.4.2 EGF Vaccines

Epidermal growth factor plays a key role in cancer development. EGFR tyrosine kinase inhibitors and EGFR-targeted monoclonals are well-established

modalities. Initial studies with recombinant EGF conjugated with P64K from *Neisseria meningitidis* along with alum and Montanide™ were evaluated in 3 pooled studies totaling 83 patients with stage III/IV NSCLC. After vaccination with EGF, the majority of patients at least doubled their anti-EGF titer (83 %) described as seroconversion. Good seroconversion was described as titer more than 1:4,000 and termed as good antibody response (GAR). Survival was better in good antibody responders versus poor responders (mean of 12.2 months *vs*. 8.07 months, respectively). Neninger Vinageras et al. conducted a phase II trial in patients with stage III/IV NSCLC (CimaVax®) who have already completed chemotherapy. The vaccine contained 50 μg equivalents of EGF given on days 1, 7, 14, and 28 and monthly thereafter. Comparing vaccinated patients ($n=37$) with non-vaccinated ($n=37$), there was a nonsignificant advantage in overall survival in favor of vaccinated patients (median of 6.5 *vs*. 5.3 months; $p=0.098$). However, interestingly, those under the age of 60 showed a significant advantage in survival (11.6 months *vs*. 5.3 months; $p=0.0124$). There was a significant correlation between overall survival and good antibody response as well as drop in EGF levels [13]. These studies prompted further studies by the Cuban teams with higher recruitment numbers.

19.4.3 MUC1 and Stimuvax®

MUC1 or mucin 1 is an extracellular glycoprotein found on the surface of normal cells and cancer cells. It normally lines the apical epithelium of lungs, intestines, stomach, and many other organs. It plays a crucial role in the defense against pathogenic infections mainly by keeping the bacteria away from the surface. MUC1 has a core protein mass of 120–220 KDa which increases to 250–500 kDa upon glycosylation. The extracellular domain contains 20-amino-acid variable number of tandem repeats (VNTR). The number of repeats varies from 20 to 120 with serine, threonine, and proline being the most common, thus allowing extensive O-glycosylation. Overexpression, aberrant intracellular localization, and defective glycosylation have been crucial to tumor propagation. Glycosylation creates a hydrophilic tumor microenvironment allowing growth factors (IGF) to stay in the vicinity, but it also creates extensive steric hindrance for immune cells as well as hindering the action of hydrophobic chemotherapeutic agents [14]. The identification of cancer-specific immunodominant epitope (HGVTSAPDTRPAPGSTAPPA), each of which has 5 O-glycosylation motifs [15], heralded the development of further studies and prompted the design of Stimuvax®.

Stimuvax®, also known as L-BLP25, was developed based on the tandem repeat motifs mentioned earlier. The motif used is STAPPAHGVTSAPDTRPAPGSTAPP-Lys(PAL)G lipopeptide. The adjuvant is monophosphoryl lipid A, while the liposomal components are cholesterol, dimyristoyl phosphatidylglycerol, and dipalmitoylphosphatidylcholine. The mechanism of action of BLP25 is postulated to be through the uptake of the lipopeptide by DCs and further presentation to TCR either through MHC I or II with further augmentation of its effect via the recruitment of cytotoxic T lymphocyte, release of cytokines, humoral factors, and NK cells as shown in Fig. 19.11 (data provided by Serono).

The clinical trials of Stimuvax® have been pioneered after successful open-label phase II study was conducted to evaluate the safety of BLP25. Twenty-two patients with unresectable stage IIIA/IIIB NSCLC received 1,000 μg every week for 8 weeks plus best supportive care. The vaccination continued for 13 weeks after the start of the trial and was given every 6 weeks until disease progression. After median follow-up of 26.7 months, the 1-year survival rate was 82 % (95 % CI, 66–98 %), while the 2-year survival rate was 64 % (95 % CI, 44–84 %) [16]. This heralded two further phase III clinical trials START and INSPIRE. START is a multicenter, randomized, double-blind, placebo-controlled phase III trial aimed at unresectable stage III NSCLC patients with previous exposure to platinum chemotherapy and radiotherapy. START recruited 1,500 patients in 33 countries.

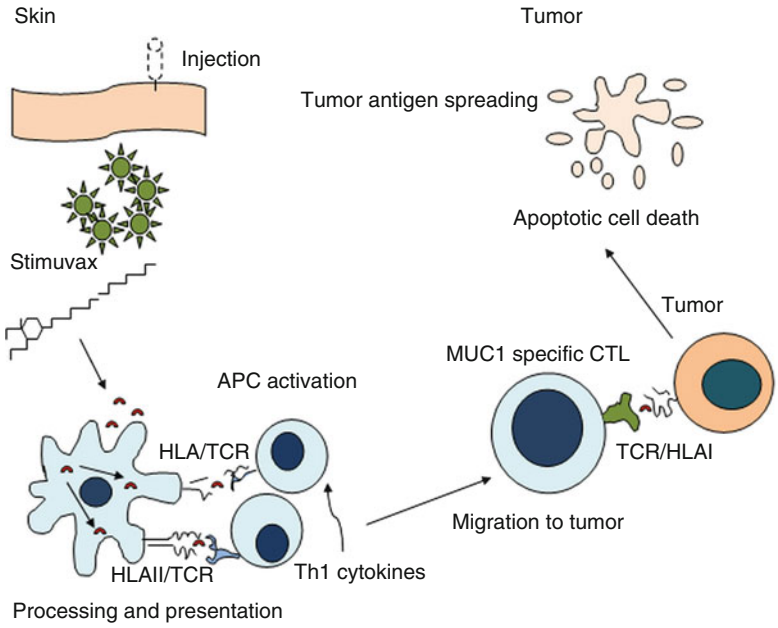


Fig. 19.11 Mechanism of action of Stimuvax/ L-BLP-25

Unfortunately, the trial was stopped due to failure of reaching primary endpoint of overall survival (Merck KGaA/Oncothyreon). The INSPIRE trial [17] is a similar trial aimed at patients with Asian background, aimed at recruiting 420 patients with unresectable IIIA/IIIB NSCLC who have at least responded with two cycles of platinum chemotherapy or have stable disease. The trial finished recruiting; however, yet again, the trial failed to reach significance. Another trial, the STOP trial, randomized 532 patients with stage III/IV NSCLC to receive vaccine versus placebo after doublet chemotherapy. The trial yet again did not show any benefit in terms of primary endpoint of overall significance. However, a subgroup of patients that had received chemotherapy 12 weeks earlier did benefit as well as those who had received chemoradiotherapy. Despite these setbacks, Stimuvax might still have a role to play if tailored appropriately.

19.4.4 Polyclonal Tumor Vaccines

19.4.4.1 IDM-2101

IDM-2101 is a ten-epitope T-cell vaccine composed of several tumor-associated antigens (TAAs). The concept behind this vaccine was based on the fact that tumors have multiple tumor-associated antigens with multiple epitopes. The main TAAs of NSCLC are carcinoembryonic antigen, p53, MAGE-2, HER2/neu, and MAGE-3. The epitopes are composed of ten peptides of the abovementioned antigens, nine of which are CTL epitopes while the tenth epitope is HLA-DR which is intended to augment the CTL response. A recent phase II study by Barve and colleagues with 63 HLA-A2-positive patients with NSCLC demonstrated no significant adverse events. A total of 13 doses was administered over a period of 2 years. One-year survival was 60 % and median survival was 17.3 months. Among the responders, there were one complete response and one partial response. A phase III trial if any is awaited [18].

19.4.5 Whole-Cell Tumor Vaccines

19.4.5.1 GVAX®

GVAX® is a vaccine composed of whole-cell tumors transfected with a non-replicating adenoviral vector engineered to secrete GM-CSF. In phase I studies with patients with stage IV NSCLC, the vaccine showed only grade 1 and 2

toxicities. During the initial phase I trial, seven patients with stable disease achieved a durable remission of more than 40 months. This prompted a further cohort study with stage IB, II, II, or IV NSCLC. In the 33 patients with advanced disease, only 3 had durable complete response lasting more than 6, 18, and 22 months. Of particular note was longer survival in patients secreting GM-CSF more than 40 ng/24 h/10^6 (median survival of 17 months, 95 % CI, 6–23 months) compared to those secreting less GM-CSF (median survival 7 months, 95 % CI, 4–10 months) ($p=0.028$) [19]. This clearly suggested an increased advantage in survival with increased secretion of GM-CSF. A subsequent trial evaluated unmodified tumor cells combined with genetically modified allogenic human erythroleukemia cell lines (K562). This combination is referred to as bystander GVAX. Forty-nine patients with advanced NSCLC received the vaccine; however, unlike the previous vaccine, none of the patients achieved a partial or complete response. It was postulated that the GM-CSF prompted the growth of myeloid-derived stem cells and impaired antigen presentation of antigen to T cells. Further studies with other tumors did not demonstrate improved survival, thus confining this treatment modality to obscurity.

19.4.5.2 Belagenpumatucel-L

Belagenpumatucel-L, also marketed as Lucanix® by NovaRx, is an allogenic whole tumor cell vaccine isolated from four irradiated and cryopreserved cell lines. The cells have been modified with antisense TGF-β2, thus causing an abrogation in the secretion of TGF which acts as a local immunosuppressant. The cell lines are NCI-H-460, NCI-H-520, SK-LU-1, and RH2. The initial success of the phase II trial heralded further analysis into Lucanix®. The phase II trial randomized three cohorts to receive three separate doses of treatment as shown in Fig. 19.12.

The results have been quite satisfying; 75 patients received a total of 550 vaccine doses as described above. The pooled stage IIIB and IV subgroup achieved an overall 5-year survival of 19 %, while stages II and IIIA reached a survival of 23 % ($n=61$) [20]. Additionally, among the responders, there was an increased release of interferon gamma as demonstrated by ELISA or ELISPOT reactions to belagenpumatucel. Within the advanced stage subgroup ($n=61$), there was increase in the overall survival as well as in the high-dose subgroups (2.5 and 5.0×10^7) [20]. In a comparative data from NovaRx, between Lucanix® and historic survivors matched to the same disease progression, the 1-year disease survival comparing Lucanix® versus topotecan as second-line single-agent maintenance therapy was 55 % versus 9 %, while the 5-year survival was 10 and 0 %, respectively, in favor of Lucanix® (analysis by Daniel Shawler, vice president of NovaRx operations) [21]. These results further prompted the phase III STOP trial which has already randomized 504 patients to receive either Best Medical Therapy (placebo) or 2.5×10^7 cells of belagenpumatucel. The injection protocol will involve once-monthly treatment intradermally for 18 months and then once at 21 and 24 months in the absence of disease. The primary endpoint is overall survival with several secondary endpoints including PFS, QOL, time to tumor progression (TTP), best tumor response, and immunological parameters

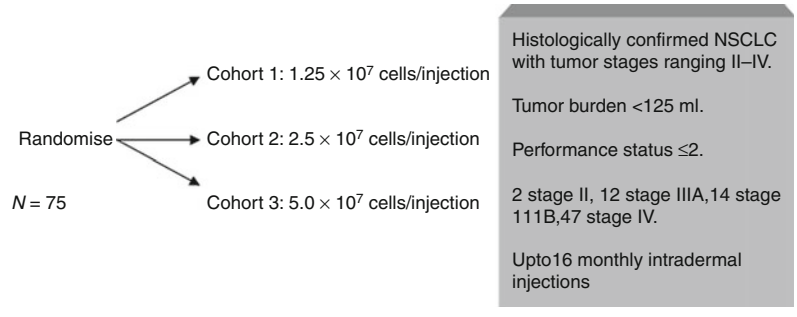


Fig. 19.12 Demonstrating the randomization and inclusion criteria of the trial

including cytokines (IFN-γ ELISPOT CD8), chemokines, and *in vitro* proliferation assays (ClinicalTrial.gov identifier NCT00676507). The study is still open; however, there have been conflicting information coming out from unblinding of some of the data which does not favor or disfavor the treatment yet.

19.4.6 TGFβ2-Antisense+rhGMCSF

This genetically designed vaccine is not unique for lung tumors only; however, the vaccine's unique design would definitely prompt mentioning in this chapter. The design of this vaccine is based upon the fact that silencing TGFβ2 and enhancing the release of GM-CSF would allow the tumor to break tolerance and allow native CTL to identify tumor cells. The vaccine was constructed from autologous tumor cells, harvested and disaggregated, and then electrocorporated with plasmid gene [24]. The resulting vaccine was subsequently irradiated and then preserved until the time of injection. Of 38 patients harvested for vaccine injection, eventually 23 patients got treated with no grade 3 or 4 adverse events. Of the ten alive patients, eight have stable disease (SD) while one patient with metastatic malignant melanoma had complete response (CR) and one patient was deemed not fit for evaluation. Additionally, the vaccine effectively knocked down TGFβ2, but not TGFβ1 with consistent expression of GM-CSF [22].

19.5 Other Treatment Modalities

19.5.1 Dendritic Cells

There are numerous articles that have used DC therapy as a mechanistic action in priming T cells as described in Table 19.7. In this chapter, I will not discuss each and every one of them. The mechanism of antigen uptake and display by MHC II and cross presentation to a more robust MHC I/CD8 system has already been well discussed (see cross presentation in previous chapters). Of particular note has been one trial undertaken by Hirschowitz et al. which demonstrated a non-conclusive correlation between immune response and tumor progression in a group of 16 patients with stage I–III NSCLC which proves the complexity of DC trials. To improve the clinical outcomes, DCs have been loaded with peptides specific to the expression profile of tumors: MUC1 in MUC1-expressing tumors or adding TRICOM (triad of co-stimulatory molecules; B7.1, ICAM-1, and LFA-3) or the use of co-inhibitory molecule inhibitors (PD-1 and CTLA-4).

19.6 Mutations

Genetic mutations in NSCLC are becoming more recognizable. Roughly 50 % of lung tumors have been attributed to a mutation, which leaves a sizeable portion of tumors still without a genetic driver mutation. This research is based from data pro-

Table 19.7 Demonstrating some dendritic cell vaccine trials in NSCLC

Author	Trial design	Result
Morisaki et al. [23]	Polyvalent, multiple-epitope vaccine against multiple tumors, RCC, NSCLC, myeloma, melanoma	Good release of interferon by ELISPOT
Hao et al.[24]	Dendritic cell-based exosomes	Proliferation-based assay shows a good CD8 titer
Hirschowitz et al. [25]	Immunization of NSCLC patients with antigen-pulsed immature DC cells	Good immune responses yet the outcome was anecdotal
Chiappori et al. [26]	Dendritic cell-based p-53 vaccine against SCLC	Good immune response and enhanced sensitivity to chemotherapy
Perroud et al. [27]	Dendritic cell-based vaccine against NSCLC	The lymphoproliferation assays were satisfactory; the response was not durable

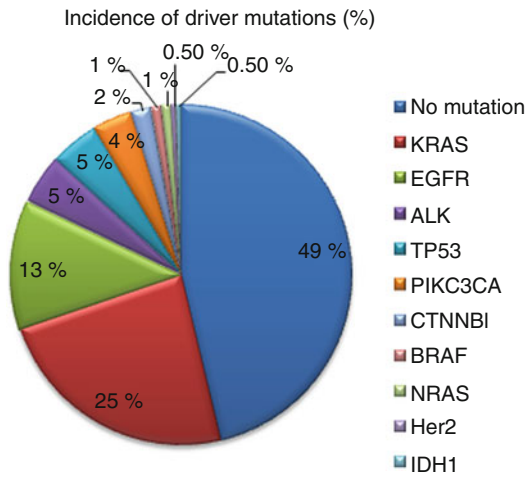


Fig. 19.13 A pie chart showing the incidence of driver mutations. *ALK* anaplastic lymphoma kinase, *PIKC* phosphoinositol kinase C, *EGFR* epidermal growth factor receptor, *IDH* isocitrate dehydrogenase, *Her2* human epidermal growth factor receptor, *NRAS* neuroblastoma RAS oncogene, Kirsten RAS GTPase, *CTTNB1* beta-catenin gene, *BRAF* B-raf, *TP53* tumor protein 53

duced by Massachusetts General Hospital which demonstrated out of 550 NSCLCs, the majority are adenocarcinomas with an identifiable mutation. A further replication of data is being undertaken by the Lung Cancer Mutation Consortium (LCMC) which aims at looking into 1,000 adenocarcinoma specimens.

The identification of a driver mutation is what makes NSCLCs or SCLCs more amenable to target therapies and hence could potentially render an unresectable tumor into a stable progression-free status and hence gives immunotherapy an opportunity. The incidence of driver mutations is shown in Fig. 19.13.

19.7 Chemoprevention

There have been several attempts to develop immunomodulatory agents, but the majority has not gone past the phase II level of investigation. In this chapter, we will focus on specific agents that have undergone at least phase III trials.

The chapter will not be able to detail the vast amount of literature relating to chemoprevention; however, I think a brief scholarly discussion will prompt the reader to look into this interesting topic further.

Chemoprevention is described as a strategy aimed at preventing tumor progression before irreversible changes to the proteome are in full progress [28]. Primary chemoprevention is defined as intervention intended to delay or prevent the development of cancer in healthy individuals. Secondary chemoprevention is aimed at patients who have been diagnosed with premalignant or dysplastic lesion, whereas tertiary chemoprevention is targeted against patients who have been diagnosed and treated for lung tumors. So far, we do not have an exact mechanistic view of tumorigenesis for lung tumors similar to colorectal carcinomas, and certainly the adenoma-carcinoma sequence or the Vogelstein model [29] would not hold in this respect. However, the evidence that substantiates the role of cigarette smoking is clear albeit not in all cases. Surrogate endpoint biomarkers as described in Table 19.8 have been described by some authors as potential niches or key markers which can be statistically measured,

Table 19.8 Classifies the surrogate endpoints according to biochemical, genetic, cellular, histopathologic, and molecular classes. These key features could be further used for disease modulation

Biochemical
IGF1 levels
Genetic
KRAS mutations, EGFR expression, HER2 expression, c-myc, p53, chromosomal loss or gain (3p,5q,11q,13q,17p)
Cellular
Proliferation markers: Ki-67
Differentiation markers: retinoic acid receptor, lectin, loss of high molecular weight cytokeratins, heterogeneous nuclear ribonucleoprotein A2/B1
Apoptosis markers: Bcl-2/Bax
Histopathologic
Carcinoma in situ, squamous dysplasia, other atypical features
Molecular
DNA methylation: promoter CpG island methylation (p16, ECAD, DAPK, MGMT, GSTP1)

ECAD E-cadherin, *DAPK* death-associated protein kinase, *MGMT* methylguanine-DNA-methyltransferase, *GSTP1* glutathione S-transferase P1, *EGFR* epidermal growth factor

and hence, interventions can be made to modulate disease progression [30]. These key endpoints are not necessarily chemopreventive agents or proteins, but their expression profile, whether genomic or proteomic, will give clues about the mechanism of tumor behavior and will promote the development of targeted therapies. The NSAIDs have become more or less consolidated in colorectal chemoprevention but that cannot be said about lung tumors.

It is worth noting that the only trial that confirms the use of chemopreventive agents is by Pastorino and colleagues, whereby 307 patients with early-stage lung tumors were randomly assigned to receive either 300,000 IU of retinyl palmitate or no treatment. After a median 46-month follow-up, the rate of second primary lung tumors (SPTs) was 39 % in the treatment group versus 48 % in the no-treatment group ($p = 0.045$ in favor of treatment progression) [31]. This further heralded the EUROSCAN trial with vitamin A and N-acetylcysteine in patients with resected pulmonary tumors, the results of which were disappointing. However, yet again, these trials were all secondary and tertiary chemoprevention trials which give us an idea that the mechanism of injury from dysplasia/metaplasia to carcinoma of the lung is a result of multiple prolonged insults across time. However, it might be that a cocktail of drugs might be required to prevent chemoprevention [32].

19.8 Concluding Remarks

This chapter will not cover the entire clinical trials that have been performed so far, but the discussion is just enough to understand the main treatment modalities that are being pioneered. As we speak, there are three major clinical trials that are awaiting results to be published. However, compared to the early days of dendritic cell therapy in the late 1990s and the current trends, we have at least two monoclonal antibodies—ipilimumab and BMS 936558—that have changed the clinical outcome of at least some cancers. Revisiting this chapter, we can clearly say that lung cancer arises from a series of insults superimposed on background mutations, treatment of which will involve tackling the mutational cascade and mounting an immune response. It is the immune response that we are focused upon, but maybe we should be expanding our search further in combining our treatment with surgery and mutation-specific chemotherapy, similar to what we do in estrogen-positive breast cancer and anastrozole therapy and obviously polyfactorial immunotherapy that involve not only the tumor cells but also the microenvironment and the immune cells. Finally, I would urge the readers to follow up on the clinical trials and possibly try to educate themselves from other tumors.

References

1. Minami S, Kijima T, Shiroyama T, Okafuji T, Hirashima K, et al. Randomized phase II trial of paclitaxel and carboplatin followed by gemcitabine switch-maintenance therapy versus gemcitabine and carboplatin followed by gemcitabine continuation-maintenance therapy in previously untreated advanced non-small cell lung cancer. BMC Res Notes. 2013;6:3.
2. Ramlau R, Gorbunova V, Ciuleanu TE, Novello S, Ozguroglu M, et al. Aflibercept and docetaxel versus docetaxel alone after platinum failure in patients with advanced or metastatic non-small-cell lung cancer: a randomized, controlled phase III trial. J Clin Oncol. 2012;30:3640–7.
3. Varey AH, Rennel ES, Qiu Y, Bevan HS, Perrin RM, et al. VEGF 165 b, an antiangiogenic VEGF-A isoform, binds and inhibits bevacizumab treatment in experimental colorectal carcinoma: balance of pro- and antiangiogenic VEGF-A isoforms has implications for therapy. Br J Cancer. 2008;98:1366–79.
4. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med. 2006;355:2542–50.
5. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol. 2010;28:3167–75.
6. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366:2443–54.

7. Pirker R, Pereira JR, von Pawel J, Krzakowski M, Ramlau R, et al. EGFR expression as a predictor of survival for first-line chemotherapy plus cetuximab in patients with advanced non-small-cell lung cancer: analysis of data from the phase 3 FLEX study. Lancet Oncol. 2011;13:33–42.
8. Abdullah SE, Haigentz Jr M, Piperdi B. Dermatologic toxicities from monoclonal antibodies and tyrosine kinase inhibitors against EGFR: pathophysiology and management. Chemother Res Pract. 2012;351210.
9. Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res. 2009;15:7412–20.
10. Lynch TJ, Bondarenko I, Luft A, Serwatowski P, Barlesi F, et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non-small-cell lung cancer: results from a randomized, double-blind, multicenter phase II study. J Clin Oncol. 2012;30:2046–54.
11. Parikh PM, Vaid A, Advani SH, Digumarti R, Madhavan J, et al. Randomized, double-blind, placebo-controlled phase II study of single-agent oral talactoferrin in patients with locally advanced or metastatic non-small-cell lung cancer that progressed after chemotherapy. J Clin Oncol. 2011;29:4129–36.
12. Kim SH, Lee S, Lee CH, Lee MK, Kim YD, et al. Expression of cancer-testis antigens MAGE-A3/6 and NY-ESO-1 in non-small-cell lung carcinomas and their relationship with immune cell infiltration. Lung. 2009;187:401–11.
13. Neninger Vinageras E, de la Torre A, Osorio Rodriguez M, Catala Ferrer M, Bravo I, et al. Phase II randomized controlled trial of an epidermal growth factor vaccine in advanced non-small-cell lung cancer. J Clin Oncol. 2008;26:1452–8.
14. Singh PK, Hollingsworth MA. Cell surface-associated mucins in signal transduction. Trends Cell Biol. 2006;16:467–76.
15. Tarp MA, Sorensen AL, Mandel U, Paulsen H, Burchell J, et al. Identification of a novel cancer-specific immunodominant glycopeptide epitope in the MUC1 tandem repeat. Glycobiology. 2006;17:197–209.
16. Butts C, Murray RN, Smith CJ, Ellis PM, Jasas K, et al. A multicenter open-label study to assess the safety of a new formulation of BLP25 liposome vaccine in patients with unresectable stage III non-small-cell lung cancer. Clin Lung Cancer. 2010;11:391–5.
17. Wu YL, Park K, Soo RA, Sun Y, Tyroller K, et al. INSPIRE: a phase III study of the BLP25 liposome vaccine (L-BLP25) in Asian patients with unresectable stage III non-small cell lung cancer. BMC Cancer. 2011;11:430.
18. Barve M, Bender J, Senzer N, Cunningham C, Greco FA, et al. Induction of immune responses and clinical efficacy in a phase II trial of IDM-2101, a 10-epitope cytotoxic T-lymphocyte vaccine, in metastatic non-small-cell lung cancer. J Clin Oncol. 2008;26:4418–25.
19. Nemunaitis J, Jahan T, Ross H, Sterman D, Richards D, et al. Phase 1/2 trial of autologous tumor mixed with an allogeneic GVAX vaccine in advanced-stage non-small-cell lung cancer. Cancer Gene Ther. 2006;13(6):555–62.
20. Neumanitis J, Dillman RO, Schwartzenberger PO, Senzer N, Cunningham C, et al. Phase II study of belagenpumatucel-L, a transforming growth factor beta-2 antisense gene-modified allogeneic tumor cell vaccine in non-small-cell lung cancer. J Clin Oncol. 2006;24:4721–30.
21. Stewart DJ. Update on the role of topotecan in the treatment of non-small cell lung cancer. Oncologist. 2004;9:43–52.
22. Olivares J, Kumar P, Yu Y, Maples PB, Senzer N, et al. Phase I trial of TGF-beta 2 antisense GM-CSF gene-modified autologous tumor cell (TAG) vaccine. Clin Cancer Res. 2011;17:183–92.
23. Morisaki T, Matsumoto K, Onishi H, Kuroki H, Baba E, et al. Dendritic cell-based combined immunotherapy with autologous tumor-pulsed dendritic cell vaccine and activated T cells for cancer patients: rationale, current progress, and perspectives. Hum Cell. 2003;16:175–82. Accession num: 15147037.
24. Hao S, Bai O, Yuan J, Qureshi M, Xiang J. Dendritic cell-derived exosomes stimulate stronger CD8+ CTL responses and antitumor immunity than tumor cell-derived exosomes. Cell Mol Immunol. 2006;3:205–11.
25. Hirschowitz EA, Foody T, Hidalgo GE, Yannelli JR. Immunization of NSCLC patients with antigen-pulsed immature autologous dendritic cells. Lung Cancer. 2007;57:365–72.
26. Chiappori AA, Soliman H, Janssen WE, Antonia SJ, Gabrilovich DI. NGN-225: a dendritic cell-based p53 vaccine (Ad.p53-DC) in small cell lung cancer: observed association between immune response and enhanced chemotherapy effect. Expert Opin Biol Ther. 2010;10:983–91.
27. Perroud Jr MW, Honma HN, Barbiero AS, Gilli SC, Almeida MT, et al. Mature autologous dendritic cell vaccines in advanced non-small cell lung cancer: a phase I pilot study. J Exp Clin Cancer Res. 2011;30:65.
28. Boghossian SM, Hawash A. Chemoprevention in colorectal cancer–where we stand and what we have learned from twenty year's experience. Surgeon. 2011;10:43–52.
29. Arends JW. Molecular interactions in the Vogelstein model of colorectal carcinoma. J Pathol. 2000;190(4):412–6.
30. Cohen V, Khuri FR. Chemoprevention of lung cancer: concepts and strategies. Expert Rev Anticancer Ther. 2005;5:549–65.
31. Pastorino U, Infante M, Maioli M, Chiesa G, Buyse M, et al. Adjuvant treatment of stage I lung cancer with high-dose vitamin A. J Clin Oncol. 1993;11:1216–22.

32. Moody TW, Switzer C, Santana-Flores W, Ridnour LA, Berna M, et al. Dithiolethione modified valproate and diclofenac increase E-cadherin expression and decrease proliferation of non-small cell lung cancer cells. Lung Cancer. 2009;68:154–60.
33. Vansteenkiste J, Zielinski M, Linder A, Dahabreh J, Gonzalez EE, Malinowski W, Lopez-Brea M, Vanakesa T, Jassem J, Kalofonos H, Perdeus J, Bonnet R, Basko J, Janilionis R, Passlick B, Treasure T, Gillet M, Lehmann FF, Brichard VG. Adjuvant MAGE-A3 immunotherapy in resected non-small-cell lung cancer: phase II randomized study results. J Clin Oncol. 2013;31(19):2396–403.

Immunotherapy of Renal and Bladder Cancers

20

Faouzia Ajili

Contents

F. Ajili, PhD
Laboratory of Human and Experimental Pathology, Pasteur Institute of Tunis, Tunis, Tunisia
e-mail: faouziaagili@yahoo.fr

20.1 Introduction

Urothelial carcinoma of the bladder leads to significant morbidity and mortality worldwide, accounting for about 5 % of all cancer deaths in humans [1]. Immunotherapy with Bacillus Calmette-Guérin (BCG) has become a first-line treatment after performing transurethral resection (TUR) on high-grade, nonmuscle, invasive bladder cancer (NMIBC) [2]. In addition, BCG reduces the risk of progression in high-risk NMIBC [3]. Moreover, immunotherapeutic approaches involving cytokines are being used to treat renal cancer.

Nonetheless, the efficacy of immunotherapy in the treatment of both renal and bladder cancer is variable. In this chapter, after briefly discussing the subtypes and staging of bladder cancers in addition to renal cancer, attention is given to the role of immunotherapy in the treatment of bladder and renal cancer and the challenges involved are discussed.

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_20, © Springer-Verlag Berlin Heidelberg 2015

20.2 Histological subtypes and staging

Approximately 90 % of bladder tumors are transitional cell carcinoma (TCC), 5–10 % are squamous, and less than 2 % are adenocarcinoma. Bladder carcinomas are heterogeneous, ranging from superficial papillary tumors to invasive carcinomas.

20.2.1 Nonmuscle Invasive Bladder Tumor

Nonmuscle invasive bladder tumors are carcinomas that do not infiltrate the bladder muscle and represent 70–80 % of bladder tumors. They consist of stage Ta or T1 papillary tumors and carcinoma in situ (CIS). Stage Ta tumors reach the epithelial layer of the bladder, while T1 stage tumors slightly infiltrate the lamina propria. T1 stage tumors are of greater concern than Ta stage, especially stage T1 grade 3 tumors, which are likely to recur quickly and move to a higher stage at recurrence. The CIS shows a high recurrence rate and is often a sign of rapid progress toward infiltration. CIS is in fact known as the superficial type, the most damaging of all types of non-muscle, invasive bladder cancer (NMIBC). However, it is rare and accounts for less than 10 % of NMIBCs diagnosed.

20.2.2 Invasive Bladder Tumor

These tumors invade the lamina propria and reach at least the bladder muscle (stage T2). They can extend to the perirenal fat bladder (stage T3), or even invade nearby organs such as the prostate (stage T4). They require more aggressive therapeutic measures, such as radical cystectomy with or without systemic chemotherapy. Immunotherapy has no valid effect on this type of tumor.

20.3 Clinical Use of BCG Immunotherapy for NMIBC

Bacillus Calmette–Guerin (BCG) is the most common intravesical therapy for treating NMIBC. BCG is a live attenuated strain of *Mycobacterium bovis* developed in 1921 as a vaccine for tuberculosis, and BCG has since been given to people as vaccination against tuberculosis. Since its first use in 1976, *Mycobacterium bovis* BCG has been established as the most effective adjuvant treatment for preventing local recurrence and tumor progression, following transurethral resection (TUR) of NMIBC. However, its effectiveness has been both variable and unpredictable. Despite nearly 40 years of clinical use, the mechanism(s) by which the intravesical administration of BCG results in elimination of bladder tumors remains undefined. Although BCG is currently regarded as the most effective treatment available for the management of NMIBC, up to 40 % of patients do not respond to treatment and are at risk of disease recurrence and progression. Unfortunately, predictive markers for recurrence and progression are lacking. In patients with intermediate to high-risk bladder cancer, such as those with high-grade Ta/T1 or CIS, BCG is often given due to the higher risk of disease recurrence and progression. The clinical efficacy of BCG has been demonstrated in a number of randomized trials and meta-analyses. Adjuvant administration of BCG after TUR has been shown to prevent both recurrence and progression compared with TUR alone or TUR with intravesical chemotherapy.

20.3.1 History

Bacillus Calmette–Guérin was used for the first time in 1921 as a vaccine against tuberculosis, and its mycobacterial antitumor effect was observed in 1929 by Pearl [4]. In fact, TB patients developed fewer malignancies than the general population. Coe and Feldman in 1966 [5] showed that the bladder might be the site of delayed hypersensitivity reactions in addition to the skin.

In 1970, BCG was used to treat cases of metastatic melanoma [6]; then, in 1974, Hanna et al. [7] demonstrated the antitumor effect of BCG on the hepatocarcinoma. They defined the foundations of local immunotherapy: a sufficient quantity of live bacilli; direct and prolonged contact between BCG and the tumor; the tumor volume after resection should be as small as possible.

In 1975, by using local BCG therapy, deKernion reported the first treatment of a bladder tumor, a metastatic malignant melanoma. This result led Morales et al. [8] and Martinez-Pineiro et al. [9] in 1976 to test the prophylactic effect of BCG on superficial bladder tumors.

These encouraging results were confirmed by a study by a group of doctors from Laval University [10]. The first controlled study confirming the efficacy of BCG was reported by Lamm et al. [11]. Since then, treatment with intravesical instillation of BCG has proved to be the most effective therapeutic agent in treating superficial bladder tumors, especially carcinoma in situ (CIS) [12]. This efficiency has led its approval by the US Food and Drug Administration agency in 1990.

Arbitrarily, it was decided to perform this treatment as six weekly intravesical instillations. The mode of administration of the treatment has been constantly optimized; nonetheless, the ideal mode has not yet been determined. In addition, it was found that the peak of the immune stimulation was located after the fourth week of instillation, also suggesting that cycles of four instillations might be sufficient instead of six [13]. Maintenance treatment, adding cycles of three instillations every 6 months after the first normal cycle of six instillations showed good results on both progression and recurrence [14, 15].

20.3.2 Effectiveness

Bacillus Calmette–Guérin immunotherapy has particularly improved disease-free survival time and reduced tumor progression, but a subset of patients remains refractory. In these patients, treatment with BCG has no effect on relapse or progression and may instead be a source of lost time in the indication of cystectomy. BCG is a complex organism whose introduction into the body leads to a significant and nonspecific stimulation of the immune system. Viability, the instilled dose, and the schedule of instillations all have an impact on the immune response after BCG administration.

Six independent studies involving a total of 585 patients showed that the recurrence was less frequent among those who had undergone resection and treatment with BCG compared with those who have had a resection (a recurrence rate of 29 % instead of 67 %) [16].

A study by Lamm [17] comparing the response to treatment according to BCG strain, comprising 1,496 patients with CIS, showed a complete response to 1,082 of these (72 %). A controlled study by the South West Oncology Group (SWOG), comparing the use of doxorubicin and BCG, showed that progression was reduced by the latter from 37 to 15 % [18].

A long-term study lasting 10 years [17] showed that the increase went from 63 % for the group treated only by TUR to 38 % for the group treated with the TUR combined with BCG ($p=0.0063$) group. Mortality among these same groups decreased from 45 to 25 % ($p=0.03$) [19].

20.3.3 Side Effects

Bacillus Calmette–Guérin consists of a pathogenic strain, which, although attenuated, causes an infection that has mainly mild or moderate side effects such as cystitis (67 %), hematuria (23 %), moderate fevers (25 %), and high and increased urinary frequency (71 %) [16].

A study by Lamm [20] on 1,278 patients treated with BCG, reported a frequency of acute cystitis of 91 %; side effects can be serious and in rare cases cause a systemic infection, especially if instillation is administered in the presence of residual sores from the TUR that have not completely healed. Granulomatosis, multivisceral, lung, liver, kidney, or otherwise, may occur on rare occasions. It is not known whether this reflects a real bacillary sepsis infection or if it is the reflection of an immunological reaction of delayed hypersensitivity.

A clinical phase III study comparing treatment response depending on the dose of BCG (75 or 150 mg) showed that a lower dose led to a similar antitumor effect, while minimizing side effects [21, 22]. In the same vein, Lamm et al. [23] showed that increasing the dose of BCG was accompanied by a decrease in the antitumor

effect in a murine model; therefore, the relationship between the dose of BCG and the antitumor effect was bell-shaped.

In summary, the current consensus indicates that BCG is administered as primary treatment for CIS, which are the most aggressive superficial bladder tumors, but against which treatment with BCG gives the best results. For tumors with a low risk of recurrence and progression, including low tumor grade and stage, TUR is the best treatment, with or without intravesical instillation of mitomycin C or adriamycin in the hours after TUR.

In the event of recurrence, BCG treatment is recommended, especially when accompanied by a maintenance cycle of three instillations every 3 months. For potentially recurrent tumors, treatment with BCG still yields better results than intravesical chemotherapy, but the side effects are more pronounced.

20.3.4 Mechanism of the Antitumor Effect

The BCG infection causes the sloughing of superficial cells off both the normal and the cancerous bladder. It has now been agreed to take into consideration that the antitumor activity of BCG is run by the local nonspecific immune response of immunocompetent cells [24]. Several immunogenic aspects have been studied after BCG instillations, such as infiltration of the cell wall by the effector cells [25], the involvement of Cytotoxic T lymphocytes [26], the major histocompatibility complex or expression of adhesion molecules on the urothelial cells [27], and the secretion of cytokines. None has been clearly implicated in the antitumor activity of BCG.

Instillation of BCG results in an increase in the granulocytes in the bladder wall followed by the T cells, mainly $CD4^+$ T cells. The proinflammatory phenotype of Thl (IL-2, IL-12, IFN) dominates after stimulation with BCG [27] and it is assumed that this phenotype accompanies a favorable response [28]. Macrophages and other antigen-presenting cells are stimulated after treatment with BCG; it appeared that urothelial cells also internalize mycobacteria and were involved in antigen presentation and cytokine secretion [29].

Another study evaluating the lymphocyte response after instillation of a high or low dose of BCG, or low, but accompanied by IFN-α-2b, showed no difference in the quality or quantity of the immune response driven by these three types of treatment [30]. Forty percent of people who do not respond to BCG have been successfully treated with instillation of IFN-α-2b [31] and more side effects brought about by this type of treatment are less pronounced with the addition of BCG [32].

All cytokines produced after treatment are of the IL-6 type, TNF-α, and IL-1-P, a chemokine (IL-8), and a growth factor (granulocyte macrophage colony-stimulating factor [GM-CSF]). On the other hand, no cytokine associated with the Th pathway was constitutively produced after stimulation. This is because the Th cytokines are produced mainly by immune cells after stimulation. BCG and IFN-α-2b seem to have different and independent stimulatory effects on cytokine production by tumor cells in the bladder [33].

In the mouse model, activated macrophages from the strain susceptible to BCG infection, produce more IL-10 than macrophages activated in the strain resistant to infection. The induction of IL-10 by the pathogen is likely to reduce its immunogenic response to the infected host [34]. In addition, a synergistic effect was observed in the antitumor effect when immunotherapy was performed with BCG/IFN-α blending (murine model). While both types of treatment led to increased levels of $CD4^+$ and $CD8^+$ T cells, the mixture resulted in the most significant increase in αβ T cells [30].

In addition, Hara et al. [35] showed that the antitumor effect was canceled in a mouse model deficient in $CD4^+$ T cells, but not in $CD8^+$ T cell-deficient models, implying that $CD4^+$ T cells are absolutely necessary, unlike $CD8^+$ T cells. In addition, BCG increases the production of the chemokines MCP-1 and RANTES, two chemoattractants that stimulate the cytostatic response of memory T cells against tumor and the release of lysosomal enzymes [36].

20.3.5 Action of BCG

The interaction of BCG with the luminal surface of the urothelium is the first stage of infection. Accumulation of BCG near the bladder wall and its adhesion factors limit an adequate clinical response. Modulation of the adhesion of BCG to the bladder influences the response induced in mice [37].

This membrane adhesion may be due to nonspecific physicochemical interactions between the wall of the urothelium and BCG or involving specific interactions between receptors and their ligands, such as fibronectin and glycosaminoglycans. For example, the attachment of BCG to the bladder involving fibronectin was blocked by antifibronectin antibodies or by the addition of soluble fibronectin [38]. When adhesion is blocked, there are no hypersensitivity reactions and no tumor rejection. From this contact, mycobacteria are either phagocytosed by macrophages or internalized in tumor or urothelial cells [29].

The local immune response is closely related to the interaction of three systems: the host (the patient), BCG (mycobacteria), and tumor. This interaction leads to a cascade of immunological events, some of which are essential for the protective effect of BCG against recurrence and progression. It is currently thought that there are three phases in the immune response to BCG.

20.3.6 The Role of the Host

The immune response to infection by intracellular germs such as BCG varies and is probably related to the host. In mice, a resistance gene to BCG vaccination has been identified. This natural resistance-associated macrophage protein (*Nramp 1*) gene is involved in T cell response to vaccination by mycobacteria [39]. The product of this gene expressed by the macrophages plays a role in molecular expression, MHC class II, antigen-presenting in humans and potentially in the inflammatory response [39, 40]. In addition, Nramp1 could control the replication of intracellular bacteria through the cell produced by infected phagosomes [41]. It may also inhibit the development of mycobacteria by promoting the production of nitric oxide (NO), a potent antimycobacterial agent [42].

In humans, the functions encoded by Nramp 1 in the mice would be under the control of many genes, some of which have been isolated on the short arm of chromosome 2 (2q35) [43]. The identification of these genes and their polymorphism may be useful in the future for the prediction of response to BCG. This polymorphism is interesting, as HLA class II antigens are involved in the response to mycobacteria [44].

20.3.7 The Immune Response to Mycobacteria and BCG

The immune response to mycobacteria is related to infection of antigen-presenting cells (monocytes, macrophages, DCs) and is associated with the production of cytokines such as IFN, IL-12, and IL-15. These cytokines are involved in the activation of T helper cells ($CD4^+$) and may be involved in the production of T cell helper 1 (Th1) cells (producing IFN and IL-2) associated with a localized form of TB or T helper 2 (Th2) cells (producing IL-4 and IL-10) and associated with a generalized form of the disease [45]. During intravesical BCG instillation, three phases can be distinguished: initiation, the internalization phase of antigen presentation, and the cytotoxic phase. These last two correspond to the effector phase.

The induction phase is characterized by the contact between BCG and the urothelium. The increased binding to fibronectin may increase the activity of BCG. This mycobacteria adhesion can also be carried out by glycosaminoglycans. From this contact, the bacteria may be phagocytosed by macrophages that belong to the group of antigen-presenting cells or internalized in urothelial cells or tumor cells [46]. This stage involving the adhesion, penetration, and activation of antigen-presenting cells is an important step in the response to mycobacteria and the response to BCG [47].

The effector phase is characterized by the presentation by antigen-presenting cells (APC) to T helper cells, certain proteins of BCG produced by degradation (immunogenic protein), followed by

the activation of cytotoxic cells. After mycobacteria infection, macrophages associated with other antigen-presenting cells, which include the urothelial cells [47, 48], manufacturent antigens and a number cytokines (IL-1, IL-6, IL-8, IL-10, IL-12, TNF, IFN) are activated [49, 50]. These cytokines are critical to the recruitment of immune cells (T lymphocytes, macrophages, and neutrophils), which infiltrate the bladder wall in large numbers during instillation [51] in parallel with the over-expression of adhesion molecules (ICAM-1) and co-stimulatory B7 molecules. These cytokines probably amplify the phenomena of antigen presentation. Soluble forms of these adhesion molecules (ICAM-1) are also found in the urine after instillation of BCG, in addition to the over-expression of molecules of major histocompatibility complex (MHC) class I and II by urothelial cells [52]. The MHC class I and II molecules are involved in the phenomena of antigen presentation, as exogenous antigens are usually presented by MHC molecules and class II antigens are expressed by endogenous molecules of MHC class I. The MHC class II molecules are expressed only by APC (macrophages, monocytes, B lymphocytes, DCs, endothelial cells), while MHC class I molecules are expressed by all other cells (except red blood cells and oocytes). These exogenous antigens are degraded by lysosomes and then present on the surface of antigen-presenting cells, bound to the MHC class II molecules. This antigen peptide and MHC class II molecules are then presented to $CD4^+$ (T helper) [53].

In this system, IFN stimulates the power of phagocytosing macrophages and their production of endotoxins. These phenomena are associated with the over-expression of adhesion molecules ICAM-1, LFA3 (APC) and co-stimulatory B7-1 and B7-2 (APC), CD28 (T cells); they probably amplify the response associated with the phenomena of antigen presentation [54]. The antigens are linked to endogenous MHC class I molecules after being prepared in the endoplasmic reticulum and sent to the cell surface, where they are recognized by CD8 lymphocytes [55]. Cytokines thus promote the cytotoxic action of lymphocytes [56], and, in addition to the cytotoxic activity, they protect lymphocytes against tumor cells [57, 58].

Mycobacteria preferentially induces cytokine responses corresponding to a Th1 (IL-2, IL-12, IFN), which is a favorable response to the development of cellular immunity [59, 60]. The Th1 promotes the expansion and proliferation of cytotoxic cells and is characterized by the expression of certain cytokines such as IL-2 or IFN. This Th1 (IL-2, IFN) was detected in the urine of patients after intravesical BCG instillation and is related to the prognosis of the disease [51]. Similarly, after intravesical BCG instillation, overproduction of the RNA messenger for IL-2 occurs in peripheral cells, a phenomenon which correlates with good response to BCG [61]. However, the response to BCG is probably not linear. In addition, it probably not only correlates with instillation doses, but is also related to the instillation protocol.

Some animal experiments suggest that high doses might be capable of inducing immunosuppression and production of Th2-type cytokines [14]. Experimentally, the increase doses cause an inversion of the response, probably associated with the suppressive response of Th2 cells characterized by the production of IL-4, IL-10, IL-5, and IL-6, an immune response to favorable humoral immunity [61]. Also, the production of IL-4 could promote the growth of B lymphocytes, activate complement (C3a, C5), and reduce the expression of IL-1 and TNF.

Cytokines induced by BCG regulate cellular response to cytotoxic vocation, wish is an integral part of the effector phase. Cytotoxic cells most frequently described in the bladder wall after intravesical instillations are $CD8^+$ T cells. Experimentally, lymphocytes ($CD4^+$ and $CD8^+$) are essential to the development of a response against mycobacteria [62]. $CD8^+$ cells seem to have a cytotoxic effect through adhesion molecules (ICAM-1) and/or via the Fas system present on target tumor cells. The population of $CD4^+/CD8^+$ was increased in the bladder after intravesical instillation, with a predominance of $CD4^+$ T cells. $CD4^+$ lymphocytes produce cytokines capable of inducing the maturation of Cytotoxic T lymphocytes. The antitumor activity

is related to $CD4^+$ and the interaction between Fas and CD40 ligands. Indeed, the interaction between CD40 (membrane glycoprotein of the TNF receptor family) and its ligand may play a major role in the activation of cytotoxic T lymphocytes and promote the Th1 response [63]. Expression of the CD40 ligand on the surface of T lymphocytes may increase monocyte survival by protecting apoptotic phenomena at sites of inflammation [63]. Moreover, the expression of CD40 at the tumor cell surface could act as a replacement to antigen-presenting cells by promoting apoptosis induced by $CD4^+$ cells expressing CD40 ligand on its surface. Similarly, the expression of Fas ligand on the surface of $CD4^+$ lymphocytes is able to induce tumor apoptosis.

Several other cytotoxic cells have been obvious: neutrophils, NK cells, BAK, LAK cells, and gamma delta (γδ). Neutrophils are the cells that are more abundant in the bladder wall after intravesical instillation of BCG [64]. These cells are capable of producing cytokines or soluble cytokine receptors, such as interleukin 1. Soluble IL-1 receptor is capable of reducing the production of IL-1, IL-8, and TNF-α; therefore, the immune response is decreased [64]. Other cells (BAK, LAK) play a direct cytotoxic role against urothelial tumor cells *in vitro* [65]. These cells co-expressed $CD8^+$ and $CD56^+$ markers on their surface with the possibility of producing IL-12 and initiating an effective antitumor response. Their mechanism of action may involve the Fas-L/Fas system or perforin/granzyme A and B.

In vitro study of the urothelium after BCG treatment has highlighted the role of NK cells and has led to the hypothesis that cytotoxic effector cells are probably of a different nature [66]. Thus, lymphocytes, which are specifically activated by mycobacteria and have cytolytic activity against tumor cells *in vitro*, could play this role [67]. These lymphocytes do not usually express the $CD4^+$ or $CD8^+$ phenotype and their ability to recognize the antigen is not restricted by MHC. This cytolytic activity seems to be reactivated in a second contact with BCG and may be involved in the quality of response to BCG by a memory-associated phenomenon. In addition, these cells have the ability to stimulate other lymphocyte populations ($CD4^+$, $CD8^+$) in response to antigenic stimulation [68]. Some authors have shown that BCG was able to induce the maturation of DCs from circulating mononuclear cells and modulate the expression of the CD40 molecule on the cell surface of urothelial tumors [69]. The expression of CD40 participates in the activation of T helper cells and sensitizes tumor cells to apoptosis via mechanisms involving the Fas-L/Fas system; over-expression of CD40 and activation of the CD40/CD40-L system may also contribute to the activation of B lymphocytes and NK cells.

20.3.8 The Role of Tumors

There are probably resistance mechanisms developed by the tumor, allowing it to escape immune host surveillance, and also treatment with BCG. In a functional immune system, Cytotoxic T lymphocytes (NK, CTL, CD8 LAK, CD4) are capable of inducing tumor apoptosis through the perforin and granzyme A or B systems via the Fas-L/Fas system. These systems are sometimes nonfunctional. Thus, Cytotoxic T lymphocytes may have perforin, granzyme A or B, and Fas-L/Fas system deficiency and may not be active against the tumor [70]. The tumor can also escape from this system by reducing the co-stimulation molecules (B7) or accession (ICAM-1) on its surface molecules required for tumor antigen presentation to Cytotoxic T lymphocytes. It can also escape the lower antigen MHC class I system at its surface (abnormal transport proteins TAP-1), depriving cells of their cytotoxic potency. The loss of normal function of P53 involved in cell apoptosis and DNA damage repair [71] can also prevent the natural phenomena of apoptosis initiated by Cytotoxic T lymphocytes and interfere with the activity of BCG. Nonetheless, the tumor may also attack the immune system. The production of immunosuppressive cytokines such as IL-10 or TGF-β1 or molecules such as Fas ligand, which are capable of inducing apoptosis of activated T cells could promote tumor growth [70]. This is a better understanding of the principles and mechanisms involved in the

antitumor response of BCG, which can help to guide the clinician toward local immunotherapy.

20.3.9 Scheme of Optimal Therapy

The optimal treatment BCG scheme remains to be defined. Indeed, many protocols have been proposed (six instillations, eight instillations, 6+6, and finally the use of maintenance therapy). There are more variations owing to the additional routes of administration (intradermal) or related to the different BCG strains used. Initial studies seeking to validate the interest in maintenance therapy did not show any significant difference. They were involved low numbers or had a period of poor monitoring. The results reported by Dr Lamm have reignited the debate on maintenance therapy and confirm that treatment with six weekly instillations is not the optimal scheme. The use of maintenance therapy with three additional weekly instillations added at 3, 6, 12, 18, 24, 30, and 36 months may improve the outcome in terms of survival without recurrence. This effect is mainly discernible in the mean time until recurrence (36 months without maintenance therapy and 77 months with maintenance therapy) [72].

20.3.10 Predictors of the Outcome of Nonmuscle Invasive Bladder Cancer

Nonmuscle invasive bladder cancer carries a high risk of recurrence and a 10–30 % disease progression rate. Multiplicity, tumor size, and prior recurrence rates are the most important predictors of recurrence, while tumor grade, stage, and CIS are the most important predictors of progression. Although BCG is currently regarded as the most effective treatment available for the management of NMIBCs, up to 40 % of patients do not respond to treatment and are at risk of disease progression. Unfortunately, predictive markers for recurrence and progression are lacking. Prediction of recurrence or progression would be of great clinical benefit. It is the combination of clinical, pathological, molecular, and immunological markers that will allow us to more accurately predict the risk of BCG failure before commencing treatment.

20.3.11 The Clinicopathological Factors Predicting Recurrence and Progression

Although tumor grade and stage are the most accurate prognostic factors in the evaluation of NMIBCs, they cannot always predict the true biological potential of the tumor, as superficial tumors of the same stage and grade may have completely different clinical courses. The European Organisation for Research and Treatment of Cancer (EORTC) scoring system and the risk tables were adopted by the European Association of Urology (EAU) guidelines to better stratify the patients at risk of recurrence and progression and to aid future treatment options by using factors that can be easily applied clinically. The risk calculator is available at the EORTC website at www.eortc.be/tools/bladdercalculator. The EORTC scoring system gives scores to factors such as the number of tumors, tumor size, the prior recurrence rate, stage, the presence of CIS, and grade, and then totals the scores. Efforts have been made to identify other potential prognostic markers that may better stratify and identify the true malignant potential of bladder cancer.

20.3.12 Grade

Although high tumor grade has always been associated with worse outcome after BCG immunotherapy, in most reports this factor did not correlate with the time to tumor recurrence or progression in either univariate or multivariate analysis [73, 74]. On the other hand, Ajili et al. [75] failed to find a significant association between grade and response to BCG immunotherapy.

20.3.13 Stage

Several studies, both univariate and multivariate analyses, have shown that high tumor stage is associated with a poor BCG immunotherapy

response. Some authors showed a correlation with time to recurrence [76]. Other studies showed a correlation with time to tumor progression (TTP) [77]. However, other large studies failed to find any association between tumor stage and the BCG immunotherapy response [73, 78]. Ajili et al. [79] showed that high tumor stage is significantly associated with a worse BCG immunotherapy response in univariate analysis ($p=0.009$). In addition, Kaplan–Meier survival curves show reduced relapse-free survival (RFS) for patients with a pT1 tumor (log-rank test $p=0.004$).

20.3.14 Multiplicity

Multiplicity is a classical prognostic factor for the recurrence of NMIBCs, but its predictive value for BCG immunotherapy response is controversial. Some studies, including Ajili et al. [75, 80, 81], have suggested that multiplicity might be an independent factor for recurrence after BCG treatment. However, most studies that showed no correlation were probably underpowered [82].

20.3.15 Gender

Several studies with larger cohorts have suggested an association between male gender and a more favorable response to BCG immunotherapy, but the gender difference was never statistically significant [81]. However, Fernandez-Gomez et al. [83] found an association between gender and BCG response in multivariate analysis, in such a way that male patients had a significantly longer time before recurrence than female patients.

20.3.16 Age

Although age has been the patient characteristic most frequently associated with BCG immunotherapy response [84], several studies have not shown any influence of age [80, 85]. Joudi et al. [86] reported that aging appears to be associated with a decreased response to BCG immunotherapy and is particularly apparent in patients older than 80 years. Cho et al. [87] have maintained that younger patients appear to have a more favorable prognosis.

20.4 Markers Predicting Response to BCG

20.4.1 Cell Cycle Regulators

20.4.1.1 P53 Protein

The most frequent molecular events in human NMIBC are mutations of the *p53* gene. The p53 protein plays a vital role in the regulation of the cell cycle and is important for genetic stability, cell proliferation, apoptosis, and angiogenesis [88]. A defect in p53 leads to the loss of p53-dependent apoptosis and gives a proliferative advantage. Some studies found that altered p53 gradually increased from normal urothelium to NMIBC to CIS to MIBC [89, 90]. However, there are some contradictory reports regarding the prognostic value of p53 in bladder cancer [91, 92]. Moreover, p53 expression was significantly associated with tumor stage, grade, and disease recurrence.

20.4.1.2 P27 Protein

The cyclin-dependent kinase (Cdk) inhibitor p27 (also known as KIP1) regulates cell proliferation, cell motility, and apoptosis. In cancers, p27 is inactivated through impaired synthesis, accelerated degradation, and by mislocalization. Moreover, studies on several tumor types have indicated that p27 expression level has both prognostic and therapeutic implications. In patients with NMIBCs, p27 has limited predictive value [93, 94].

20.4.1.3 The Retinoblastoma

The retinoblastoma protein (pRB) is a tumor suppressor involved in cell cycle control. The predictive power of pRB may be inferior to other cell cycle regulators in NMIBCs [94]. Esuvaranathan et al. [95] found that the low expression of pRB in patients treated with BCG and IFN-α is associated with a high recurrence rate. In a homogeneous population of T1G3 patients, Cormio et al. [96] demonstrated that an altered RB was associated with decreased progression-free and disease-free survival. Overall,

altered RB expression may serve as a predictive maker of BCG treatment outcome.

20.4.2 Apoptotic Markers

Apoptosis is the distinctive form of programmed cell death that complements cell proliferation in maintaining normal tissue homeostasis. Several proteins are involved in the regulation of apoptosis and their abnormal expression is associated with an altered balance between cell growth and cell death. The significance of constitutive apoptosis in the recurrence of NMIBCs has yet to be investigated. So far, little attention has been paid to the potential role of apoptotic protein expression in superficial bladder tumors treated by BCG immunotherapy.

20.4.2.1 Bcl-2 Protein

Bcl-2 is an apoptotic marker that controls ion channels, caspase status, and cytochrome c location. Bcl-2, caspase-3, p53, and survivin have a cooperative effect on the progression of bladder cancer. In published urothelial carcinoma data, the expression of Bcl-2 varies considerably. Its incidence ranges from 69 % to less than 2 % in some studies, including muscle-invasive tumors [97]. Taking into consideration the association between the over-expression of this anti-apoptotic protein and the tumor characteristics, including stage and grade, the reported data are contradictory [25, 26, 30]. Gonzalez-Campora et al. [98] found that Bcl-2 over-expression was associated with low-grade NMIBCs, while none of the high-grade superficial tumors expressed Bcl2. On the other hand, in multivariate Cox regression analysis, Bcl-2 was found to be an independent factor for recurrence [99].

20.4.2.2 Bax Protein

Bax protein is known to play a pro-apoptotic role. Its expression varies considerably in human tumors and the significance of its role in tumor progression and outcome remains generally unknown. In nonmuscle invasive bladder cancer, it was shown that Bax over-expression was an independent predictor of overall survival [98]. No data are available on the clinical outcome of NMIBCs with regard to the Bax status in tumor urothelial cells treated by local immunotherapy. In univariate analysis, Ajili et al. [99] showed that decreased or absent Bax expression was associated with low-grade tumors and a favorable outcome after treatment. To select the best predictors of recurrence among all the aforementioned variables, multivariate Cox regression analysis was performed. It showed that decreased or absent Bax expression was a significantly favorable independent factor for response to BCG therapy.

20.4.2.3 Survivin

Survivin is an important apoptotic marker. Some authors found that survivin expression analysis might identify patients with NMIBC at high risk of disease recurrence and progression [100]. Moreover, survivin over-expression increased gradually from NMIBC to advanced bladder cancer to metastatic lymph node tissue [100].

20.4.3 Angiogenesis Markers

Angiogenesis is the formation of new capillaries from the existing vascular network and is essential for tumor growth. This process is tightly regulated by angiogenic stimulators, such as fibroblast growth factor, and some angiogenic inhibitors. In various tumor types, angiogenesis is a prognostic factor determining the biological behavior. Indeed, various mechanisms are involved in the angiogenic process, with convergence of these signals permitting transduction and subsequent activation of pathways that promote tumor proliferation, migration, invasion, and ultimately, survival and metastasis.

Several angiogenic markers, including thrombospondin-1 (TSP-1), CD34, and vascular endothelial growth factor (VEGF), are currently thought to be of clinical importance for bladder cancer.

20.4.3.1 Vascular Endothelial Growth Factor

Vascular endothelial growth factor promotes endothelial mitogenesis and migration, extracellular matrix remodeling, increased vascular permeability, and the maintenance of newly formed vasculature. Higher VEGF expression was

associated with increasing tumor stage, grade, progression, and recurrence in patients treated with TUR [101]. These findings support the role of VEGF in bladder tumorigenesis and further support it as a potential target for therapy [102].

20.4.3.2 Thrombospondin-1

Thrombospondin-1 is a potent inhibitor of angiogenesis that is independently associated with disease recurrence [101, 102]. Grossfeld et al. [103] previously reported that tumors with p53 alterations are associated with low TSP-1 expression, and that these tumors are more likely to demonstrate high microvessel density (MVD) counts.

20.4.3.3 Platelet/Endothelial Adhesion Molecule

Platelet/endothelial adhesion molecule, also known as CD34, is an endothelial antigen that has been used to highlight the density of intra-tumorous vessels as a direct marker of the degree of MVD of neoangiogenesis. It has been investigated in many other malignancies and is thought to be an important prognostic factor in some locations; however, there are only a few studies dealing with it in urothelial carcinoma. In bladder urothelial carcinomas, most reports are related to muscle-invasive tumors. Indeed, MVD has been extensively investigated in these tumors as a prognostic tool and has been associated with a poor outcome. Ajili et al. [79] showed that MVD was a significantly unfavorable independent factor for response to BCG immunotherapy. Indeed, recurrence was lowest for those with the lowest MVD count and highest for those with the highest MVD. MVD, a surrogate marker for angiogenesis, has been demonstrated to be a prognostic marker associated with the highest risk of recurrence and failure of BCG immunotherapy in patients with NMIBCs.

20.4.4 Inflammatory Markers

20.4.4.1 Dendritic Cells

Dendritic cells have been suggested to play an important role in the response to mycobacteria. Indeed, BCG provokes inflammation involving the contribution of cells associated with the innate immune response. A high number of urinary DCs seem to have a positive effect on the outcome of BCG treatment [104]. On the other hand, the risk of recurrence decreases with high RNA expression of antigen-presenting molecules in normal urothelial cells [105]. In patients with a weak initial immune response, determined by low levels of $CD83^+$ tumor-infiltrating DCs, maintenance BCG proved to be highly effective by activating immune cells [106].

20.4.4.2 Tumor-Infiltrating Macrophages

Extensive infiltration of tumor-infiltrating macrophages has been reported to correlate with a good prognosis in various types of cancer [107] and in nonmuscle invasive bladder cancer. High numbers of TAMs seem to play a negative role in BCG response [106]. Ajili et al. [108] showed that the increased TAM was a significantly unfavorable, independent factor of response to BCG immunotherapy. Indeed, patients with a high TAM count showed a higher risk of recurrence than those with a low TAM count. These data suggest that determination of TAM count might be of value for predicting clinical outcome or prognosis, and that patients with NMIBCs expressing a high TAM count might be less sensitive to BCG immunotherapy. On the other hand, other studies have reported that patients with a high level of infiltration by $CD68^+$ TAMs do not respond to BCG immunotherapy either [106].

20.5 Immunotherapy of Renal Cancer

Immunotherapy has provided the basis for experimental strategies that have introduced new indications for cytoreductive surgery in patients with metastatic renal cell cancer.

20.5.1 General Treatments for Metastatic Renal Cancer

Considering the low efficiency of even aggressive, local treatments, improvement in the prognosis of

metastatic renal cancer is inevitably highlighted by the development of treatments with a general aim.

20.5.1.1 Hormonotherapy

The therapeutic option of hormonotherapy is based on experimental findings obtained by using progesterone to block kidney tumors induced in hamsters. Apart from a few isolated results [109], hormonal treatment using progesterone has been proven ineffective as adjunctive therapy for metastatic kidney cancer [110].

20.5.1.2 Chemotherapy

Up to now, no cytotoxic chemotherapy has revealed regular efficiency, either as mono- or as chemotherapy [111]. Current efforts tend to reduce the toxicity of chemotherapies, as well as to increase their effectiveness. The issuance of chemotherapy through implantable pumps may be programmed to limit toxic effects [112]. Based on the principles of chronobiology, this method has been used in the treatment of metastasized kidney cancer with encouraging results: 7.1 % complete response and 12.5 % partial response in a series of 56 patients, confirming the efficacy of treatment with continuous systemic infusion of floxuridine (FUDR), with few side effects.

20.5.1.3 Traditional Immunotherapy

The importance of the relationship between the host and the tumor has long been emphasized in kidney cancer, in particular because of evidence of the spontaneous regression of metastases.

These relationships play prominent roles leading to the development of therapeutic immunotherapy. Traditional immunotherapy is active in that it exogenously stimulates the host immune system. This type of immunotherapy may be specific, directed against a tumor-specific antigen, or nonspecific.

To increase nonspecific host immunity, several molecules have been used on metastasized kidney cancer. The main, active, nonspecific immunotherapy trials have mostly used BCG [113]. Other molecules that have immunomodulatory action were used with polyinosinic-polycytidylic acid (poly I:C), which increases the cytotoxicity of lymphocytes by inducing the production of interferon [10], or with coumarin, which affects mitogen on lymphocytes, and cimetidine, which has an inhibitory effect on T lymphocyte suppressor [114]. The results of this active immunotherapy do not vary depending on the specific molecules used: little effect for some (poly I:C) or few answers for others (BCG).

Good results have been obtained with the coumadin–cimetidine combination, with 3 complete responses and 11 partial responses in a series of 42 patients (33 % overall response), but the results obtained in this preliminary study have not been confirmed by other teams, and seem to have no impact on survival, as in all cases, this tumor regression is transient [114].

Specific active immunotherapy seems very effective. However, conflicting results were obtained. A significant gain in survival was shown in a group of 71 patients who had nephrectomy followed by monitored tumor vaccinations associated with particular dietary supplementation [115], but these results have not been confirmed, and other studies have shown inefficiency in terms of immunotherapy-specific active survival.

Among them, in a study involving 33 patients receiving tumor vaccines in the form of injections of irradiated tumor cells, either autologous or not, no difference in survival was shown between the two groups of patients, i.e., responders (24 % partial response, no response complete), and nonresponders [116]. Thus, whether specific or nonspecific, traditional immunotherapy can be considered an effective treatment for metastasized kidney cancer.

20.5.1.4 New Developments in Immunotherapy

New approaches to the immunotherapy of metastasized kidney cancer apply cytokines, which are molecules involved in the regulation of the immune system. They may be in the form of monokines (such as TNF or IL-1), or lymphokines (ITN and IL-2). These cytokines can be used alone, or in synergistic associations with certain chemotherapeutics: vinblastine [117], 5-fluorouracil and mitomycin [118], or VP16 [119].

Adoptive immunotherapy uses the lymphocytes activated by the patient in *in vitro* IL-2. It may be circulating lymphocytes (lymphokine

activated killer) or intra-tumor lymphocytes (tumor infiltrating lymphocyte).

20.5.2 Prognostic Systems in Metastatic Kidney Cancer

Different prognostic models developed as predictive models are mainly based on response to immunotherapy. One model that has proved its usefulness is that of the French Immunotherapy Group. It is schematically based on circulating neutrophils, the interval between the initial diagnosis and the onset of metastases, the presence of liver metastases, and the number of metastatic sites. The main utility of this model was to classify the first patients for clinical immunotherapy trials and it has clearly. It demonstrated that patients with poor or intermediate prognosis do not benefit from receiving IFN and IL-2. Objectively, this model is mostly considered as a tool for evaluating reply to immunotherapy, rather than a prognostic model.

20.6 Concluding Remarks

Significant advances have been made in introducing novel immunotherapeutic approaches, but future studies are warranted.

References

1. Murta-Nascimento C, Schmitz-Dräger BJ, Zeegers MP, Steineck G, Kogevinas M, Real FX, et al. Epidemiology of urinary bladder cancer: from tumor development to patient's death. World J Urol. 2007;25(3):285–95.
2. Babjuk M, Burger M, Zigeuner R, Shariat SF, van Rhijn BW, Compérat E, et al. EAU guidelines on non–muscle-invasive urothelial carcinoma of the bladder: update 2013. Eur Urol. 2013;64(4):639–53.
3. Böhle A, Bock P. Intravesical bacille Calmette-Guerin versus mitomycin C in superficial bladder cancer: formal meta-analysis of comparative studies on tumor progression. Urology. 2004;63(4):682–6.
4. Pearl R. Cancer and tuberculosis. Am J Epidemiol. 1929;9(1):97–159.
5. Coe J, Feldman J. Extracutaneous delayed hypersensitivity, particularly in the guinea-pig bladder. Immunology. 1966;10(2):127.
6. Grant R, Cochran A, Hoyle D, Mackie R, Murray E, Ross C. Results of administering BCG to patients with melanoma. Lancet. 1974;304(7889):1096–100.
7. Hanna M, Snodgrass M, Zbar B, Rapp H. Histopathology of tumor regression after intralesional injection of mycobacterium bovis. IV. Development of immunity to tumor cells and BCG. J Natl Cancer Inst. 1973;51(6):1897–908.
8. Morales A, Eidinger D, Bruce A. Intracavitary Bacillus Calmette-Guerin in the treatment of superficial bladder tumors. J Urol. 1976;116(2):180–3.
9. Martinez-Pineiro J, Muntanola P, Martin M, Hidalgo L. Fluorescence urinary cytology in bladder cancer. Eur Urol. 1976;3(3):142–4.
10. Douville Y, Pelouze G, Roy R, Charrois R, Kibrite A, Martin M, et al. Recurrent bladder papillomata treated with bacillus Calmette-Guerin: a preliminary report (phase I trial). Cancer Treat Rep. 1978;62(4):551–2.
11. Lamm D, Thor D, Harris S, Reyna J, Stogdill V, Radwin H. Bacillus Calmette-Guerin immunotherapy of superficial bladder cancer. J Urol. 1980;124(1):38–40.
12. Talic R, Hargreave T, Bishop M, Kirk D, Prescott S. Intravesical Evans bacille Calmette-Guérin for carcinoma in situ of the urinary bladder. Br J Urol. 1994;73(6):645–8.
13. Zlotta A, Van Vooren J-P, Huygen K, Drowart A, Decock M, Pirson M, et al. What is the optimal regimen for BCG intravesical therapy? Eur Urol. 2000;37(4):470–7.
14. Lamm D. Long-term results of intravesical therapy for superficial bladder cancer. Urol Clin N Am. 1992;19(3):573–80.
15. Lamm DL, Bluemenstein BA, Crissman JD, Montie JE, Gottesman JE, Lowe BA, et al. Maintenance bacillus Calmette-Guerin immunotherapy for recurrent TA, T1 and carcinoma in situ transitional cell carcinoma of the bladder: a randomized Southwest Oncology Group Study. J Urol. 2000;163(4):1124–9.
16. Shelley MD, Court J, Kynaston H, Wilt TJ, Fish RG, Mason M. Intravesical Bacillus Calmette-Guerin in 154, Ta and Tl bladder cancer. Cochrane Database Syst Rev. 2000;(4):CD001986.
17. Lamm D. BCG immunotherapy for transitional-cell carcinoma in situ of the bladder. Oncology (Huntingt). 1995;9:947–52.
18. Lamm DL, Blumenstein BA, Crawford ED, Montie JE, Scardino P, Grossman HB, et al. A randomized trial of intravesical doxorubicin and immunotherapy with bacille Calmette–Guerin for transitional-cell carcinoma of the bladder. N Engl J Med. 1991;325(17):1205–9.
19. Herr HW, Schwalb DM, Zhang Z-F, Sogani PC, Fair WR, Whitmore W, et al. Intravesical bacillus Calmette-Guérin therapy prevents tumor progression and death from superficial bladder cancer: ten-year follow-up of a prospective randomized trial. J Clin Oncol. 1995;13(6):1404–8.
20. Lamm D. Optimal BCG, treatment of superficial bladder cancer as defined by American trials. Eur Urol. 1991;21:12–6.
21. Bassi P, Spinadin R, Carando R, Balta G, Pagano F. Modified induction course: a solution to side-effects? Eur Urol. 2000;37 Suppl 1:31–2.

22. Cheng C, Ng M, Chan S, Sun W. Low dose BCG as adjuvant therapy for superficial bladder cancer and literature review. ANZ J Surg. 2004;74(7):569–72.
23. Lamm DL, Reichert D, Harris S, Lucio R. Immunotherapy of murine transitional cell carcinoma. J Urol. 1982;128(5):1104–8.
24. Alexandroff AB, Jackson AM, O'Donnell MA, James K. BCG immunotherapy of bladder cancer: 20 years on. Lancet. 1999;353(9165):1689–94.
25. Rubenstein M, Muchnik S, Chet M, Shaw M, McKiel C, Guinan P. Tumor infiltrating lymphocytes: the effect of bacillus Calmette Guerin on helper/suppressor-T cell ratios of treated and untreated tumors. J Urol. 1991;146(6):1650–3.
26. Shemtov MM, Cheng DL-W, Kong L, Shu W-P, Sassaroli M, Droller MJ, et al. LAK cell mediated apoptosis of human bladder cancer cells involves a pH-dependent endonuclease system in the cancer cell: possible mechanism of BCG therapy. J Urol. 1995;154(1):269–74.
27. Jackson AM, Alexandroff A, Kelly RW, Skibinska A, Esuvaranathan K, Prescott S, Chisholm GD, James K. Changes in urinary cytokines and soluble intercellular adhesion molecule-1 (ICAM-1) in bladder cancer patients after bacillus Calmette-Guerin (BCG) immunotherapy. Clin Exp Immunol. 1995; 99(99):369–75.
28. Yamada H, Luo Y, Matsumoto T, O'DONNELL MA. A novel expression of macrophage derived chemokine in human bladder cancer. J Urol. 2005; 173(3):990–5.
29. Bevers R, De Boer E, Kurth K, Schamhart D. BCG-induced interleukin-6 upregulation and BCG internalization in well and poorly differentiated human bladder cancer cell lines. Eur Cytokine Net. 1998;9(2):181–6.
30. Gan Y-H, Mahendran R, James K, Lawrencia C, Esuvaranathan K. Evaluation of lymphocytic responses after treatment with bacillus Calmette-Guerin and interferon-α 2b for superficial bladder cancer. Clin Immunol. 1999;90(2):230–7.
31. Sargent E, Williams R. Immunotherapeutic alternatives in superficial bladder cancer. Interferon, interleukin-2, and keyhole-limpet hemocyanin. Urol Clin N Am. 1992;19(3):581–9.
32. Glashan R. A randomized controlled study of intravesical alpha-2b-interferon in carcinoma in situ of the bladder. J Urol. 1990;144(3):658–61.
33. Zhang Y, Khoo HE, Esuvaranathan K. Effects of bacillus Calmette-Guerin and interferon alpha-2B on cytokine production in human bladder cancer cell lines. J Urol. 1999;161(3):977–83.
34. Smit JJFG, Nijkamp FP. Ramp-ing up allergies: Nramp1 (Slc11a1), macrophages and the hygiene hypothesis. Trends Immunol. 2004;25:342–7.
35. Hara I, Sato N, Miyake H, Muramaki M, Hikosaka S, Kamidono S. Introduction of 65 kDa antigen of mycobacterium tuberculosis to cancer cells enhances anti-tumor effect of BCG therapy. Microbiol Immunol. 2004;48(4):289–95.
36. Reale M, Intorno R, Tenaglia R, Feliciani C, Barbacane RC, Santoni A, et al. Production of MCP-1 and RANTES in bladder cancer patients after bacillus Calmette-Guerin immunotherapy. Cancer Immunol Immunother. 2002;51(2):91–8.
37. Hudson M, Brown E, Ritchey J, Ratliff T. Modulation of fibronectin-mediated Bacillus Calmette-Guerin attachment to murine bladder mucosa by drugs influencing the coagulation pathways. Cancer Res. 1991;51(14):3726–32.
38. Kavoussi LR, Brown EJ, Ritchey JK, Ratliff TL. Fibronectin-mediated Calmette-Guerin bacillus attachment to murine bladder mucosa. Requirement for the expression of an antitumor response. J Clin Invest. 1990;85(1):62.
39. Lang T, Prina E, Sibthorpe D, Blackwell JM. Nramp1 transfection transfers Ity/Lsh/Bcg-related pleiotropic effects on macrophage activation: influence on antigen processing and presentation. Infect Immun. 1997;65(2):380–6.
40. Smith Jr JA, Labasky RF, Cockett AT, Fracchia JA, Montie JE, Rowland RG. Bladder cancer clinical guidelines panel summary report on the management of nonmuscle invasive bladder cancer (stages Ta, T1 and TIS). J Urol. 1999;162(5):1697–701.
41. Gruenheid S, Pinner E, Desjardins M, Gros P. Natural resistance to infection with intracellular pathogens: the Nramp1 protein is recruited to the membrane of the phagosome. J Exp Med. 1997; 185(4):717–30.
42. Arias M, Rojas M, Zabaleta J, Rodríguez JI, París SC, Barrera LF, et al. Inhibition of virulent Mycobacterium tuberculosis by Bcgr and Bcgs macrophages correlates with nitric oxide production. J Infect Dis. 1997;176(6):1552–8.
43. Kadhim SA, Chin JL, Batislam E, Karlik SJ, Garcia B, Skamene E. Genetically regulated response to intravesical bacillus Calmette Guerin immunotherapy of orthotopic murine bladder tumor. J Urol. 1997;158(2):646–52.
44. Chopin D, PJJ, Saint F, Bari R, Velotti F, Abbou C. Bases et principes de l'immunothérapie locale utilisant le bacille Calmette Guérin dans le carcinome urothélial vésicale. Implications pratiques. Prog Urol. 1998;8(Suppl 2):8–12.
45. Sousa AO, Lee FK, Freiji R, Lagrange PH, André N. Human immunodeficiency virus infection alters antigen-induced cytokine responses in patients with active mycobacterial diseases. J Infect Dis. 1998;177(6):1554–62.
46. Becich M, Carroll S, Ratliff T. Internalization of bacille Calmette-Guerin by bladder tumor cells. J Urol. 1991;145(6):1316–24.
47. Fleischmann J, Park M-C, Hassan M. Fibronectin expression on surgical specimens correlated with the response to intravesical bacillus Calmette-Guerin therapy. J Urol. 1993;149(2):268–71.
48. Lattime EC, Gomella LG, McCue PA. Murine bladder carcinoma cells present antigen to BCG-specific CD4+ T-cells. Cancer Res. 1992;52(15):4286–90.

49. Böhle A, Nowc C, Ulmer A, Musehold J, Gerdes J, Hofstetter A, et al. Elevations of cytokines interleukin-1, interleukin-2 and tumor necrosis factor in the urine of patients after intravesical bacillus Calmette-Guerin immunotherapy. J Urol. 1990;144(1):59–64.
50. De Boer E, De Jong W, Steerenberg P, Aarden L, Tetteroo E, De Groot E, et al. Induction of urinary interleukin-1 (IL-1), IL-2, IL-6, and tumour necrosis factor during intravesical immunotherapy with bacillus Calmette-Guerin in superficial bladder cancer. Cancer Immunol Immunother. 1992;34(5): 306–12.
51. de Reijke TM, de Boer EC, Kurth KH, Schamhart DH. Urinary cytokines during intravesical bacillus Calmette-Guerin therapy for superficial bladder cancer: processing. Stability and prognostic value. J Urol. 1996;155(2):477–82.
52. Stavropoulos N, Ioachim E, Pavlidis N, Pappa L, Kalomiris P, Agnantis N. Local immune response after intravesical interferon gamma in superficial bladder cancer. Br J Urol. 1998;81:875–9.
53. Allen PM, Strydom DJ, Unanue ER. Processing of lysozyme by macrophages: identification of the determinant recognized by two T-cell hybridomas. Proc Natl Acad Sci. 1984;81(8):2489–93.
54. Costello RT, Gastaut J-A, Olive D. What is the real role of CD40 in cancer immunotherapy? Immunol Tod. 1999;20(11):488–93.
55. Buus SSA, Colon S, Miles C, Grey HM. The relation between major histocompatibility complex (CMH) restriction and the capacity of Ia to bind to immunogenic peptides. Science. 1987;235:1353–8.
56. Cui JST, Kawato T, Sato H, Kondo E, Toura I, Kaneko Y, Hoseki H, Kanno M, Tanigshi M. Requirement for V14 NKT cells in IL12 mediated rejection of tumours. Science. 1997;278:1623–6.
57. Jackson A, James K. Understanding the most successful immunotherapy for cancer. Immunologist. 1994;2(6):208.
58. Hawkyard S, Jackson A, James K, Prescott S, Smyth J, Chisholm G. The inhibitory effects of interferon gamma on the growth of bladder cancer cells. J Urol. 1992;147(5):1399–403.
59. Patard JJ, Saint F, Izadifar V, Muscatelli Groux B, Maille P, Abbou CC, Chopin D. Valeur pronostique de l'IL2 et de l'IFN gamma urinaires après traitement par BCG endovesical. Prog Urol. 1996;6:19A
60. Haanen J, de Waal MR, Kraakman EM, Ottenhoff T, de Vries R, Spits H. Selection of a human T helper type 1-like T cell subset by mycobacteria. J Exp Med. 1991;174(3):583–92.
61. Kaempfer R, Gerez L, Farbstein H, Madar L, Hirschman O, Nussinovich R, et al. Prediction of response to treatment in superficial bladder carcinoma through pattern of interleukin-2 gene expression. J Clin Oncol. 1996;14(6):1778–86.
62. Ratliff T, Ritchey J, Yuan J, Andriole G, Catalona W. T-cell subsets required for intravesical BCG immunotherapy for bladder cancer. J Urol. 1993;150(3): 1018–23.
63. Grewal IS, Flavell RA. A central role of CD40 ligand in the regulation of $CD4^+$ T-cell responses. Immunol Tod. 1996;17(9):410–4.
64. Cassatella MA. The production of cytokines by polymorphonuclear neutrophils. Immunol Tod. 1995;16(1):21–6.
65. Campbell S, Tanabe K, Alexander J, Edinger M, Tubbs R, Klein E. Intercellular adhesion molecule-1 expression by bladder cancer cells: functional effects. J Urol. 1994;151(5):1385–90.
66. Saint F, Patard J, Groux Muscatelli B, Belda L, de Medina Gil Diez S, Abbou C, et al. Evaluation of cellular tumour rejection mechanisms in the peritumoral bladder wall after bacillus Calmette-Guérin treatment. BJU Int. 2001;88(6):602–10.
67. Wang MH, Chen YQ, Gercken J, Ernst M, Böhle A, Flad HD, et al. Specific activation of human peripheral blood γ/δ+T lymphocytes by sonicated antigens of Mycobacterium tuberculosis: role in vitro in killing human bladder carcinoma cell lines. Scand J Immunol. 1993;38(3):239–46.
68. Hoft DF, Brown RM, Roodman ST. Bacille Calmette-Guérin vaccination enhances human γδ T cell responsiveness to mycobacteria suggestive of a memory-like phenotype. J Immunol. 1998;161(2): 1045–54.
69. Thurnher M, Ramoner R, Gastl G, Radmayr C, Böck G, Herold M, et al. Bacillus Calmette-Guerin mycobacteria stimulate human blood dendritic cells. Int J Cancer. 1997;70(1):128–34.
70. Graubert TA, Ley TJ. How do lymphocytes kill tumor cells? Clin Cancer Res. 1996;2(5):785–9.
71. Cooke PW, James ND, Ganesan R, Wallace M, Burton A, Young LS. CD40 expression in bladder cancer. J Pathol. 1999;188(1):38–43.
72. Sternberg CN, Donat SM, Bellmunt J, Millikan RE, Stadler W, De Mulder P, et al. Chemotherapy for bladder cancer: treatment guidelines for neoadjuvant chemotherapy, bladder preservation, adjuvant chemotherapy, and metastatic cancer. Urology. 2007; 69(1):62–79.
73. Akaza H, Hinotsu S, Aso Y, Kakizoe T. Bacillus calmette-Guérin treatment of existing papillary bladder cancer and carcinoma in situ of the bladder. Four-year results. Cancer. 1995;75(2):552–9.
74. Cookson MS, Sarosdy MF. Management of stage T1 superficial bladder cancer with intravesical bacillus Calmette-Guerin therapy. J Urol. 1992;148(3): 797–801.
75. Ajili F, Manai M, Darouiche A, Chebil M, Boubaker S. Tumor multiplicity is an independent prognostic factor of non-muscle-invasive bladder cancer treated with bacillus Calmette-Guerin immunotherapy. Ultrastruct Pathol. 2012;36(5):320–4.
76. Zlotta AR, Noel J-C, Fayt I, Drowart A, Van Vooren J-P, Huygen K, et al. Correlation and prognostic significance OF p53, $p21^{WAF1/CIP1}$ and Ki-67 expression in patients with superficial bladder tumors treated with Bacillus Calmette-Guerin intravesical therapy. J Urol. 1999;161(3):792–8.

77. Martinez-Pineiro J, Flores N, Isorna S, Solsona E, Sebastian J, Pertusa C, et al., for CUETO (Club Urológico Español De Tratamiento Oncológico) Long-term follow-up of a randomized prospective trial comparing a standard 81 mg dose of intravesical bacille Calmette-Guérin with a reduced dose of 27 mg in superficial bladder cancer. BJU Int. 2002; 89(7):671–80.
78. Davis JW, Sheth S, Doviak MJ, Schellhammer PF. Superficial bladder carcinoma treated with Bacillus Calmette–Guerin: progression-free and disease specific survival with 10-years. J Urol. 2002;167:494–501.
79. Ajili F, Kacem M, Tounsi H, Darouiche A, Enayfer E, Chebi M, et al. Prognostic impact of angiogenesis in nonmuscle invasive bladder cancer as defined by microvessel density after immunohistochemical staining for CD34. Ultrastruct Pathol. 2012;36(5): 336–42.
80. Takashi M, Wakai K, Hattori T, Furuhashi K, Ono Y, Ohshima S, et al. Multivariate evaluation of factors affecting recurrence, progression, and survival in patients with superficial bladder cancer treated with intravesical bacillus Calmette-Guérin (Tokyo 172 strain) therapy: significance of concomitant carcinoma in situ. Int Urol Nephrol. 2002;33(1):41–7.
81. Boorjian SA, Zhu F, Herr HW. The effect of gender on response to bacillus Calmette-Guérin therapy for patients with non-muscle-invasive urothelial carcinoma of the bladder. BJU Int. 2010;106(3):357–61.
82. Pansadoro V, Emiliozzi P, de Paula F, Scarpone P, Pansadoro A, Sternberg CN. Long-term follow-up of G3T1 transitional cell carcinoma of the bladder treated with intravesical bacille Calmette-Guerin: 18-year experience. Urology. 2002;59(2):227–31.
83. Fernandez-Gomez J, Solsona E, Unda M, Martinez-Piñeiro L, Gonzalez M, Hernandez R, et al. Prognostic factors in patients with non-muscle-invasive bladder cancer treated with bacillus Calmette-Guérin: multivariate analysis of data from four randomized CUETO trials. Eur Urol. 2008; 53(5):992–1002.
84. Saint F, Salomon L, Quintela R, Cicco A, Hoznek A, Abbou CC, et al. Do prognostic parameters of remission versus relapse after Bacillus Calmette-Guérin (BCG) immunotherapy exist?: analysis of a quarter century of literature. Eur Urol. 2003;43(4):351–61.
85. Yuge K, Kikuchi E, Matsumoto K, Takeda T, Miyajima A, Oya M. Could patient age influence tumor recurrence rate in non-muscle-invasive bladder cancer patients treated with BCG immunotherapy? Jpn J Clin Oncol. 2011;41(4):565–70.
86. Joudi FN, Smith BJ, O'Donnell MA, Konety BR. The impact of age on the response of patients with superficial bladder cancer to intravesical immunotherapy. J Urol. 2006;175(5):1634–40.
87. Cho KS, Hwang T-K, Kim BW, Yoon DK, Chang S-G, Kim SJ, et al. Differences in tumor characteristics and prognosis in newly diagnosed Ta, T1 urothelial carcinoma of bladder according to patient age. Urology. 2009;73(4):828–32.e1.
88. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature. 2000;408(6810):307–10.
89. Shariat SF, Zlotta AR, Ashfaq R, Sagalowsky AI, Lotan Y. Cooperative effect of cell-cycle regulators expression on bladder cancer development and biologic aggressiveness. Mod Pathol. 2007;20(4): 445–59.
90. Shariat SF, Ashfaq R, Sagalowsky AI, Lotan Y. Correlation of cyclin D1 and E1 expression with bladder cancer presence, invasion, progression, and metastasis. Hum Pathol. 2006;37(12):1568–76.
91. Malats N, Bustos A, Nascimento CM, Fernandez F, Rivas M, Puente D, et al. P53 as a prognostic marker for bladder cancer: a meta-analysis and review. Lancet Oncol. 2005;6(9):678–86.
92. Bolenz C, Lotan Y. Molecular biomarkers for urothelial carcinoma of the bladder: challenges in clinical use. Nat Clin Pract Urol. 2008;5(12):676–85.
93. Shariat SF, Ashfaq R, Sagalowsky AI, Lotan Y. Predictive value of cell cycle biomarkers in nonmuscle invasive bladder transitional cell carcinoma. J Urol. 2007;177(2):481–7.
94. Shariat SF, Karam JA, Lerner SP. Molecular markers in bladder cancer. Curr Opin Urol. 2008;18(1): 1–8.
95. Esuvaranathan K, Chiong E, Thamboo TP, Chan YH, Kamaraj R, Mahendran R, et al. Predictive value of p53 and pRb expression in superficial bladder cancer patients treated with BCG and interferon-alpha. Cancer. 2007;109(6):1097–105.
96. Cormio L, Tolve I, Annese P, Saracino A, Zamparese R, Sanguedolce F, et al. Altered p53 and pRb expression is predictive of response to BCG treatment in T1G3 bladder cancer. Anticancer Res. 2009;29(10): 4201–4.
97. Amirghofran Z, Monabati A, Khezri A, Malek-Hosseini Z. Apoptosis in transitional cell carcinoma of bladder and its relation to proliferation and expression of p53 and bcl-2. Pathol Oncol Res. 2004;10(3):154–8.
98. Gonzalez-Campora R, Davalos-Casanova G, Beato-Moreno A, Garcia-Escudero A, Pareja Megia MJ, Montironi R, et al. BCL-2, TP53 and BAX protein expression in superficial urothelial bladder carcinoma. Cancer Lett. 2007;250(2):292–9.
99. Ajili F, Kaabi B, Darouiche A, Tounsi H, Kourda N, Chebil M, et al. Prognostic value of Bcl-2 and Bax tumor cell expression in patients with non muscle-invasive bladder cancer receiving bacillus Calmette-Guerin immunotherapy. Ultrastruct Pathol. 2012;36(1):31–9.
100. Karam JA, Lotan Y, Ashfaq R, Sagalowsky AI, Shariat SF. Survivin expression in patients with non-muscle-invasive urothelial cell carcinoma of the bladder. Urology. 2007;70(3):482–6.
101. Youssef RF, Mitra AP, Bartsch Jr G, Jones PA, Skinner DG, Cote RJ. Molecular targets and targeted therapies in bladder cancer management. World J Urol. 2009;27(1):9–20.
102. Shariat SF, Youssef RF, Gupta A, Chade DC, Karakiewicz PI, Isbarn H, et al. Association of

angiogenesis related markers with bladder cancer outcomes and other molecular markers. J Urol. 2010;183(5):1744–50.
103. Grossfeld GD, Ginsberg DA, Stein JP, Bochner BH, Esrig D, Nichols PW, et al. Thrombospondin-1 expression in bladder cancer: association with p53 alterations, tumor angiogenesis, and tumor progression. J Natl Cancer Inst. 1997;89(3):219–27.
104. Beatty JD, Islam S, North ME, Knight SC, Ogden CW. Urine dendritic cells: a noninvasive probe for immune activity in bladder cancer? BJU Int. 2004; 94(9):1377–83.
105. Videira PA, Calais FM, Correia M, Ligeiro D, Crespo HJ, Calais F, et al. Efficacy of bacille Calmette-Guerin immunotherapy predicted by expression of antigen-presenting molecules and chemokines. Urology. 2009;74(4):944–50.
106. Ayari C, LaRue H, Hovington H, et al. Bladder tumor infiltrating mature dendritic cells and macrophages as predictors of response to bacillus Calmette-Guerin immunotherapy. Eur Urol. 2009;55:1386–96.
107. Talmadge JE, Donkor M, Scholar E. Inflammatory cell infiltration of tumors: Jekyll or Hyde. Cancer Metastasis Rev. 2007;26(3–4):373–400.
108. Ajili F, Kourda N, Darouiche A, Chebil M, Boubaker S. Prognostic value of tumor-associated macrophages count in human non-muscle-invasive bladder cancer treated by BCG immunotherapy. Ultrastruct Pathol. 2013;37(1):56–61.
109. Dreikorn K, Terwey B, Drings P, Horsch R, Palmtag H, Rössler W. Complete regression of multiple pulmonary metastases in a patient with advanced renal cell carcinoma treated by occlusion of the renal artery with subsequent radical nephrectomy and progesterone. Eur Urol. 1982;9(4):254–6.
110. Pizzocaro G, Piva L, Di Fronzo G, Giongo A, Cozzoli A, Dormia E, et al. Adjuvant medroxyprogesterone acetate to radical nephrectomy in renal cancer: 5-year results of a prospective randomized study. J Urol. 1987;138(6):1379–81.
111. Swanson D. Systemic treatment for renal cell carcinoma. EORTC Genitourinary Group Monograph. 1990;9:201.
112. Hrushesky W, von Roemeling R, Lanning RM, Rabatin JT. Circadian-shaped infusions of floxuridine for progressive metastatic renal cell carcinoma. J Clin Oncol. 1990;8(9):1504–13.
113. Martinez-Pineiro L, De La Pena J, Picazo M, Jiménez LJ, Beneitez M, Martinez-Pineiro J. Spontaneous regression of pulmonary metastases and long-term survival of a patient with metastatic renal cell carcinoma, after immunostimulation with bacillus Calmette-Guerin and extirpation of brain and contralateral lung metastases. Eur Urol. 1987; 15(1–2):146–9.
114. Marshall ME, Mendelsohn L, Butler K, Riley L, Cantrell J, Wiseman C, et al. Treatment of metastatic renal cell carcinoma with coumarin (1, 2-benzopyrone) and cimetidine: a pilot study. J Clin Oncol. 1987;5(6):862–6.
115. Tallberg T, Tykkä H, Mahlberg K, et al. Active specific immune ~therapy with supportive measures in t he treatment of palliatively nephrectomized, renal adenocarcinoma patients. Eur Urol. 1985;11:233.
116. Kurth K, Marquet R, Zwartendijk J, Warnaar S. Autologous anticancer antigen preparation for specific immunotherapy in advanced renal cell carcinoma. Eur Urol. 1986;13(1–2):103–9.
117. Schornagel J, Verweij J, Bokkel Huinink WW, et al. Phase II study of recombinant interferon alpha-2A and vinblastine in advanced renal cell carcinoma. J Urol. 1989;142:253.
118. Sella A, Logothetis C, Fitz K, Dexeus F, Amato R, Kilbourn R, et al. Phase II study of interferon-alpha and chemotherapy (5-fluorouracil and mitomycin C) in metastatic renal cell cancer. J Urol. 1992;147(3):573–7.
119. Burgers J, Marshall F, Isaacs J. Enhanced anti-tumor effects of recombinant human tumor necrosis factor plus VP-16 on metastatic renal cell carcinoma in a xenograft model. J Urol. 1989;142(1):160–4.

21 Immunopathology of Specific Cancers in Males and Females and Immunotherapy of Prostate and Cervical Cancer

Maurizio Chiriva-Internati, Fabio Grizzi, Gianluigi Taverna, Leonardo Mirandola, Scott W. Dahlbeck, Marjorie R. Jenkins, Diane D. Nguyen, Martin J. D'Souza, Everardo Cobos, and Jose A. Figueroa

Contents

21.1 Introduction

It is now recognized that human carcinogenesis is a dynamic process that depends on a large number of variables and is regulated at multiple *spatial* and *temporal* scales [1–4]. Cancer is a highly heterogeneous disease: more than 100 distinct types of human cancer have been described, and various tumor subtypes can be found within specific organs. In addition, tumors have somatic mutations and epigenetic changes, many of which are specific to the individual neoplasm [5]. It is

M. Chiriva-Internati, PhD (✉)
Division of Oncology and Hematology, Department of Internal Medicine, Texas Tech University Health Sciences Center, Lubbock, TX 79430, USA

Kiromic LLC, Lubbock, TX 79407, USA

Laura W. Bush Institute for Women's Health and Center for Women's Health and Gender-Based Medicine, Amarillo, TX 79106, USA
e-mail: maurizio.chiriva@ttuhsc.edu

F. Grizzi, PhD
Department of Immunology and Inflammation, Humanitas Research and Clinical Center, Milan, Rozzano 20089, Italy

G. Taverna, MD
Department of Immunology and Inflammation, Humanitas Research and Clinical Center, Milan, Rozzano 20089, Italy

L. Mirandola, PhD
Department of Oncology and Hematology, Texas Tech University Health Sciences Center, Lubbock, TX 79409, USA

S.W. Dahlbeck, MD • D.D. Nguyen, MD
E. Cobos, MD • J.A. Figueroa, MD
Department of Oncology and Hematology, Texas Tech University Health Sciences Center, Lubbock, TX 79409, USA

Kiromic LLC, Lubbock, TX 79409, USA

M.R. Jenkins, MD
Laura W. Bush Institute for Women's Health and Center for Women's Health and Gender-Based Medicine, Amarillo, TX 79106, USA

M.J. D'Souza, MD
Department of Pharmaceutical Sciences, Mercer University, College of Pharmacy, Atlanta, GA 30341, USA

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_21,

accepted that this genetic and phenotypical variability primarily determines the self-progressive growth, invasiveness and metastatic potential of neoplastic cells, and their response or resistance to therapy, and it seems that the "multilevel complexity" of cancer explains the clinical diversity of histologically similar neoplasia [6, 7]. It is known that cancer therapy is designed to specifically integrate distinct treatment modalities in the most effective way to achieve the highest cure rate. Surgery and radiation therapies are mainly applied for locoregional disease control, whereas systemic therapies are used to treat micrometastatic or widespread metastatic cancers and hematologic malignancies. While systemic therapies have historically been given after local measures have been undertaken to eradicate the primary tumor, they are increasingly used prior to definitive local treatment both to achieve systemic disease control earlier and to evaluate the responsiveness of the tumor to treatment. Therapies that induce an immune response to tumors – immunotherapies – have been investigated for over 100 years as attractive strategies for cancer treatment. While initially tested alone, newer researches demonstrate that immunotherapy can complement standard cancer treatments. A new era of effectively exploiting the immune system to treat and prevent cancer has begun. Two distinct immune-based therapies are now approved for cancer treatment: the first cancer vaccine for advanced prostate cancer, and the first immune checkpoint inhibitor for advanced melanoma. These early successes have led to heightened interest and activity in developing new strategies for tipping the balance of the host-tumor interaction toward definitive tumor rejection. It is clear that strategically integrating immune-based therapies with current cancer treatments, mainly chemotherapy and radiotherapy, have the potential to reengineer the overall host *milieu* and the tumor microenvironment to disrupt pathways of immune tolerance and suppression [8]. Here, the clinical experiences and results achieved with immunotherapy for treating prostate and cervical cancers, two of the most significant malignancies in human male and females, respectively, are briefly reviewed.

21.2 Prostate Cancer: Past, Present, and Future

Prostate cancer is the most prevalent malignancy in men worldwide and is a leading cause of cancer death [9, 10]. Several men with localized prostate cancer will never suffer any symptoms or adverse effects of the disease, but because of the difficulties in identifying this subgroup of patients, the majority receive radical local treatment, which can mainly result in erectile dysfunction, incontinence, and other subsequent impairments [11, 12]. The still open question for clinicians is deciding which men have "fast-growing" or aggressive cancers that need essential treatment and which men have insignificant or "slow-growing" cancers that will never become symptomatic [13]. Prognostic markers may help to avoid unnecessary invasive procedures or treatments and identify patients who would benefit from more aggressive therapies [14–17]. Based on the exponential aging of the population and the increasing life expectancy in industrialized Western countries, prostate cancer in elderly men is becoming a disease of increasing significance [18–20].

It has been ascertained that the human prostate is the site of origin for the two most prevalent diseases: benign prostatic hyperplasia and prostate cancer [21, 22]. Benign prostatic hyperplasia is a more common form of lower urinary tract symptoms and is due to the excessive growth of both stromal and epithelial cells of the prostate. Prostate cancer is a highly heterogeneous disease encompassing a wide variety of pathological entities and a range of different clinical behaviors [23]. This is underpinned at the molecular level by a complex array of genetic alterations that affect cell processes, thus determining the dynamic progression of neoplastic disease [24, 25]. Genomic alterations with a potential involvement in prostate cancer include somatic mutations, gene deletions or amplifications, and chromosomal rearrangements [25–29]. Epigenetic changes, more specifically DNA methylation, are the most common alterations in prostate cancer [30]. These changes are associated with transcriptional silencing of genes, leading to an altered cellular behavior.

The glutathione S-transferase p1 gene (*GSTP1*) has been proposed for the early diagnosis and prognosis of prostate cancer. Other markers have a strong body of scientific data supporting their role in prostate cancer diagnosis, most notably Adenomatosis polyposis coli (APC), retinoic acid receptor beta (RARB), RAS association domain family protein 1 (RASSF1), CDH1, CDKN2A (p16), and the O(6)-methylguanine-DNA methyltransferase (MGMT) [30, 31]. Prostate cancer clinical phenotypes range from indolent or clinically insignificant to locally aggressive or metastatic [32–34]. A high number of gene expression profiling studies have been carried out to attempt the establishment of a "molecular" staging system, but the identification of genetic markers that predict aggressive disease has not yet been clinically demonstrated [35–40]. The optimal treatment for localized prostate cancer remains unknown, and various reports concerning the best treatment modality are still conflicting [32]. Histopathologic examination reveals that like other tumors, prostate cancer is associated with diverse immune cell infiltrates and that in the cancer context epithelial cells coexist with extracellular matrix components and nonneoplastic cell types, which collectively form the tumor stroma [41–43]. Several lines of evidence support the concept that tumor stromal cells are not merely a scaffold, but rather they influence growth, survival, and invasiveness of cancer cells, dynamically contributing to the tumor microenvironment, together with immune cells [42, 44–47]. It is known that interactions between epithelium and the surrounding stroma are required to maintain organ function and that these interactions provide proliferative and migratory restraints that define anatomical and positional information, mediated by several growth factors and extracellular matrix components [48]. When cancer develops, transformed cells lose these constraints, while stroma adapts and coevolves to support the "function" of the tumor [42]. The prostate represents an example of an organ that relies on its surrounding stroma during normal development and cancer progression [42]. Jia et al. [49] compared Affymetrix gene expression profiles in stroma near tumor and identified a set of 115 probe sets for which the expression levels were significantly correlated with time to relapse. The authors compared patients that relapsed shortly after prostatectomy (<1 year) and patients that did not relapse in the first 4 years after prostatectomy and identified 131 differentially expressed microarray probe sets between these two categories. They concluded that tumor-adjacent prostate cancer stroma contains numerous changes in gene expression at the time of diagnosis that correlate with the chance of relapse following prostatectomy [49]. It is likely that the differences in RNA expression are often reflected in differences in chromatin modification, DNA methylation, and protein levels, which could also serve as stromal markers for progression [50]. In an *in vitro* study, Reinertsen et al. [51] showed that cocultivation with the human prostate cancer PC-3 cells seems to make the cancerous and hyperplastic fibroblasts more alike each other, as the number of differentially expressed genes decrease [51]. The cells of the immune system that are commonly found infiltrating prostate cancer include IL-17^{+} macrophages [52, 53], neutrophils [54], mast cells [55], and natural killer (NK) cells [56], as well as cells associated with an adaptive immune response, i.e., T and B lymphocytes [57–61]. Although it is thought that an immune response localized to the tumor inhibits cancer growth, it is now clear that some types of tumor-associated inflammatory cells may also exert an opposite action, at least at some point of prostate cancer natural history [62].

21.3 Immunotherapy of Prostate Cancer

The goals of any cancer therapy are to improve disease control, palliate pain, and improve overall survival [63]. In 2010, the American Food and Drug Administration (FDA) approved the first therapeutic cancer vaccine, called sipuleucel-T, for the treatment of castration refractory prostate cancer [64, 65]. Different from the currently adopted chemotherapy drugs that produce widespread cytotoxicity to kill tumor cells, anticancer

vaccines and immunotherapies focus on empowering the immune system to overcome the tumor. It has been shown that prostate cancer is an ideal model for cancer vaccine development. This is mainly due to its humoral and cellular immunity to a range of cancer antigens, which are good candidates for vaccine therapy to generate a robust antitumor response. Recently, Cheema et al. suggested the potential applications of BORIS (i.e., a cancer-testis antigen normally present at high levels in the testis and aberrantly expressed in various tumors and cancer cell lines) as a biomarker for prostate cancer diagnosis, an immunotherapy target, and, potentially, a prognostic marker of aggressive prostate cancer [66]. Additionally, the ability of BORIS to activate the androgen receptor gene indicates its involvement in the growth and development of prostate tumors [66]. Chiriva-Internati et al. first reported the aberrant expression of the cancer-testis antigen A-kinase anchor protein 4 (AKAP4) in prostate cancer, which will potentially be developed as a biomarker in prostate cancer. They also provide evidence that AKAP4 is a potential target for prostate cancer adoptive immunotherapy or antitumor vaccination [67]. Beginning in the early 1990s, several tumor-associated antigen genes including the cancer-testis antigens were identified that exhibited tumor-specific expression. Cancer-testis antigens are a group of proteins that are typically restricted to the testis in the normal adult but are aberrantly expressed in cancers of unrelated histologic origin [68]. Hudolin et al. observed MAGE-A1 in 10.8 % of carcinoma samples, whereas multi-MAGE-A and NY-ESO-1/LAGE-1 stained 85.9 and 84.8 % of samples using immunohistochemistry, suggesting that a panel of CT antigens rather than individual ones may be more valuable biomarkers [69]. Smith et al. suggested that multiple synovial sarcoma X chromosome breakpoint (SSX) proteins are expressed in metastatic prostate cancers, which are amenable to simultaneous targeting [70].

Enzalutamide, a second-generation androgen antagonist, has recently been approved by the FDA for castration-resistant prostate cancer treatment. Ardiani et al. showed that enzalutamide mediated immunogenic modulation in TRAMP-C2 cells. *In vivo*, enzalutamide mediated reduced genitourinary tissue weight, enlargement of the thymus, and increased levels of T-cell excision circles. Because no changes were seen in T-lymphocyte function, as determined by $CD4^+$ T-lymphocyte proliferation and Treg functional assays, enzalutamide was determined to be immune inactive [71]. They concluded that the combination of enzalutamide and immunotherapy is a promising treatment strategy for castration-resistant prostate cancer. Recently, there is a renewed interest in prostatic acid phosphatase (i.e., a nonspecific phosphomonoesterase synthesized in prostate epithelial cells, which level proportionally increases with prostate cancer progression) because of its usefulness in prognosticating intermediate- to high-risk prostate cancers and its success in the immunotherapy of prostate cancer [72]. Based on the good prognostic value of prostatic acid phosphatase and the potential usefulness of prostatic acid phosphatase as an antigen, an immunotherapy employing autologous prostatic acid phosphatase-loaded dendritic cells was initiated [73]. Wada et al. used a well-described genetically engineered mouse, autochronous prostate cancer model to explore the relative sequencing and dosing of anti-cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antibody when combined with a cell-based, granulocyte macrophage colony-stimulating factor (GM-CSF)-secreting vaccine [74]. These experiments corroborate recent clinical data, which suggest that the combination of CTLA-4 blockade and cell-based, GM-CSF-secreting vaccines may have significant antitumor effects in men with prostate cancer. These data also indicate that the "therapeutic window" of such an approach may be maximized through meticulous study of various dosing regimens. Additionally, future clinical studies may find that the addition of cyclophosphamide to this treatment strategy allows for reduction in the dose of anti-CTLA-4, potentially limiting autoimmune toxicity. In a recent phase I trial, Perez et al. demonstrated that the AE37 vaccine is safe and induces HER-2/neu-specific immunity in a heterogeneous population of $HER\text{-}2/neu^+$ prostate cancer patients [75]. Clusterin is a cytoprotective

chaperone protein that is overexpressed in many tumor types and is upregulated in response to cellular stress caused by cancer treatments, including hormonal manipulation, radiation, and chemotherapy. Custirsen is a second-generation antisense oligonucleotide that is complementary to clusterin mRNA and potently suppresses clusterin expression in preclinical models of prostate cancer as well as in clinical trials. The innovative first-in-human phase I neoadjuvant trial demonstrated dose-dependent plasma and prostate tissue concentrations of custirsen, which was well tolerated at all dose levels [76]. Results from the clinical trials (i.e., sipuleucel-T-based vaccine, GVAX-PCa, viral prostate cancer vaccines, DNA-based vaccines, and gene-mediated cytotoxic immunotherapy) indicate that prostate cancer vaccines are generally safe and, encouragingly, capable of generating tumor-specific T-lymphocyte responses. It is becoming evident that prostate cancer patients with early-stage disease may be those who obtain the main benefits from vaccines.

21.4 Cervical Cancer: What We Know and What We Need to Know

Female genital tract malignancies have worldwide distribution but vary from one region to another. The acquired immune deficiency syndrome has considerably altered the pattern of female genital cancers [77]. Studies have found that human immunodeficiency virus (HIV)-seropositive women are at least five times as likely to be infected with human papillomavirus (HPV) as seronegative controls. In immunocompromised HIV-seropositive women, the risk of cervical intraepithelial neoplasia (CIN) is almost as high as in women with squamous intraepithelial lesions on their Pap smear. Cervical cancer is the second most common malignant tumor among women worldwide [78]. In developed countries, the introduction of routine screening and treatment for premalignant lesions of the cervix has lead to a dramatic fall in the incidence and mortality of cervical cancer over the past five decades [79]. Cervical cancer is the most common female genital cancer in the developing countries. Its distribution and presentation is unique in the developing countries because most cases present at the advanced stages of the disease. The initiating event of cervical cancer is the infection with certain types of HPV. Rössler et al. investigated 290 cases of high-grade cervical intraepithelial neoplasia (CIN2, CIN3) or adenocarcinoma in situ of the cervix and clearly showed that HPV 16 is the predominant type in high-grade CIN, the immediate precursor lesions of cervical cancer [80]. More than 60 % of the lesions were associated with HPV 16 and 18 and more than 85 % with HPV 16, 18, 31, 33, or 45 [80]. The prevalence of adenocarcinoma in situ is underestimated, since its detection is difficult with current cytology screening. The infection is usually clinically silent with an absence of common genital symptoms, but it can manifest with a spectrum of lesions from genital warts to invasive cancer [81]. *In vivo*, the virus does not bind directly to the cells, but it requires contact with the basement membrane. This contact can be accomplished by microabrasions in the cervical surface, which reveal the basement membrane [81]. It is well known that the most vulnerable sites to tumorigenesis are where cell transformation occurs. Both cervix and anus belong to this category in contrast to the vulva and vagina where no metaplasia occurs. The transformation zone is the most common site of squamous intraepithelial lesion. Many researchers have tried to clarify the differences in the milieu and the immunosurveillance between the transitional zone and the exocervix [82]. In the transformation zone compared to the exocervix, significantly decreased numbers of Langerhans cells are observed. Dendritic cells recognize special patterns on the pathogens utilizing their Toll-like receptors and use major histocompatibility complex (MHC) to present the antigens to the T lymphocytes, sometimes assisted by inflammatory agents such as chemokines and cytokines. However, even in the absence of lesions, Langerhans cells of the epidermis do not produce a sufficient T-cell response, compared to the dendritic cells of the dermis, due to the lack of appropriate co-stimulatory microenvironment.

Consequently, Langerhans cells may be unable to elicit a successful immune response and become a part of the virus tolerance tactics. Accordingly, potential vaccines should avoid using Langerhans cells as a presenting agent without using co-stimuli. Interestingly, viral oncogene expression is necessary but not per se sufficient to promote cervical cancer, and other factors are involved in neoplastic progression. Thus, major research efforts should be focused to identify novel cocarcinogenic factors and to understand the mechanisms played into tumor development. Besides HPV, multiple additional risk factors related to the onset of cervical cancer are early-age sexual activities, high numbers of sexual partners (which is the most salient risk factor), suppression and alteration of the immune status, long-term use of oral contraceptives, and other hormonal influences [83, 84]. Although cervical screening procedures have been successful in reducing the disease burden associated with HPV infection because of a lack of resources or inadequate infrastructure, many countries have failed to reduce cervical cancer mortality. Therefore, prevention may be a valuable strategy for reducing the economic and disease burden of HPV infection. Although the diagnosis and treatment of cervical cancer has been developed recently, there are important consequences from the disease and its treatment among survivors, especially the impact on quality of life. Also, functional disorders may result from therapies such as surgery, which could involve the female genital anatomy; radiotherapy which could damage the vaginal mucosa and epithelium; and chemotherapy which could induce some side effects like nausea, vomiting, diarrhea, constipation, microsites, weight changes, and hormonal changes [85]. The classical management of invasive cervical cancer involves evaluating tumor extent which includes tumor size, depth of invasion, microvascular space tumor invasion, spread to regional lymph nodes, and grade of differentiation [86]. The treatment of cervical cancer is predicated on the evaluation of the clinical stage of tumor according to the classification of the International Federation of Gynecology and Obstetrics (FIGO). For early stages (FIGO I–IIA), either surgery or radiotherapy is employed, whereas for late stages (FIGO IIB–IV), chemotherapy is indicated [86]. However, clinical staging has certain limitations due to variables such as interobserver variability. Imaging technologies, such as computed tomography and ultrasound, have been adopted to improve the clinical staging accuracy of cervical cancer. Metastatic disease or recurrent lesions not amenable to radical local excision or regional radiation have a poor prognosis and are treated with palliative platinum-based chemotherapy. A thorough molecular characterization of cervical cancer remains crucial for a rationale implementation of targeted agents and companion biomarkers [87]. Alternative clinical trial designs may also be necessary to optimize the clinical development of new drugs for cervical cancer.

21.5 The Immunotherapy of Cervical Cancer

Given that cervical cancers are caused by HPV, the prospect of therapeutic vaccination to treat existing lesions and prophylactic vaccination to prevent persistent infections with the virus is high and may be implemented in the near future [77, 88–90]. Powell et al. have recently reported that prophylactic vaccines targeting HPV types 16 and 18 could potentially prevent up to 80.9 % of invasive cervical cancers and that cancers associated with HPV types 16, 18, and 45 were diagnosed at younger ages, supporting the hypothesis of faster progression than for tumors caused by other HPV types [91]. Several strategies of HPV therapeutic vaccines have been evaluated to reverse the effect of immunosuppression in the tumor microenvironment, including inhibition of HPV oncoproteins, activation of the host-specific immune response against HPV antigens by co-stimulatory molecule expression, and administration of T-lymphocyte helper 1 cytokines to activate the T-lymphocyte-mediated immune response [92]. Preventive vaccination against high-risk HPV types 16 and 18 is of widespread use. Current vaccines utilize the major capsid protein L1 and minor capsid protein L2 on virus-like particles [93, 94]. It has been

ascertained that L1-overexpressed proteins spontaneously self-assemble into virus-like particles that resemble the conformation of authentic virions, are neither infectious nor oncogenic, and induce high levels of type-specific neutralizing antibodies. A number of therapeutic vaccines are now in clinical trials all over the world. In contrast to preventive vaccines, therapeutic ones target the oncogenetic proteins E6 and E7, which are continuously expressed in cells throughout the HPV infection [95–97]. Contrary to the stimulation of humoral immunity by prophylactic vaccines, therapeutic vaccines develop cell-mediated immunity in order to control the infection. An approach is the use of viral or bacterial live vectors, like *Listeria monocytogenes*, adenovirus, and vaccinia virus, which are highly immunogenic and broadcast the antigens to many APCs for process, stimulating activation of both $CD4^+$ and $CD8^+$ T lymphocytes through MHC II and MHC I, respectively [98–101]. Although long-term protection is a key point in evaluating HPV vaccines over time, there is currently inadequate information on the duration of HPV vaccine-induced immunity and on the mechanisms related to the activation of immune memory [102]. As reported by Mariani et al. [102], the importance of vigorous and prolonged immune protection over time is related to the following: (a) the risk of HPV infection remains as long as women remain sexually active, (b) it is crucial to test the utility of HPV vaccination programs as public health interventions, and (c) it displays the maximum benefits of cervical cancer and other HPV-related cancers. Nevertheless, it should also be highlighted that long-term protection is not fully predictable at the introduction of any vaccine, because it varies according to many variables that are not strictly related to immune response only [102]. Recently, Zhang et al. [103] provides insights for further development of CD146 (i.e., endothelial cell adhesion molecule which is overexpressed in various types of malignant cancer) monoclonal antibodies in the detection of gynecological malignant cancer types and implies that a combined treatment strategy of anti-CD146 immunotherapy with other traditional chemo- or radiotherapy treatments may be a promising approach against cervical cancer. Agarwal et al. reported that *AKAP4* gene and protein expression was detected in 86 % of total patients with cervical cancer [104]. They concluded that *AKAP4* has a putative role in early tumorigenesis and may be implicated as a biomarker and immunotherapeutic target for cervical cancer. Sperm protein 17, a member of the cancer-testis antigens, has been found highly expressed in human cervical cancers in a heterogeneous pattern [105]. Although the expression frequency of Sp17 is not correlated with the histological subtype, the staining pattern may help to define cervical cancers. As stated by the authors themselves, Sp17 targeted immunotherapy of tumors needs more accurate validation. Previously, the *SSX* genes (i.e., members of the cancer-testis antigens family) were found by serological analysis of antigens by recombinant expression cloning (SEREX) in gynecological malignancies, including cervical cancer [106].

21.6 Concluding Remarks

An effective immune response to cancers should result in the regression of established tumors and should also be able to prevent recurrence [8]. However, multiple factors present a barrier to the antitumor immune response. Because tumors are frequently perceived by the immune system as "self," the mechanisms that control the development of autoreactive immune responses (and thus, autoimmune disease) also serve to preclude the development of an effective immune response to cancer [8]. A variety of immune cells that promote tumor growth and inhibit tumor-associated immune responses, or both, accumulate within the tumor and its locoregional draining lymph nodes. In particular, these include $CD4^+CD25^+FOXP3^+$ regulatory T lymphocytes, $CD4^+$ interleukin-17-producing T helper lymphocytes, myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs). Additional features of the tumor microenvironment further silence the antitumor immune response, including high levels of suppressive intra-tumoral cytokines, including

transforming growth factor-β (TGF-β), tumor necrosis factor (TNF), and interleukin-10 (IL-10), the constitutive or induced expression of immune checkpoint molecules by the tumor cells, and various other phenotypic alterations that lead to immune escape (the loss of tumor antigens and other molecules essential for antigen processing and presentation). Because surgery, radiotherapy, chemotherapy, and targeted therapies are widely used to treat most established cancers, the optimal integration of immune-based therapies with these standard modalities to minimize antagonistic interactions between different treatment modalities is of great importance. Immunotherapy approaches have, for several decades, been tested against several tumors, most often against malignant melanoma [107]. The development of molecular methods and an improved understanding of tumor immunosurveillance have led to novel immunotherapy approaches in the last few years. Randomized phase III trials have proven that immunotherapy can prolong the survival of patients with metastatic melanoma or prostate cancer [107]. The concept that elements of the immune system contribute to cancer control was already proposed more than 100 years ago by the German immunologist and Nobel Prize winner Paul Ehrlich. Immunotherapy is now a routine part of the treatment of some tumors, and novel immunotherapies against cancer will be available soon. New developments are expected in the field of modulating T-cell activity by interfering with co-stimulatory or co-inhibitory pathways and in adoptive immunotherapy using CAR T cells. Treatment options for advanced prostate cancer have improved considerably in the last 2 years. The immunotherapy sipuleucel-T, the cytotoxic cabazitaxel, the androgen biosynthesis inhibitor abiraterone acetate, the radioisotope radium-223, and the antiandrogen enzalutamide have all been shown to improve survival in randomized phase III studies for patients with metastatic castration-resistant prostate cancer [108]. It is also clear that in the future, radiation therapy approaches designed to optimize immune stimulation at the level of dendritic cells (DC), lymphocytes, tumor, and stroma effects could be evaluated specifically in clinical trials. High prevalence and mortality rates of cervical cancer create an imperative need to clarify the uniqueness of HPV infection, which serves as the key causative factor in cervical malignancies. Currently, immunotherapeutic strategies include vaccination with peptide, viral vectors, carbohydrates, and anti-idiotypic antibodies. HPV infection alone is not enough to induce cervical cancer, and so other risk factors also have a role such as smoking, prolonged oral contraceptive use, coinfections, multiparity, and immune-related factors which appear to lead the way on the path toward carcinogenesis. Understanding the immunological details and the microenvironment of the infection can be a useful tool for the development of novel therapeutic interventions.

Acknowledgments *This study is* partially supported by Rush Endowed Chair for Excellence in Women's Health and Oncology and the Division of Hematology Oncology.

References

1. Grizzi F, Di Ieva A, Russo C, Frezza EE, Cobos E, Muzzio PC, et al. Cancer initiation and progression: an unsimplifiable complexity. Theor Biol Med Model. 2006;3:37.
2. Enderling H, Hahnfeldt P, Hlatky L, Almog N. Systems biology of tumor dormancy: linking biology and mathematics on multiple scales to improve cancer therapy. Cancer Res. 2012;72:2172–5.
3. Deisboeck TS, Wang Z, Macklin P, Cristini V. Multiscale cancer modeling. Ann Rev Biomed Eng. 2011;13:127–55.
4. Chakrabarti A, Verbridge S, Stroock AD, Fischbach C, Varner JD. Multiscale models of breast cancer progression. Ann Biomed Eng. 2012;40:2488–500.
5. Taddei ML, Giannoni E, Comito G, Chiarugi P. Microenvironment and tumor cell plasticity: an easy way out. Cancer Lett. 2013;341:80–96.
6. Grizzi F, Chiriva-Internati M. Cancer: looking for simplicity and finding complexity. Cancer Cell Int. 2006;6:4.
7. Wang E, Zou J, Zaman N, Beitel LK, Trifiro M, Paliouras M. Cancer systems biology in the genome sequencing era: part 2. Evolutionary dynamics of tumor clonal networks and drug resistance. Semin Cancer Biol. 2013;23:286–92.
8. Chen G, Emens LA. Chemoimmunotherapy: reengineering tumor immunity. Cancer Immunol Immunother. 2013;62:203–16.

9. Siegel R, DeSantis C, Virgo K, Stein K, Mariotto A, Smith T, et al. Cancer treatment and survivorship statistics, 2012. CA Cancer J Clin. 2012;62:220–41.
10. Leitzmann MF, Rohrmann S. Risk factors for the onset of prostatic cancer: age, location, and behavioral correlates. Clin Epidemiol. 2012;4:1–11.
11. Daly T, Hickey BE, Lehman M, Francis DP, See AM. Adjuvant radiotherapy following radical prostatectomy for prostate cancer. Cochrane Database Syst Rev. 2011(12):CD007234.
12. Moskovic DJ, Miles BJ, Lipshultz LI, Khera M. Emerging concepts in erectile preservation following radical prostatectomy: a guide for clinicians. Int J Impot Res. 2011;23:181–92.
13. Lawrentschuk N, Trottier G, Kuk C, Zlotta AR. Role of surgery in high-risk localized prostate cancer. Curr Oncol. 2010;17 Suppl 2:S25–32.
14. Miyake H, Fujisawa M. Prognostic prediction following radical prostatectomy for prostate cancer using conventional as well as molecular biological approaches. Int J Urol. 2012;20:301–11.
15. Lumen N, Fonteyne V, De Meerleer G, De Visschere P, Ost P, Oosterlinck W, et al. Screening and early diagnosis of prostate cancer: an update. Acta Clin Belg. 2012;67:270–5.
16. Carlsson S, Vickers AJ, Roobol M, Eastham J, Scardino P, Lilja H, et al. Prostate cancer screening: facts, statistics, and interpretation in response to the US preventive services task force review. J Clin Oncol. 2012;30:2581–4.
17. Eylert MF, Persad R. Management of prostate cancer. Br J Hosp Med (Lond). 2012;73(2):95–9.
18. Spahn M, Haeni K. Approach to prostate cancer in men older than 75 years: active or passive? Urologe A. 2012;51:1368–74.
19. Kessler ER, Flaig TW. Optimal management of recurrent prostate cancer in older patients. Drugs Aging. 2012;29:871–83.
20. Albrand G, Mottet N, Ruffion A, Flechon A, Bensadoun RJ, Gigante M, et al. Prostate cancer in elderly subjects: how diagnosis should be made, why and how geriatric assessment should be implemented. Prog Urol. 2012;22 Suppl 2:S55–63.
21. Lee CH, Akin-Olugbade O, Kirschenbaum A. Overview of prostate anatomy, histology, and pathology. Endocrinol Metab Clin N Am. 2011;40:565–75.
22. Orsted DD, Bojesen SE. The link between benign prostatic hyperplasia and prostate cancer. Nat Rev Urol. 2012;10:49–54.
23. Mackinnon AC, Yan BC, Joseph LJ, Al-Ahmadie HA. Molecular biology underlying the clinical heterogeneity of prostate cancer: an update. Arch Pathol Lab Med. 2009;133:1033–40.
24. Liu W, Laitinen S, Khan S, Vihinen M, Kowalski J, Yu G, et al. Copy number analysis indicates monoclonal origin of lethal metastatic prostate cancer. Nat Med. 2009;15:559–65.
25. Aschelter AM, Giacinti S, Caporello P, Marchetti P. Genomic and epigenomic alterations in prostate cancer. Front Endocrinol (Lausanne). 2012;3:128.
26. Holcomb IN, Young JM, Coleman IM, Salari K, Grove DI, Hsu L, et al. Comparative analyses of chromosome alterations in soft-tissue metastases within and across patients with castration-resistant prostate cancer. Cancer Res. 2009;69:7793–802.
27. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science. 2005;310:644–8.
28. Nakagawa H, Akamatsu S, Takata R, Takahashi A, Kubo M, Nakamura Y. Prostate cancer genomics, biology, and risk assessment through genome-wide association studies. Cancer Sci. 2012;103:607–13.
29. Cazares LH, Drake RR, Esquela-Kirscher A, Lance RS, Semmes OJ, Troyer DA. Molecular pathology of prostate cancer. Cancer Biomark. 2010;9: 441–59.
30. Van Neste L, Herman JG, Otto G, Bigley JW, Epstein JI, Van Criekinge W. The epigenetic promise for prostate cancer diagnosis. Prostate. 2012;72: 1248–61.
31. Richiardi L, Fiano V, Grasso C, Zugna D, Delsedime L, Gillio-Tos A, et al. Methylation of APC and GSTP1 in non-neoplastic tissue adjacent to prostate tumour and mortality from prostate cancer. PLoS One. 2013;8:e68162.
32. Wilt TJ, Brawer MK, Jones KM, Barry MJ, Aronson WJ, Fox S, et al. Radical prostatectomy versus observation for localized prostate cancer. N Engl J Med. 2012;367:203–13.
33. Barbieri CE, Demichelis F, Rubin MA. Molecular genetics of prostate cancer: emerging appreciation of genetic complexity. Histopathology. 2012;60: 187–98.
34. Cheng L, Montironi R, Bostwick DG, Lopez-Beltran A, Berney DM. Staging of prostate cancer. Histopathology. 2012;60:87–117.
35. Luo J, Solimini NL, Elledge SJ. Principles of cancer therapy: oncogene and non-oncogene addiction. Cell. 2009;136:823–37.
36. Sun J, Turner A, Xu J, Gronberg H, Isaacs W. Genetic variability in inflammation pathways and prostate cancer risk. Urol Oncol. 2007;25:250–9.
37. Foye A, Febbo PG. Cancer gene profiling in prostate cancer. Methods Mol Biol. 2010;576:293–326.
38. Golias C, Charalabopoulos A, Stagikas D, Giannakopoulos X, Peschos D, Batistatou A, et al. Molecular profiling and genomic microarrays in prostate cancer. Exp Oncol. 2007;29:82–4.
39. Konishi N, Shimada K, Ishida E, Nakamura M. Molecular pathology of prostate cancer. Pathol Int. 2005;55:531–9.
40. Reynolds MA. Molecular alterations in prostate cancer. Cancer Lett. 2008;271:13–24.
41. Yates C. Prostate tumor cell plasticity: a consequence of the microenvironment. Adv Exp Med Biol. 2011;720:81–90.
42. Franco OE, Hayward SW. Targeting the tumor stroma as a novel therapeutic approach for prostate cancer. Adv Pharmacol. 2012;65:267–313.

43. Barron DA, Rowley DR. The reactive stroma microenvironment and prostate cancer progression. Endocr Relat Cancer. 2012;19:R187–204.
44. Iacopino F, Angelucci C, Sica G. Interactions between normal human fibroblasts and human prostate cancer cells in a co-culture system. Anticancer Res. 2012;32:1579–88.
45. Parrinello S, Coppe JP, Krtolica A, Campisi J. Stromal-epithelial interactions in aging and cancer: senescent fibroblasts alter epithelial cell differentiation. J Cell Sci. 2005;118:485–96.
46. Goldstein AS, Witte ON. Does the microenvironment influence the cell types of origin for prostate cancer? Genes Dev. 2013;27:1539–44.
47. Gharaee-Kermani M, Kasina S, Moore BB, Thomas D, Mehra R, Macoska JA. CXC-type chemokines promote myofibroblast phenoconversion and prostatic fibrosis. PLoS One. 2012;7:e49278.
48. Singer E, Linehan J, Babilonia G, Imam SA, Smith D, Loera S, et al. Stromal response to prostate cancer: nanotechnology-based detection of thioredoxin-interacting protein partners distinguishes prostate cancer associated stroma from that of benign prostatic hyperplasia. PLoS One. 2013;8:e60562.
49. Jia Z, Rahmatpanah FB, Chen X, Lernhardt W, Wang Y, Xia XQ, et al. Expression changes in the stroma of prostate cancer predict subsequent relapse. PLoS One. 2012;7:e41371.
50. Jia Z, Wang Y, Sawyers A, Yao H, Rahmatpanah F, Xia XQ, et al. Diagnosis of prostate cancer using differentially expressed genes in stroma. Cancer Res. 2011;71:2476–87.
51. Reinertsen T, Halgunset J, Viset T, Flatberg A, Haugsmoen LL, Skogseth H. Gene expressional changes in prostate fibroblasts from cancerous tissue. APMIS. 2012;120:558–71.
52. Vykhovanets EV, Maclennan GT, Vykhovanets OV, Gupta S. IL-17 expression by macrophages is associated with proliferative inflammatory atrophy lesions in prostate cancer patients. Int J Clin Exp Pathol. 2011;4:552–65.
53. Fang LY, Izumi K, Lai KP, Liang L, Li L, Miyamoto H, et al. Infiltrating macrophages promote prostate tumorigenesis via modulating androgen receptor-mediated CCL4-STAT3 signaling. Cancer Res. 2013;73:5633–46.
54. Okada K, Kojima M, Naya Y, Kamoi K, Yokoyama K, Takamatsu T, et al. Correlation of histological inflammation in needle biopsy specimens with serum prostate- specific antigen levels in men with negative biopsy for prostate cancer. Urology. 2000;55:892–8.
55. Pittoni P, Colombo MP. The dark side of mast cell-targeted therapy in prostate cancer. Cancer Res. 2012;72:831–5.
56. Frankel TL, Burns W, Riley J, Morgan RA, Davis JL, Hanada K, et al. Identification and characterization of a tumor infiltrating CD56(+)/CD16 (-) NK cell subset with specificity for pancreatic and prostate cancer cell lines. Cancer Immunol Immunother. 2010;59:1757–69.
57. Kryvenko ON, Jankowski M, Chitale DA, Tang D, Rundle A, Trudeau S, et al. Inflammation and preneoplastic lesions in benign prostate as risk factors for prostate cancer. Mod Pathol. 2012;25:1023–32.
58. Sfanos KS, De Marzo AM. Prostate cancer and inflammation: the evidence. Histopathology. 2012;60:199–215.
59. Flammiger A, Bayer F, Cirugeda-Kuhnert A, Huland H, Tennstedt P, Simon R, et al. Intratumoral T but not B lymphocytes are related to clinical outcome in prostate cancer. APMIS. 2012;120:901–8.
60. McDowell KL, Begley LA, Mor-Vaknin N, Markovitz DM, Macoska JA. Leukocytic promotion of prostate cellular proliferation. Prostate. 2010;70:377–89.
61. Liu Y, Saeter T, Vlatkovic L, Servoll E, Waaler G, Axcrona U, et al. Dendritic and lymphocytic cell infiltration in prostate carcinoma. Histol Histopathol. 2013;28:1621–8.
62. Lakshmi Narendra B, Eshvendar Reddy K, Shantikumar S, Ramakrishna S. Immune system: a double-edged sword in cancer. Inflam Res. 2013;62:823–34.
63. Snyder A, Tepper JE, Slovin SF. Perspectives on immunotherapy in prostate cancer and solid tumors: where is the future? Semin Oncol. 2013;40:347–60.
64. Michael A, Relph K, Annels N, Pandha H. Prostate cancer vaccines. Exp Rev Vacc. 2013;12:253–62.
65. Hammerstrom AE, Cauley DH, Atkinson BJ, Sharma P. Cancer immunotherapy: sipuleucel-T and beyond. Pharmacotherapy. 2011;31:813–28.
66. Cheema Z, Hari-Gupta Y, Kita GX, Farrar D, Seddon I, Corr J, et al. Expression of the cancer-testis antigen BORIS correlates with prostate cancer. Prostate. 2013;74(2):164–76.
67. Chiriva-Internati M, Yu Y, Mirandola L, D'Cunha N, Hardwicke F, Cannon MJ, et al. Identification of AKAP-4 as a new cancer/testis antigen for detection and immunotherapy of prostate cancer. Prostate. 2012;72:12–23.
68. Simpson AJ, Caballero OL, Jungbluth A, Chen YT, Old LJ. Cancer/testis antigens, gametogenesis and cancer. Nat Rev Cancer. 2005;5:615–25.
69. Hudolin T, Juretic A, Spagnoli GC, Pasini J, Bandic D, Heberer M, et al. Immunohistochemical expression of tumor antigens MAGE-A1, MAGE-A3/4, and NY-ESO-1 in cancerous and benign prostatic tissue. Prostate. 2006;66(1):13–8.
70. Smith HA, Cronk RJ, Lang JM, McNeel DG. Expression and immunotherapeutic targeting of the SSX family of cancer-testis antigens in prostate cancer. Cancer Res. 2011;71:6785–95.
71. Ardiani A, Farsaci B, Rogers CJ, Protter AA, Guo Z, King TH, et al. Combination therapy with a second-generation androgen receptor antagonist and a metastasis vaccine improves survival in a spontaneous prostate cancer model. Clin Cancer Res. 2013;19:6205–18.

72. Kong HY, Byun J. Emerging roles of human prostatic acid phosphatase. Biomol Therapeut. 2013;21:10–20.
73. Drake CG. Prostate cancer as a model for tumour immunotherapy. Nat Rev Immunol. 2010;10:580–93.
74. Wada S, Jackson CM, Yoshimura K, Yen HR, Getnet D, Harris TJ, et al. Sequencing CTLA-4 blockade with cell-based immunotherapy for prostate cancer. J Transl Med. 2013;11:89.
75. Perez SA, Anastasopoulou EA, Tzonis P, Gouttefangeas C, Kalbacher H, Papamichail M, et al. AE37 peptide vaccination in prostate cancer: a 4-year immunological assessment updates on a phase I trial. Cancer Immunol Immunother. 2013;62:1599–608.
76. Higano CS. Potential use of custirsen to treat prostate cancer. OncoTarg Ther. 2013;6:785–97.
77. Brinkman JA, Caffrey AS, Muderspach LI, Roman LD, Kast WM. The impact of anti HPV vaccination on cervical cancer incidence and HPV induced cervical lesions: consequences for clinical management. Eur J Gynaecol Oncol. 2005;26:129–42.
78. Di Domenico F, De Marco F, Perluigi M. Proteomics strategies to analyze HPV-transformed cells: relevance to cervical cancer. Exp Rev Proteom. 2013;10(5):461–72.
79. Okeke T, Onah N, Ikeako L, Ezenyeaku C. The frequency and pattern of female genital tract malignancies at the university of Nigeria teaching hospital, enugu. Niger Ann Med Health Sci Res. 2013;3:345–8.
80. Rossler L, Reich O, Horvat R, de Souza SC, Holl K, Joura EA. Human papillomavirus in high-grade cervical lesions: Austrian data of a European multicentre study. Wien Klin Wochenschr. 2013;125:591–9.
81. Deligeoroglou E, Giannouli A, Athanasopoulos N, Karountzos V, Vatopoulou A, Dimopoulos K, et al. HPV infection: immunological aspects and their utility in future therapy. Infect Dis Obstet Gynecol. 2013;2013:540850.
82. Giannini SL, Hubert P, Doyen J, Boniver J, Delvenne P. Influence of the mucosal epithelium microenvironment on Langerhans cells: implications for the development of squamous intraepithelial lesions of the cervix. Int J Cancer. 2002;97:654–9.
83. Asiaf A, Ahmad ST, Mohammad SO, Zargar MA. Review of the current knowledge on the epidemiology, pathogenesis, and prevention of human papillomavirus infection. Eur J Cancer Prev. 2013;23:206–24.
84. Gadducci A, Barsotti C, Cosio S, Domenici L, Riccardo GA. Smoking habit, immune suppression, oral contraceptive use, and hormone replacement therapy use and cervical carcinogenesis: a review of the literature. Gynecol Endocrinol. 2011;27:597–604.
85. Fernandes WC, Kimura M. Health related quality of life of women with cervical cancer. Rev Lat Am Enfermagem. 2010;18:360–7.
86. Amaro-Filho SM, Golub JE, Nuovo GJ, Cunha CB, Levi JE, Villa LL, et al. A comparative analysis of clinical and molecular factors with the stage of cervical cancer in a Brazilian cohort. PLoS One. 2013;8(3):e57810.
87. Diaz-Padilla I, Monk BJ, Mackay HJ, Oaknin A. Treatment of metastatic cervical cancer: future directions involving targeted agents. Crit Rev Oncol Hematol. 2013;85(3):303–14.
88. Gravitt PE. The known unknowns of HPV natural history. J Clin Invest. 2011;121(12):4593–9.
89. Jenkins M, Chiriva-Internati M, Mirandola L, Tonroy C, Tedjarati SS, Davis N, et al. Perspective for prophylaxis and treatment of cervical cancer: an immunological approach. Int Rev Immunol. 2012;31(1):3–21.
90. Chiriva-Internati M, Kast WM, Bot A. In this issue: targeting human papilloma virus-associated disease, from cervical to head and neck carcinoma…and more. Int Rev Immunol. 2012;31(1):1–2.
91. Powell N, Cuschieri K, Cubie H, Hibbitts S, Rosillon D, De Souza SC, et al. Cervical cancers associated with human papillomavirus types 16, 18 and 45 are diagnosed in younger women than cancers associated with other types: A cross-sectional observational study in Wales and Scotland (UK). J Clin Virol Off Publ Pan Am Soc Clin Virol. 2013;58(3):571–4.
92. Peralta-Zaragoza O, Bermudez-Morales VH, Perez-Plasencia C, Salazar-Leon J, Gomez-Ceron C, Madrid-Marina V. Targeted treatments for cervical cancer: a review. OncoTargets Ther. 2012;5:315–28.
93. Rose RC, Reichman RC, Bonnez W. Human papillomavirus (HPV) type 11 recombinant virus-like particles induce the formation of neutralizing antibodies and detect HPV-specific antibodies in human sera. J Gen Virol. 1994;75(Pt 8):2075–9.
94. Roden RB, Ling M, Wu TC. Vaccination to prevent and treat cervical cancer. Hum Pathol. 2004;35(8):971–82.
95. Morrow MP, Yan J, Sardesai NY. Human papillomavirus therapeutic vaccines: targeting viral antigens as immunotherapy for precancerous disease and cancer. Expert Rev Vaccines. 2013;12(3):271–83.
96. Liu Y, Chiriva-Internati M, Grizzi F, Salati E, Roman JJ, Lim S, et al. Rapid induction of cytotoxic T-cell response against cervical cancer cells by human papillomavirus type 16 E6 antigen gene delivery into human dendritic cells by an adeno-associated virus vector. Cancer Gene Ther. 2001;8(12):948–57.
97. Chiriva-Internati M, Liu Y, Salati E, Zhou W, Wang Z, Grizzi F, et al. Efficient generation of cytotoxic T lymphocytes against cervical cancer cells by adeno-associated virus/human papillomavirus type 16 E7 antigen gene transduction into dendritic cells. Eur J Immunol. 2002;32(1):30–8.
98. Bermudez-Humaran LG, Cortes-Perez NG, Lefevre F, Guimaraes V, Rabot S, Alcocer-Gonzalez JM, et al. A novel mucosal vaccine based on live Lactococci expressing E7 antigen and IL-12 induces systemic and mucosal immune responses and protects mice against human papillomavirus type 16-induced tumors. J Immunol. 2005;175(11):7297–302.

99. Zhou L, Zhu T, Ye X, Yang L, Wang B, Liang X, et al. Long-term protection against human papillomavirus e7-positive tumor by a single vaccination of adeno-associated virus vectors encoding a fusion protein of inactivated e7 of human papillomavirus 16/18 and heat shock protein 70. Hum Gene Ther. 2010;21(1):109–19.
100. Echchannaoui H, Bianchi M, Baud D, Bobst M, Stehle JC, Nardelli-Haefliger D. Intravaginal immunization of mice with recombinant Salmonella enterica serovar Typhimurium expressing human papillomavirus type 16 antigens as a potential route of vaccination against cervical cancer. Infect Immun. 2008;76(5):1940–51.
101. Guirnalda PD, Paterson Y. Vaccination with immunotherapeutic Listeria monocytogenes induces IL-17(+) gammadelta T cells in a murine model for HPV associated cancer. Oncoimmunology. 2012; 1(6):822–8.
102. Mariani L, Venuti A. HPV vaccine: an overview of immune response, clinical protection, and new approaches for the future. J Transl Med. 2010;8:105.
103. Zhang H, Zhang J, Wang Z, Lu D, Feng J, Yang D, et al. CD146 is a potential marker for the diagnosis of malignancy in cervical and endometrial cancer. Oncol Lett. 2013;5(4):1189–94.
104. Agarwal S, Saini S, Parashar D, Verma A, Jagadish N, Batra A, et al. Expression and humoral response of a-kinase anchor protein 4 in cervical cancer. Int J Gynecol Cancer. 2013;23:650–8.
105. Li FQ, Liu Q, Han YL, Wu B, Yin HL. Sperm protein 17 is highly expressed in endometrial and cervical cancers. BMC Cancer. 2010;10:429.
106. Hasegawa K, Koizumi F, Noguchi Y, Hongo A, Mizutani Y, Kodama J, et al. SSX expression in gynecological cancers and antibody response in patients. Cancer Immun. 2004;4:16.
107. Riether C, Schurch C, Ochsenbein AF. From “magic bullets” to specific cancer immunotherapy. Swiss Med Week. 2013;143:w13734.
108. Mukherji D, Pezaro CJ, Shamseddine A, De Bono JS. New treatment developments applied to elderly patients with advanced prostate cancer. Cancer Treat Rev. 2013;39:578–83.

Immunology and Immunotherapy of Ovarian Cancer

22

Jacek R. Wilczyński and Marek Nowak

Contents

22.1 Introduction

Tumors are able to escape from the host immunosurveillance due to a wide spectrum of mechanisms which actively disturb process of tumor immunological recognition. Besides change of tumor immunogenicity, two other mechanisms play a pivotal role in cancer growth, namely, production of tumor-derived regulatory molecules and interaction of cancer cells with tumor-infiltrating immune cells. According to the "immunoediting" hypothesis, initial elimination of tumor cells by host immune system "shapes" tumor phenotype and allows for survival of immuno-resistant cancer cells [1, 2]. This process is further augmented by both chemotherapy and immunotherapy used in the management of the disease, which usually kill only sensitive cells and spare resistant ones. Genetic instability of the tumor additionally contributes to the creation of cells resistant to immune eradication [3]. From an opposite point of view, tumor itself is not only a passive member of immunological interplay with the host but actively "shapes" the pattern

J.R. Wilczyński, MD, PhD (✉) • M. Nowak, MD, PhD
Gynecology Department with Gynecologic Oncology Unit, Polish Mother's Health Center Research Institute, 281/289 Rzgowska Street, 93-338 Lodz, Poland
e-mail: jrwil@post.pl; mrn@poczta.onet.pl

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_22, © Springer-Verlag Berlin Heidelberg 2015

and intensity of host immune response [4]. Vascularization of the tumor, production of metastases, and existence of pro-inflammatory environment inside and next to the tumor are very important components of tumor propagation. All of them are regulated by cytokines originated from tumor cells or tumor-infiltrating immune cells which are forced functionally to favor cancer growth.

22.2 The Role of Cytokines in Neovascularization of Epithelial Ovarian Cancer (EOC)

22.2.1 Characterization of VEGF Function

Initially, cancer grows as an avascular tumor which takes nutrients from surrounding tissue; however, its enlargement is dependent on creation of new vessels. They usually have irregular shape and disturbed morphology and their wall may be partly composed of cancer cells (vascular mimicry). "Vascular switch" is a very important step to increase both proliferative and metastatic potential of the tumor [5–7]. A large number of tumor-derived proangiogenic factors have been identified: basic fibroblast growth factor (bFGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), platelet-derived endothelial cell growth factor (PDEGF), epidermal growth factor (EGF), angiopoietin-1, transforming growth factor-β (TGFβ), and vascular endothelial growth factor (VEGF) [5, 8]. Tumor environment also contains angiogenesis inhibitors which counteract the action of proangiogenic stimulants. Among the most important inhibitors are cytokines, mainly interferon (IFN)-α and interferon-γ, interleukin (IL)-12, IL-10, as well as angiostatin and endostatin [9, 10]. The proteins belonging to VEGF family (VEGF-A, VEGF-B, VEGF-C, VEGF-D) are extensively studied due to their key role in tumor neoangiogenesis. The dominant role for VEGF-A in tumor vascularization is mediated through binding to VEGFR-2 (KDR/Flk-1) receptor, being expressed on both endothelial and tumor cells [11]. Receptor VEGFR-1 (Flt-1) functions as a decoy receptor [12]. Induction of VEGF in the cancer cells is regulated by different signals, including mutant *p53*, *PTEN*, *Ras* and *Raf* genes, hypoxia-inducible factor (HIF)-1α, growth factors (PDGF, EGF), and cytokines (TGFβ, prostaglandin PGE_2, and interleukin IL-1β). IL-12 and IFN-γ are potent inhibitors of VEGF-dependent angiogenesis [13]. Secretion of VEGF initiates endothelial cell growth and increases expression of matrix metalloproteinases (MMPs) which stimulate endothelial cell division and spread [5, 14–17]. More importantly, overactivity of MMPs secondary to VEGF augments tumor invasiveness and potential to metastasize [18], as immature new capillaries with defective wall allow greater accessibility for migrating tumor cells [19]. VEGF also enhances tumor invasion by direct autocrine effect on tumor cells, as well as indirectly by inhibiting the functional maturation of the host dendritic cells (DCs) [20, 21]. Besides the tumor itself, there are also tumor-associated macrophages (TAMs) and $CD11^+Gr1^+$ myeloid-derived suppressor cells (MDSC), as well as cancer-associated fibroblasts (CAFs) which actively regulate neovascularization of the tumor. According to their function, tumor-associated macrophages are divided to either M1 or M2 type. Macrophages of M1 type could effectively destroy tumor cells through production of Th1 cytokines and stimulation of T $CD8^+$ CTLs. Conversely, macrophages of M2 type mainly produce IL-6, IL-10, TGFβ, and VEGF [22–24]. Hypoxic environment inside solid tumors resulting in VEGF and HIF-1α expression are the stimuli for macrophage recruitment into the tumor and contribute to proangiogenic and prometastatic TAMs activity [4, 25–28]. From the other side, M2-type TAMs enhance intratumor angiogenesis by secretion of VEGF, TGFβ, and FGF and influence extracellular matrix remodeling by MMP production [23]. Myeloid-derived suppressor cells (MDSCs) of $CD11b^+/Gr\text{-}1^+$ phenotype are a population of cells which links the mechanisms of chronic inflammation and tumor progression [29]. Besides suppressing host antitumor responses, MDSCs are also capable of augmenting both the formation of new blood

vessels and tumor metastatic potential [29]. CAFs are the residents of tumor stroma and exert their tumor-promoting activity by secretion of growth factors, including VEGF [30, 31, 32]. The population of CAFs also expresses some chemokines (CCL5, CXCL12, and CXCL14), which enhance tumor angiogenesis and metastatic potential [33, 34]. Cytokines, like TNF-α, IL-4, and IL-10, are candidates for inhibitors of proangiogenic signals originating from cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs), respectively [35].

22.2.2 VEGF in Ovarian Cancer Patients

Cytokine VEGF is not only engaged in normal ovarian function but is also implicated in the growth of highly vascularized EOC [36]. Abnormal expression of certain cytokines, including VEGF, was confirmed in many studies on EOC lines and cell cultures *in vitro* [37]. Transformation of normal ovarian epithelium into neoplastic tissue has been shown to be connected to the VEGF overexpression [36, 38]. Borderline ovarian tumors indicated variable VEGF mRNA expression, while all malignant tumors were characterized by strong VEGF mRNA expression. Higher expression of mRNA for VEGF receptors was also observed in endothelial cells lining the vessels of both borderline and malignant tumors [39]. Expression of VEGF depends also on the histological type of the tumor, serous tumors having the strongest level of expression [40, 41]. Increased concentration of VEGF (and IL-8) has been described in cystic fluid and serum of patients with ovarian endometriosis and EOC, compared to patients with serous and follicular benign cysts [42]. Most patients with EOC are diagnosed at late stages of the disease, when peritoneal dissemination of the tumor and ascites are already present. The role of VEGF has also been proved both in the tumor peritoneal dissemination and the subsequent development of malignant ascites [43–45]. Preoperative serum VEGF levels were raised in patients with EOC compared to those with benign mass and those with tumors of low-malignancy potential. Patients presenting with ascites had similarly significantly increased VEGF serum levels [46]. Patients with higher serum VEGF concentration had significantly shorter overall and disease-free survival [47]. It seems that VEGF serum concentration is an indicator of tumor proliferative activity rather than its dimension [19]. Studies on VEGF concentration in peritoneal fluid of patients with malignant and benign ovarian disease indicated that malignant ascites contained significantly higher concentrations of VEGF (together with IL-6) [37]. In addition, malignant ovarian tissue is characterized by abnormal expression of VEGF receptors [39]. Moreover, genetic studies on *VEGF* gene polymorphisms in EOC patients indicated that simultaneous carriage of three homozygous genotypes (*VEGF* 634C/C, *VEGF* 1154G/G, *VEGF* 2578C/C) was associated with increased tumor VEGF expression and had prognostic value [48].

22.2.3 Role of VEGF for Ovarian Cancer Growth, Dissemination, and Metastases

The main regulatory pathways of VEGF expression in EOC appear to be the same in general as those described in Sect. 22.2.1. It was revealed that the most important inducers of VEGF expression in ovarian tumors were growth factors (like EGF, IGF-1, PDGF), cytokines (TGF-β, TNF-α, IL-6), prostaglandins (PGE-2), factor HIF-1α, cyclooxygenase (COX), or metalloproteinases (MMPs) [49–58]. Production of VEGF is also regulated by oncogenes, i.e., HER-2/neu, which the defective function was confirmed in EOC cells [59]. VEGF-dependent angiogenesis is an early, crucial, and indispensable step in ovarian carcinogenesis [39, 60]. VEGF and its two receptors VEGFR-1 and VEGFR-2 were detected at both mRNA and protein levels in ovarian tumors [39, 61–65]. Cancer cell-derived VEGF shows stimulatory action directed toward host vessels which are remodeled in order to support tumor growth. This stimulation is mediated by upregulation of angiopoietin-2 on endothelial cells

[66]. Moreover, autocrine loop based on VEGF–VEGFR-2 interactions and synergy between VEGF and CXCL12 together augment neovascularization of ovarian tumor [49, 62]. The role of VEGF is not restricted to pro-vascularization activity, as it was shown that VEGF directly stimulates proliferative activity of tumor cells [67], and a significant correlation between the expression of VEGF/VEGF receptors and the activation status of signal transducer and activator of transcription pathway (STAT3, STAT5) was evidenced in EOC cells [68]. Besides proliferation, VEGF contributes to EOC metastasis by reprogramming the metastatic profile of tumor cells, stimulating the migration of those cells and performing a direct suppression of host antitumor cytotoxic activity [69–71]. The migration of cancer cells is enhanced by the fact that newly formed vessels are structurally and functionally abnormal, which results in the increase of vascular permeability. VEGF-mediated activation of ovarian cancer Src-family kinases [72] and metalloproteinases (MMP-2, MMP-7, MMP-9) is responsible for this phenomenon [73, 74]. Cancer growth and metastatic potential correlate with its VEGF-producing capacity and are inhibited by the suppression of VEGF function [72, 75]. Other components of the pro-metastatic tumor environment are TAM and CAF cells capable of extracellular matrix degradation [76]. Synergy exists between VEGF and growth factors, like PDGF or bFGF, according to the proliferative and metastatic abilities of EOC cells. Inhibition of bFGF and VEGF activities suppressed the growth and metastases production *in vivo* in murine model of ovarian cancer [52, 60, 77]. Similar mechanisms are responsible for VEGF-mediated intraperitoneal dissemination of EOC, development of peritoneal carcinomatosis, and finally for ascites production [61, 78, 79]. In all those pathologies, VEGF plays a pivotal role, as it was shown that growth of new peritoneal implants and production of ascitic fluid clearly depend on neovascularization and increased vessel permeability produced by VEGF, even in the areas of unchanged peritoneum [80–83]. Both ovarian cancer cells and mesothelial cells of the peritoneum were identified as VEGF producers [84]. It was shown that expression of VEGF in omental metastases correlated with preoperative CA125 levels, the extent of omental infiltration and patient's outcome [85]. Confirmation of the key role of VEGF in ascites formation comes from the studies which found that ascitic VEGF concentration correlated with fluid production and VEGF inhibition both hampered implant growth and decreased malignant ascites [86, 87]. Regulation of VEGF function in the peritoneal cavity is quite complex, and it seems that at least MMPs (MMP-2, MMP-9) and TGF-β could stimulate or inhibit VEGF expression, respectively [53, 57, 88]. Extraperitoneal metastases of EOC to the lymph nodes are also VEGF dependent (VEGF-C), and it seems that VEGF contributes to EOC metastases to the lungs and liver [72, 77, 89]. Despite the fact that VEGF is responsible for neovascularization, attempts for combining VEGF concentration with microvessel density (MVD) in the tumors have so far given conflicting results. While in some studies the correlation between VEGF and MVD was confirmed for VEGF-rich malignant tumors [90], results of other studies showed that VEGF immunostaining was not significantly stronger in high-MVD regions of malignant compared to borderline tumors [91]. The last studies seem to support the notion that no correlation exists between MVD and VEGF expression, as well as between MVD and patient's clinicopathologic parameters [92]. Therefore, although VEGF controls the tumor angiogenesis, it does not consistently contribute to higher MVD values observed in the ovarian tumors.

22.3 The Role of Pro-inflammatory Cytokines in Ovarian Cancer

22.3.1 Inflammation and Cancer: General Remarks

It is now an unquestionable opinion that an association exists between inflammation and cancer development [93, 94]. It was found that chronic inflammation may account for about 15 % of

cancers. Molecules produced by infection-activated inflammatory immune cells, like TNF-α, could initiate tumor growth by stimulation of nitric oxide and ROI, both being capable of DNA damage [95, 96]. Cytokine TNF-α is also capable of DNA repair inhibition [97, 98]. Current data support the opinion that during later steps of tumor development, chronic inflammation resulting from tumor-infiltrating immune cells does not enhance cancer eradication, but instead contributes to cancer progression [99, 100].

Activation of TLR receptors which are present on the surface of innate immune cells, mainly macrophages but also on cancer cells themselves, enhances tumor growth by various mechanisms like stimulation of growth-promoting cytokines or protection against apoptosis [101–103]. Genetic studies confirm the importance of TLRs for cancer development, as polymorphisms in the group of genes encoding TLR6 and TLR10 positively influenced the risk of some cancers [104]. One of the pro-inflammatory cytokines stimulated by TLRs is TNF-α which promotes tumor survival by stimulation of NF-κB-dependent antiapoptotic molecules, inhibiting antitumor cytotoxic reactions and augmenting tumor proliferation, neoangiogenesis, and metastatic properties [105, 106]. Genetic polymorphisms that enhance TNF-α production were connected with both greater risk of cancer and poor prognosis [107], while in mice deficient for TNF receptors, reduced incidence of tumors was noted [108]. However, high-dose management of tumors with TNF-α kills the cells and disrupts tumor vasculature [109]. Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is one of the inducers of apoptosis in tumor cells. However, its effects could be abrogated by TNF-α-mediated activation of NF-κB pathway in cancer cells [105]. TRAIL-deficient animals presented with greater predisposition to induced and spontaneous cancers [110]. In some tumors, resistance against the TRAIL-dependent apoptosis evolved, based on the lack of caspase expression, inactivation of proapoptotic proteins, overexpression of mutant *PTEN* gene, and upregulation of Akt/NF-κB intracellular pathway [111, 112].

Interleukin (IL)-6 is a pro-inflammatory cytokine which through activation of intracellular STAT3 pathway influences the function of genes engaged in cell proliferation and resistance to apoptosis [111, 113]. Genetic studies have revealed that some polymorphisms of the *IL6* gene promoter region could interfere with the IL-6 production, thus changing the risk of certain tumors and patients' prognosis [114]. *In vitro* studies showed that IL-6 augmented growth of colon carcinoma cells in a dose-dependent manner, which was further confirmed *in vivo* by an evidence that IL-6 serum levels correlated with the dimensions of the tumor in colon cancer patients [115, 116]. The IL-6-mediated signaling in colon cancer occurs mainly by soluble IL-6 receptors (sIL-6Rs) [117].

Clinical observations on prophylactic use of nonsteroid anti-inflammatory drugs in colorectal cancer and confirmation of antitumor effects which resulted from the application of selective cyclooxygenase (COX)-2 inhibitors have turned the attention to COX-2–PGE_2 tumor development pathway [14, 73]. Cyclooxygenase-2 is upregulated by pro-inflammatory cytokines, HIF-1α, and tumor promoters followed by a higher expression of PGE_2 which in turn activates EGF receptor and interacts with different intracellular pathways, including Ras/MAPK signaling pathway, PI3K/Akt pathway, and NF-κB-mediated pathway [14, 73]. Overexpression of COX-2 also stimulates VEGF, which through a positive feedback loop enhances autocrine PGE_2 secretion [118]. It was shown that COX-2 overexpression influences tumor neoangiogenesis and predicts poor survival in some cancer patients [75, 119, 120]. Prostaglandin PGE_2 indicates multidirectional influence on tumor immunological environment. It decreases homing of immune cells into the tumor by changing tumor chemokine expression, inhibits DCs maturation and migration toward tumor-draining lymph nodes, upregulates Th2 (IL-4, IL-10) cytokines, and increases tumor migratory and metastatic potential [14, 29, 121].

The relationship between the cancer and inflammation is also documented by results of studies on pro-inflammatory cytokine IL-23, which is physiologically secreted from activated DCs and macrophages upon bacterial stimulation and exerts functional effects on many immune cell

populations, including memory IL-17-secreting Th17 cells [122]. Upon stimulation by IL-23, IL-6 and TGFβ are produced by tumor cells and Th17 cells release IL-17 and other inflammatory mediators like IL-1, IL-8, TNF-α, PGE_2, as well as chemokines which produce a pro-tumor inflammatory environment. The increased expression of both IL-23 and IL-17 was observed in many malignant tumors and correlated with angiogenesis, expression of MMPs, and decrease of cytotoxic antitumor immune response [3, 123].

Interleukin-10 is considered to mainly have antitumor activity, as many studies revealed that IL-10-mediated inhibition of VEGF, TNF-α, and IL-6 resulted in tumor growth restriction [35, 124, 125]. It was also shown that IL-10-stimulated Treg cells with anti-inflammatory phenotype triggered and stimulated by recognition of gut bacteria are able to control the colon cancer initiation [126]. However, the biological effects of IL-10 are much more complex and in part are responsible for promotion of tumor growth. Activation of the STAT3 pathway by IL-10 in tumor cells upregulates *Bcl-2* gene and protects cancer cells from apoptosis [127]. IL-10 was also shown to upregulate HLA-G molecule which protects the tumor from immuno-recognition by host cells, thus enhancing "escape" stage of the tumor growth [128]. Hypoxic environment inside solid tumors provokes cancer cells to generate free adenosine which protects the tumor from cytotoxic T lymphocyte by IL-10-mediated activity of TAMs [129, 130]. Immunosuppressive IL-10 was shown to be secreted directly by tumor cells [131], thus skewing the intratumor and peripheral blood milieu for immunosuppressive phenotype (M2-type TAMs and Th2-type lymphocytes). Moreover, immunoregulatory Tr1/Th3 and $CD4^+CD25^+Foxp3^+$ Tregs produce IL-10 which inhibits host antitumor cytotoxic reactions [132–134]. Defect of Th1 activity was shown in peripheral blood of cancer patients and was correlated with tumor progression [135–137]. Expression of IL-10 was found to be increased in metastatic tumors [138].

Similar to IL-10, bidirectional activity of TGF-β for both cancer regression and promotion was shown in many studies. Cytokine TGF-β exerts direct suppressor activity against colon cancer cells and shows anti-inflammatory properties on immune cells, in part by inhibition of IL-6 secretion by Th1 cells [117, 139]. Despite antitumor activity in early tumors, TGF-β might also enhance tumor growth in later stages. The source of TGF-β could be both tumor cells and M2-type TAMs [22, 23, 131]. Tumor-derived TGFβ facilitates the recruitment of regulatory cells secreting IL-10 and TGF-β which further suppress host immunity [140]. It was confirmed that in advanced tumors, TGF-β is engaged in many mechanisms of "tumor escape," including Th17 cell differentiation, inhibition of DC maturation, and stimulation of VEGF production, generating the $CD4^+CD25^+Foxp3^+$ Tregs and decreasing the activity of NKT, T $CD8^+$, and NK cytotoxic cells [132, 134].

22.3.2 Inflammatory Reaction and the Risk of Ovarian Cancer

Epidemiological data suggest that the overall risk reduction for EOC could be attributed to increased parity, prolonged breast-feeding, and use of oral contraceptives, all of them acting as protective factors against frequent ovulation [141–144]. Exposure to toxic pro-inflammatory agents like talc or asbestos which could enter the peritoneal cavity through the tubes increases the risk of EOC, while hysterectomy and tubal ligation seem to reduce that risk [145, 146]. Therefore, local inflammation existing in the pelvic macroenvironment may increase the risk of EOC. The greater risk for ovarian carcinogenesis observed in patients with PID (particularly chronic PID with coexistence of infertility) supports these observations [147, 148]. On the contrary, prolonged use of nonsteroid anti-inflammatory drugs and acetylsalicylic acid has a protective action against EOC [149]. Ovulation traditionally considered as a major risk factor for ovarian carcinogenesis is also an inflammatory process. Preovulatory follicles contain TNF-α and metalloproteinases (MMP-1, MMP-2) which are responsible for cellular death and proteolysis leading to thinning and consecutive rupture of the follicular wall [150]. Reparation and proliferation

of ovarian surface epithelial cells during formation of corpus luteum depend on expression of growth factors (EGF, PDGF), toll-like receptors (TLR2, TLR4), and pro-inflammatory cytokines, including IL-1, IL-6, IL-8, TNF-α, COX, and prostaglandins, while TGF-β counteracts their function and exerts antiproliferative effects. Inadequate repair of the ovarian epithelium at the site of ovulation may facilitate the damage of DNA and initiate carcinogenesis [151–153]. According to "gonadotropin hypothesis," pituitary hormones could further stimulate the proliferation and malignant transformation of ovarian epithelium [154]. Similarly, endometriosis with its well-recognized inflammatory background is a risk factor for ovarian carcinogenesis, as it was found that women suffering from long-term ovarian endometriosis had 2.5–4.0-fold greater risk of EOC (especially of endometrioid and clear-cell types) compared to healthy subjects [155, 156]. It is a common knowledge that implants and peritoneal fluid of endometriotic patients contain pro-inflammatory cytokines (PGE_2, COX, IL-1β, IL-6, IL-8, TNF-α, and TGF-β) produced by activated macrophages [157, 158]. The concentration of MMP-1 and MMP-9 both in peritoneal fluid and implants was found to be elevated and correlated with VEGF expression [159, 160]. M2-type macrophages and pro-inflammatory cytokines are orchestrated to stimulate endometrial implant growth and augment their vascularization. The possible role of inflammation in origin of tubal intraepithelial carcinoma (TIC) which has been recently considered as a preclinical step for a high-grade serous EOC in women carriers of BRCA mutation and in a subset of nonfamilial serous cancer patients has been strongly suggested [161]. Inflammatory episodes inside the tubes could result from retrograde menstruation or chronic inflammation caused by bacterial agents including *Chlamydia trachomatis* [162–164]. Endometrial fluid in the cases of retrograde menstruation contains inflammatory mediators like IL-8, TNF-α, and GM-CSF, which are also elevated in EOC [165]. Moreover, epithelium of tubal fimbriae exposed to oxidative stress and cytokines produced by peritoneal macrophages activated by menstrual blood might be subjected to inflammation-dependent mutagenesis [166, 167]. Epidemiologic studies indicate that chronic inflammation from hepatitis, human papilloma virus infection, or ulcerative colitis may be connected to increased risk of tubal cancer [162].

22.3.3 Inflammation and Ovarian Cancer Growth and Dissemination

Although genetic mutations are the crucial initial step in ovarian carcinogenesis, this is an inflammation that plays a key role as a shaping environmental factor. The biochemical and immunological features of particular cancer cell populations indicate that inflammatory pathways inseparably influence their phenotype. Ovarian cancers are a heterogenic group of tumors which are characterized not only by a different histology but also because of existence of high/low malignancy potential tumors as well as by the presence of different populations of tumor cells based on their chemoresistance. Mutations of *K-ras*, *BRAF*, and *PTEN* genes are observed mainly in low-malignancy potential ovarian tumors (classified as type I tumors), while *p53* mutations are typical for high-malignancy potential type II tumors [161]. Moreover, type II cancers have different patterns of biological behavior characterized by overexpression of HLA-G molecule and inflammatory mediators (iNOS, COX, GLUT-1) [168, 169]. Based on the chemoresistance, two populations of cancer cells coexisting inside the tumor were identified, type I chemoresistant and type II chemosensitive cells. Type I cells are a slow-growing population, having constitutive pro-inflammatory phenotype with NF-κB activity and IL-6, IL-8, MCP-1, and GRO-1/α secretion and stemlike properties, while type II cells represent classical tumor cells with high proliferative activity and fast growth [170]. Proliferation and cytokine secretion of type I cells depend on a TLR-4 pathway [171] and may result in their differentiation to type II cells. Activation of TLR-4 pathway by cells disintegrated after surgery or chemotherapy may thus initiate the tumor renewal from stemlike type I cancer cells [170].

Moreover, TLR-4 expression in EOC cells was connected with resistance to paclitaxel therapy [172]. Some other TLRs were detected on malignant ovarian epithelial cells, including TLR-2, TLR-3, TLR-5, and TLR-9 [173]. Studies on their function for EOC promotion revealed that TLR-3 signaling can contribute both to tumor eradication and to its progression and neovascularization, dependent on the intracellular signaling via RIP-1/FADD or NF-κB pathways, respectively [174]. In addition, it was demonstrated that TLR-9 overexpression is associated with poor differentiation and high metastatic potential of the EOC [175].

There are strong suggestions that stromal senescent ovarian fibroblasts are capable of promoting ovarian carcinogenesis by creation of a pro-inflammatory network [176]. Upon senescence, aged fibroblasts acquire "senescence-associated secretory phenotype" (SASP) characterized by activation and production of pro-inflammatory molecules including interleukins (IL-6, IL-1β), chemokines (IL-8, MCP-1, GRO-1/α), MMPs, adhesion molecules, and integrins [176, 177]. It was shown that GRO-1/α-mediated senescence of ovarian fibroblasts was a tumor-promoting factor for ovarian epithelial cancer in nude mice [178]. In humans, the senescent stromal fibroblasts were detected in specimens of ovarian tumors in areas adjacent to malignant epithelium [178]. Cellular senescence could be triggered *in vivo* by both physiological (i.e., ovulation) and pathological (i.e., infection) inflammatory stimuli existing in tubal and/or peritoneal macroenvironment and microenvironment [176].

Interleukin-6 produced either by tumor cells themselves or by M2-shifted tumor-associated macrophages is one of the most important pro-inflammatory mediators in EOC patients [179]. Interaction of EOC cells and host immune cells produces a cooperative network (together with IL-1, TNF-α, VEGF, and chemokines) at the tumor site, responsible for its growth and progression in which IL-6 seems to be the most important [55, 180]. *In vitro* investigations showed that *p53* overexpression observed in EOC tumors could regulate IL-6 secretion [181, 182]. Genetic studies on G/C polymorphism at the position −174 of *IL6* gene brought the conclusion that patients possessing −174 G/G phenotype presented with early diagnosis and had better disease-free and overall survival [183, 184], indicating the key role for IL-6 in EOC. In women with advanced cancer, significantly higher IL-6 levels both in the serum and ascites were observed [185–187]. *In vitro* studies showed that the contact of EOC cells with autologous peripheral blood mononuclear cells (PBMC) altered the pattern of produced cytokines into pro-inflammatory profile, including increased IL-6 secretion [188]. Moreover, IL-6 was able to reciprocally stimulate *in vitro* EOC cell proliferation [189]. Although the action of IL-6 is mediated by membrane-bound IL-6R receptor, the presence of highly inflammatory conditions in the case of EOC forces the use of an alternative IL-6-signaling pathway based on soluble ("shedded") sIL-6R variant. This is the major IL-6-signaling pathway for migrating cancer cells [190, 191]. It was demonstrated that IL-6 was engaged in many aspects of EOC growth *in vivo*, starting from promotion of angiogenesis through stimulation of peritoneal metastases and finally by initiation of ascites production [190, 192]. Interaction of IL-6 with receptors leads to activation of intracellular STAT3 transcription factor, which is overexpressed in EOC tissue, thus resulting in increased tumor proliferative capacity. The correlation between STAT3 activation and both tumor-aggressive phenotype and disease advance was observed in EOC patients [193, 194]. *In vitro* studies indicated that treatment of EOC lines with EGF or exogenous IL-6 stimulated STAT3 activity and induced epithelial–mesenchymal transition and appearance of cell migratory phenotype [193]. This observation also suggests that STAT3 activation in EOC could be obtained by different pathways [195]. Paracrine effects of IL-6 could induce suppressive Th2 phenotype in tumor-infiltrating immune cells and inhibit cytotoxic T-cell activation by induction of lymphocyte apoptosis [196]. Immunosuppressive properties of ascites environment were confirmed by observation of a high CD4/CD8 ratio, reduced IL-2, and increased IL-6 and IL-10 concentrations promoting Th2 lymphocyte activity [197]. The

lower proliferative response and IL-2 production capacity observed in PBMC of advanced EOC patients may be a consequence of JAK3, STAT3, and CD3-ζ signaling abnormalities resulting from a suppressive cytokine environment [198]. Many studies confirm that notion that despite the presence of intratumoral immune cell infiltrate, the observed anticancer host response is inefficient and correlates with the stage of EOC [199, 200]. One of the most typical mechanisms observed in EOC immunoediting is facilitating of TAMs M2-type activity by tumor-derived cytokines. IL-6 and LIF present in high concentrations in EOC ascites are key players in this mechanism [201]. The multidirectional activity of IL-6 on several genes in advanced EOC results in cachexia, anemia, depressive behavior, and tumor multidrug resistance [166]. The proinflammatory environment produced by both tumor and activated immune cells, especially TAMs, results in chronic overactivity of IL-6, IL-1, and TNF-α and cancer-related anorexia/cachexia (CACS) [202, 203]. In this context, a very important observation is that of negative correlation between serum leptin concentrations and both IL-6 levels and stage of the EOC [204]. As leptin controls energy metabolism and weight balance, its function impairment associated with chronic inflammation results in development of CACS in EOC patients [166]. The administration of anti-IL-6 antibody inhibits the development of most cachectic changes which demonstrates the central role of this cytokine in the pathogenesis of CACS [205]. Pro-inflammatory status in EOC is also responsible for anemia observed in the patients, as the low hemoglobin concentration correlates with serum IL-6 and leptin levels, as well as with IL-6 concentration in malignant ovarian cysts [206, 207]. Moreover, chronic inflammation is associated with disturbed function of hypothalamic–pituitary axis and secondary hypercortisolemia observed in depression. It was shown that EOC patients with depressive symptoms were characterized by IL-6 serum elevations [208]. IL-6-mediated STAT3 activation in EOC is responsible for tumor chemoresistance, as STAT3 antagonists were able to decrease EOC invasiveness and enhanced sensitivity to paclitaxel [194]. The mechanism of chemoresistance induction is probably mediated also by IL-6-dependent activation of apoptosis-inhibitory proteins [190].

A cytokine that is actively involved in immunoregulation in EOC patients is also TGF-β. Genetic studies indicated that age-related epigenetic modifications could lead to suppression of TGF-β signaling and thus contribute to ovarian carcinogenesis [209]. Similarly, promoter hypermethylation of transforming growth factor-β-inducible gene-h3 (TGFBI) was frequently observed in EOC and associated with paclitaxel resistance [210]. Mutations of the TGF-β-receptor type I and II genes (*TGF-β RI*, *TGF-β RII*) and variants of *Smad2* gene engaged in TGF-β-mediated signal transduction pathway were identified in EOC and associated with reduced TGF-β RI and p53 protein expression [211]. In gene-based analysis of the TGF-β-signaling pathway, *SMAD6* was identified as the most significant gene associated with ovarian cancer risk [212]. Resistance to TGF-β-signaling observed in most EOC means that contrary to normal ovarian epithelium, tumor cells acquire genetic changes which in early steps of carcinogenesis help them to escape the antiproliferative effects of TGF-β (Yamada et al. 1999). TGF-β1 was detected at the mRNA level in EOC cell cultures *in vitro*, and TGF-β1 levels were elevated *in vivo* in the plasma and peritoneal fluid of ovarian cancer patients [213–215]. Overexpression of TGF-β isotypes on the EOC cells was confirmed and connected to more aggressive tumor behavior [216, 217], the fact which supports the TGF-β meaning for EOC growth, angiogenesis, and suppression of the host immunity. Tumor-derived TGF-β may contribute to peritoneal dissemination of EOC, as *in vitro* studies revealed that TGF-β enhanced specifically plasminogen activator inhibitor type 1 (PAI-1) upregulation in mesothelial cells, thus increasing the cell–cell interactions [218]. Moreover, TGF-β isotypes induced MMP secretion in EOC cell cultures, followed by loss of cell–cell junctions, downregulation of E-cadherin, upregulation of N-cadherin, and acquisition of a fibroblastoid phenotype, consistent with an epithelial-to-mesenchymal

transition. These changes were abrogated by use of *Smad3* siRNA transfection, indicating that TGF-β/Smad3 pathway positively regulates EOC metastatic potential. The correlation between high tumor Smad3 expression and poor patients' survival was a clinical confirmation of that statement [214]. Animal studies on nude mice showed that TGF-β blockade impaired tumor growth by decreasing its proliferation and angiogenesis. Additionally, TGF-β blockade abolished VEGF-dependent ascites formation and improved ascites drainage [53, 88]. TGF-β was shown to have an immunosuppressive effect on TILs from patients with ovarian cancer and to convert effector T cells into Tregs [219–221]. Tumor-derived TGF-β, together with TNF-α, induced functional alterations in tumor-associated plasmacytoid dendritic cells (TApDCs) which produced less pro-inflammatory cytokines compared to pDCs found in ascites or peripheral blood and thus created local immune tolerance for the tumor [222].

22.3.3.1 Tumor Necrosis Factor-α

The role of TNF-α for the promotion of EOC was widely studied. Both serum and cyst fluid levels of TNF-α were reported to be higher in women with ovarian carcinoma compared to healthy subjects and women with benign ovarian cysts [223, 224]. Similarly, increased TNF-α concentrations were observed in cancer tissues and ascites [225]. Cancer patients were also characterized by overexpression of receptor TNF-R2, which was further correlated with tumor stage and patient prognosis [226]. TNF-α is produced by tumor cells, and studies devoted to EOC indicated its multidirectional activity, as an autocrine stimulator of tumor progression and neovascularization as well as a downregulator of Tregs and enhancer of the host immunity against tumor [54, 227–229]. In a tumor xenograft model, TNF-α treatment converted EOC xenograft tumors to peritoneal masses with well-developed stroma [230]. According to some opinions, TNF-α together with IL-6 and stromal cell-derived factor-1 (CXCL12) induced by pro-inflammatory milieu creates an autocrine/paracrine "TNF network" functioning as a regulator of tumor growth [180]. The members of this network were constitutively expressed and co-regulated in EOC cells, and the dependency of the whole network from TNF-α was proved by downregulation obtained by the use of anti-TNF antibodies [54, 180]. Some other cytokines/chemokines like macrophage migration inhibitory factor (MIF), VEGF, and monocyte chemotactic protein-1 (MCP-1, CCL2) belong to that regulatory network [54]. The function of the CXCL12 was based on the interactions with its receptor CXCR4 and upregulated by TNF-α through a NF-κB-dependent pathway [231]. Ovarian cancers also indicated higher TRAIL expression compared to normal ovaries, and the highest TRAIL expression was correlated with favorable survival and better chemosensitivity [232–234]. A very interesting problem is an existence of functional connections between coagulation pathway and inflammation. It is a well-known finding that patients with cancer, especially in advanced stages, present with hemostatic abnormalities and pro-coagulatory state [189, 235]. TNF-α, a stimulator of tissue factor (TF) production by TAMs, upregulates the cell surface leukocyte adhesion molecules and downregulates thrombomodulin [236–238]. Ovarian cancer cells may also express TF and other coagulation components that generate local thrombin [239]. Tissue factor plays an important role in both coagulation-dependent and coagulation-independent pathways, which result not only in clot formation but could contribute to tumor progression [189]. TF was shown to promote tumor angiogenesis by activation of MAPK and protein C kinase-dependent signaling [50, 240]. The TF/PAR-2 pathway synergizes the function of growth factors to enhance the formation of metastases, and TF via activation of $P21^{Ras}$ and P42/P44 MAPK pathway mediates inhibition of EOC cell apoptosis and overexpression of chemokines [241–243].

22.3.3.2 Interleukin-10

Interleukin-10 secretion occurs predominantly in the tumor environment, EOC cells and host immune cells being the main sources of IL-10 [215, 244, 245]. Inside the tumor, the main secretors of IL-10 are endothelial cells, characterized by high expression of both IL-10 and its recep-

tor IL-10R mRNAs, which means that this cell population is also the main responder for IL-10 autocrine actions [246]. Another cell population secreting IL-10 are myeloid-derived suppressor cells (MDSC). In normal ovaries, IL-10 acts as cytokine synthesis inhibitor, but in ovarian cancer, IL-10 seems to favor tumor progression and protect EOC from cell-mediated host immunity [246–251]. Consistent with these observations, IL-10 concentrations in peritoneal fluid and serum of ovarian cancer patients were found to be higher than in patients with benign ovarian diseases, and the IL-10 levels in ascites were higher compared to serum [186, 252, 253]. Moreover, the expression of IL-10 was found to correlate with tumor aggressiveness, thus being the highest in malignant compared to borderline tumors, and IL-10 was concomitantly overexpressed with VEGF in all tumor tissues [254]. IL-10 present in malignant ascites of EOC patients was shown to promote antiapoptotic activity probably by inhibition of TRAIL-mediated apoptosis of cancer cells. Clinical studies confirmed that patients with higher IL-10 levels in ascites had shorter progression-free survival [255]. *In vitro* studies on EOC cell lines indicated that VEGF and TGF-β1 strongly interfered with DC maturation and consequently led to immature DCs, which secreted high levels of IL-10 accumulating around the tumor site. TGF-β1 and IL-10 induced Treg generation without antigen presentation in DCs [254]. *In vitro* and *in vivo* animal studies indicated, however, that IL-10 should not be considered only as a tumor-promoting factor, as IL-10 had suppressive effects on angiogenesis, tumor growth, and peritoneal dissemination of VEGF-producing ovarian cancer cells [256]. Clinical studies also indicated that only about 55 % of specimens from EOC patients expressed IL-10 [254]. Therefore, it seems reasonable to conclude that the presence of IL-10 overactivity and a precise mechanism of IL-10 action may depend on the tumor phenotype.

22.3.3.3 COX and PGE_2

Several *in vivo* and *in vitro* studies suggest that using nonsteroid anti-inflammatory drugs (NSAIDs) reduces the proliferative activity, MMP-dependent metastasis potential, and VEGF-dependent vascularization of EOC [58, 257–260]. Therefore, the role of COX and PGE_2 in ovarian tumors has been extensively studied. COX exists in two isoforms (COX-1 and COX-2). COX-1 is constitutively expressed in most tissues and plays a role in various physiologic functions, whereas COX-2 is transiently inducible by inflammatory cytokines, growth factors, oncogenes, and hormones [261–264]. The expression of both COX isoforms was observed in the epithelial lining of inclusion cysts of normal ovaries. The appearance of COX-2 in this localization might indicate an alteration of epithelial cell phenotype; as for COX-2 expression, the upregulation of C/EBPβ transcription factor is required, the phenomenon which similarly was noted in EOC and correlated with tumor malignancy [73, 265–267]. Upregulation of C/EBPβ transcription factor, probably initiated by some pro-inflammatory stimuli existing in the ovarian microenvironment, induces the activity of *cox2* gene which is one of the participants of ovarian carcinogenesis, as was proved by *in vitro* studies [265, 268]. Moreover, appearance of COX-2 accompanied to the loss of epithelial basal membrane in ovarian specimens from BRCA1/BRCA2 mutation carriers [269]. Overexpression of COX-2 in EOC was confirmed and correlated with resistance to platinum-based chemotherapy and short survival, while upregulation of COX-1 was suggested to enhance neovascularization [58, 270–275]. An increase of COX-2 expression was observed in malignant compared to borderline and benign tumors, while COX-1, microsomal prostaglandin E synthase-1 (mPGES-1), and prostaglandin E receptor-1 (EP1) were upregulated exclusively in highly malignant tumors [265]. The immunohistological staining revealed that COX-1, COX-2, mPGES-1, and EP1 were positive not only in tumor epithelial cells but also in the tumor stroma, indicating that stromal fibroblasts and/or immune cells participate in the paracrine COX/PGE_2-mediated signaling [265]. The expression of COX-1 was significantly higher in non-mucinous compared to mucinous tumors [276]. Tumors presenting with expression of both COX-1 and COX-2 simultaneously

with low density of T $CD8^+$ cytotoxic cells and high density of $CD1a^+$ DCs were characterized by the worst prognosis [277]. This means that the COX upregulation may influence the pattern of tumor infiltration by immune cells. Some studies postulate that COX-1, but not COX-2, is exclusively expressed on human EOC cells, as well as on genetically engineered tumors studied on murine model [257, 270]. In murine EOC model, COX-1 inhibitors administered together with Taxol decreased the expression of VEGF mRNA levels, reduced microvessel density (MVD), and enhanced cellular apoptotic index [278]. The observed effects of COX inhibition on EOC proliferation and apoptosis were synergistic, while using together COX-1 and COX-2 inhibitors compared to selective use of either COX-1 or COX-2 inhibitors [279] which supports the opinions of both COX-1 and COX-2 meaning for EOC. Peroxisome proliferator-activated receptor-γ (PPARγ), which activation has been linked to cellular differentiation, apoptosis, and anti-inflammatory reaction, is engaged in regulation of COX [280]. PPARγ activation could inhibit COX-2 expression in EOC cells via NF-κB pathway [281]. The xenografted mice treatment of EOC with COX-2 inhibitor and PPARγ stimulator resulted in decrease of serum and ascites PGE_2 levels, reduction of MVD, enhanced tumor apoptosis, and prolonged survival [282]. Another regulatory possibility is based on COX-1/PPARδ/ERK pathway. Peroxisome proliferator-activated receptor-δ (PPARδ) was found on the surface of EOC cells, and its inactivation resulted in tumor growth restriction. Aspirin that preferentially inhibits COX-1 could compromise PPARδ function and tumor growth by inhibition of extracellular signal-regulated kinase 1/2 (ERK1/2) signaling [283]. The next regulatory mechanism is associated with the function of insulin-like growth factors (IGFs) and their binding proteins (IGF-BPs) which were identified in ovarian tumors [284–288]. Increased serum concentrations of IGF-BP-2 were observed in EOC patients and correlated with aggressiveness of the tumor [289]. *In vitro* studies indicated that treatment of the cells with IGF-BP-2 stimulated their growth and COX-2 (but not COX-1) expression through multiple ways, namely, ERK 1/2 pathway, the stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) pathway, and the p38 MAP kinase pathway, which mediates inflammatory and stress responses [290]. Similarly, induction of COX-2 expression in EOC cells mediated by IGF-I is dependent on PI3K, MAPK, and PKC pathways [291]. The elevated levels of lysophosphatidic acid (LPA) were detected in serum and ascites of EOC patients, and it was shown that LPA was responsible for enhancement of migration and invasion of cancer cells via stimulation of MMP-2 [292–296]. It was shown that LPA-mediated MMP-2 activation resulted from the COX-2 expression induction in EOC lines by transactivation of EGF receptor and Ras/MAPK pathway [259]. Prostaglandin E_2 (PGE_2) produced upon activation of COX enzymes was found in increased amounts in ovarian tumors and was recognized as a positive regulator of proliferation and angiogenesis [297, 298]. The background for COX-dependent neovascularization of the ovarian tumors was the observation that treatment of EOC cells with endothelin-1 (ET-1) induced COX-1 and COX-2 expression, followed by PGE_2 production and interaction with EP2 and EP4 receptors that finally resulted in stimulation of VEGF [299]. The effects of ET-1 were mediated by endothelin A receptor (ET_AR), activation of p42/44 MAPK and p38 MAPK signaling pathways, and transactivation of EGF receptor [300]. Concurrent with "gonadotropin hypothesis" postulating that FSH/LH hormones can promote ovarian cancer, a recent study demonstrated that FSH/LH can upregulate COX-1 and COX-2 via PI3K/AKT signaling pathway. Upregulation of both COX isoforms was critical for gonadotropin-induced production of PGE_2 and expression of MMP-2 and MMP-9 in EOC cells [301].

22.3.3.4 Interleukin-23 and Th17 Cells

The IL-23 and its receptor IL-23R have been engaged in carcinogenesis, and *IL-23R* gene polymorphism analysis revealed that the frequency of C and A alleles in rs10889677 position differed between EOC patients and controls, as well as

between advanced and less-advanced tumors [194]. Ovarian cancer samples particularly showed high level of expression of genes encoding members and cooperates of TNF-signaling pathway, including IL-23, TGF-β, and NF-κB system [302]. Tumor cells, cancer-associated fibroblasts, TAMs, T cells, and APCs produce pro-inflammatory cytokines (IL-1β, IL-6, IL-23, TNF-α) that facilitate the expansion of Th17 cells in tumor environment [302, 303]. High numbers of Th17 cells have been identified among ovarian tumor TILs, and IL-17 was consistently detectable in both serum and ascites of EOC patients [302–304]. Using a murine model of EOC, it was found that TNF-α/TNF-R1 signaling maintained IL-17 secretion by T $CD4^+$ cells and led to myeloid cell recruitment into the tumor. Consistent with this observation, treatment with anti-TNF antibody reduced serum IL-17 levels in EOC patients and downregulated expression of IL-1R and IL-23R in T $CD4^+$ $CD25^-$ cells isolated from tumor ascites [302]. Similarly, treatment with anti-IL-1 antibody alone or a combination of anti-IL-1 and anti-IL-6 antibodies reduced the ability of tumor cells to expand memory Th17 cells [303]. It seems that chronic inflammation in tumor localization contributes to tumor-promoting actions mediated by IL-17. However, IL-17, due to its functional ambiguity, could also contribute to the effective host antitumor immune response. In tumors characterized by abundant TIL infiltrate, it was shown that Th17 cells secreting IFN-γ and IL-17 were able to upregulate CXCL9 (monokine induced by interferon-γ, MIG) and CXCL10 (interferon-γ-induced protein-10, IP10) chemokines, thus leading to chemoattraction of NK and T cytotoxic cells. It was correlated with patients' better prognosis [305].

22.3.3.5 Macrophage Migration Inhibitory Factor

Macrophage migration inhibitory factor (MIF) was originally described as a product of activated T cells capable of inhibition of macrophage migration [306]. Further studies revealed that MIF is a potent inducer of pro-inflammatory cytokines TNF-α and IL-10 in macrophages and could be produced by a variety of tissues including EOC cells [307, 308]. MIF could also protect both tumor cells and macrophages from apoptosis by suppressing the p53-dependent pathway [309, 310]. MIF is overexpressed in borderline and malignant ovarian tumors and is present in ascites-derived tumor cells, as well as in ascitic fluid [311, 312]. The main source of MIF in cancer patients is EOC cells, while contribution of MIF originating from TAMs appears to be small. Overexpression of MIF correlates with tumor invasiveness, and higher levels are associated with bad prognosis [312]. Murine studies indicated that knockdown of MIF in EOC cells resulted in decreased proliferation and increased cancer cell apoptosis and downregulation of TNF-α, IL-6, and IL-10 in the ascitic environment. It also reduced the expression of VEGF. The results of these investigation sustain the statement that autocrine production of MIF by EOC cells promotes creation and neovascularization of peritoneal implants [311]. Paracrine functions of MIF concentrate on downregulation of NKG2D receptor expression on NK and $CD8^+$ T cytotoxic cells, the fact which was proved by *in vitro* experiments using recombinant human MIF and by blocking of cytotoxic NK cells and T lymphocytes with serum and ascites of EOC patients [312].

22.3.3.6 Macrophage Colony-Stimulating Factor

Macrophage colony-stimulating factor (CSF-1) was originally described as a stimulator of macrophage differentiation, and it was shown that its activity was mediated through a tyrosine kinase receptor encoded by c-*fms* proto-oncogene [313, 314]. Compared to benign and low-malignancy potential ovarian tumors, CSF-1/c-*fms* is abundantly expressed by the malignant EOC epithelium [315, 316]. Extremely high expression of both CSF-1 and c-*fms* was observed in EOC metastases and was a sign of poor prognosis [315]. Elevated serum and ascites fluid CSF-1 levels were described in EOC patients and were connected to shortened survival [317–319]. CSF-1 signaling was correlated with invasion of extracellular matrix by EOC cells, and it was discovered that CSF-1-stimulated invasiveness was mediated by interactions of urokinase plasmin-

ogen activator (uPA) with its receptor (uPAR) [320]. Overexpression of uPA was demonstrated in EOC and was found to be a bad prognostic factor [321–323]. The CSF-1-dependent uPA/uPAR pathway can enhance the EOC metastasis formation potential. It was shown by *in vivo* animal studies that intraperitoneal injection of CSF-1-overexpressing EOC cells produced a robust disease dissemination [313, 324]. CSF-1 also stimulated TAMs from EOC patients to secrete IL-8, IL-6, and TNF-α [325].

22.3.3.7 Chemokines

Chemokines are key mediators of inflammation and in EOC are responsible for two main functions: mediation of proangiogenic and proliferative signals and recruitment and activation of immune cells toward tumor and ascites.

Interleukin-8 (IL-8, CXCL8) is a chemokine secreted by macrophages, neutrophils, and endothelial and tumor cells. It possesses proangiogenic activity and acts through binding to CXCR1 and CXCR2 receptors present on both tumor and endothelial cells [86, 326, 327]. Increased IL-8 levels in ovarian cyst fluid, ascites, and serum from ovarian cancer patients were associated with decreased patient survival [42, 328–331]. Overexpression of IL-8 was confirmed on EOC cells and attributed to advanced and high-grade tumors; in addition, it was associated with increased tumor vascularity and tumor cell proliferation both *in vivo* and *in vitro* [73, 86, 332]. Secretion of IL-8 seems to be the indispensable feature of EOC cells, as genetic modification of ovarian epithelial cells by disruption of the p53, retinoblastoma (Rb), and RAS signaling pathways produced functional cancer cells that showed elevated expression of several inflammatory cytokines and IL-8 [333]. A potent stimulator of IL-8 expression on EOC in ovarian cancer is hypoxia [334]. The mechanism of hypoxia-induced IL-8 upregulation depends on the *Ras* oncogene overexpression and activation of PI3K/Akt and p38 MAPK pathways which enhance *IL-8* gene transcription [335]. Transcription factor NF-κB also participates in regulation of IL-8 expression, as NF-κB signaling blockade significantly inhibited *in vitro* and *in vivo* expression of VEGF and IL-8 in cultured cells and in cells implanted into the peritoneal cavity of nude mice [336]. In murine EOC model, *IL-8* gene silencing with liposome-encapsulated siRNA led to the reduction of mean tumor weight and decrease of MVD of the tumors, while combination of liposome-encapsulated siRNA and docetaxel resulted in reduction of tumor growth and proliferation [332]. IL-8 is a participant of an intraperitoneal regulation network which augments neoangiogenesis and peritoneal spreading of EOC. Studies on murine model of EOC discovered that activation of protease-activated receptor-1 (PAR-1) by MMP-1 stimulated tumor angiogenesis and metastases through paracrine regulation of IL-8 and GRO-1/α (CXCL1) which bound to CXCR1 and CXCR2 receptors and caused endothelial cell proliferation, vessel formation, and migration [337]. Moreover, IL-8 and CXCR1 receptor were co-expressed on peritoneal macrophages and T $CD3^+$ cells suggesting that IL-8 is engaged in recruiting certain immune cells into the peritoneum, where they contribute to tumor spread and formation of ascites [73]. Ascites of EOC patients also contained plasmacytoid dendritic cells (PDCs) attracted by CXCL12 and showing proangiogenic activity through upregulated production of IL-8 and TNF-α [338]. The role of CXCR receptors in ovarian tumorigenesis is supported by animal studies showing that knockdown of CXCR2 expression reduced tumor growth. CXCR2 acted as an inhibitor of EOC cell apoptosis by suppression of p53 and Bcl-xS and simultaneous activation of Bcl-xL and Bcl-2 proteins [339]. IL-8 was also shown to block TRAIL-induced cancer cell apoptosis [340]. Signaling through CXCR2 also increased expression of VEGF on tumor cells. CXCR2 was found to be highly expressed in EOC patients and served as a bad prognostic factor [339]. The antiapoptotic IL-8 action is probably engaged in chemoresistance of ovarian cancer. *In vitro* studies on isolated EOC cell lines indicated that chemoresistant populations were characterized by increased expression of IL-6 and IL-8 [341].

Stromal cell-derived factor-1 (SDF-1, CXCL12) belongs to chemokines and interacts with its receptor CXCR4. Human EOC expresses

CXCL12 following exposure to hypoxia [49]. Both CXCL12 and CXCR4 were also expressed in malignant ascites [342]. Functional network of CXCL12/CXCR4 is important for migration of cells in the tumor environment. The CXCL12/CXCR4 network is involved in the formation of intraperitoneal and lymph node metastases [343–345] and an enhancement of integrin β_1 and VEGF expression on EOC [346]. CXCL12 also mediates the trafficking of plasmacytoid DCs into the tumor [347]. The relevant role for CXCL12/CXCR4 pathway in EOC was further confirmed in immune-competent mice with peritoneal ovarian cancer. The studies found that siRNA-mediated knockdown of CXCL12 resulted in reduced EOC proliferation *in vitro* and *in vivo*. Moreover, the use of a selective CXCR4 antagonist increased tumor cell apoptosis and, more interestingly, caused reduction of Tregs in tumor environment, thus enhancing host T-cell-mediated antitumor response [348]. However, according to other studies, blocking of CXCR4 was not sufficient to completely prevent Tregs trafficking into a tumor. This could be accomplished by blocking macrophage-derived chemoattractant CCL22 chemokine, which was shown to be produced by cancer cells and TAMs [338]. Tumors characterized by upregulation of CXCL12/CXCR4 pathway and CXCL22 and consecutive high Treg infiltrations are characterized by negative patient outcomes [305, 349]. Similarly, the overexpression of CXCL12/CXCR4 together with CXCL16 (small inducible cytokine subfamily B member 16 – SCYB16) and its receptor CXCR6 was related to formation of ascites and was considered as a poor prognostic factor [350]. On the contrary, chemokines CXCL10 and CXCL11 (interferon-inducible T-cell α-chemoattractant, I-TAC) and their receptor CXCR3 (G protein-coupled receptor 9, GPR9) were shown to be upregulated on high-grade serous EOC possessing "immunoreactive" phenotype. They function as regulators of Th1 $CD4^+$ T cells and also $CD8^+$ T cells and NK cells trafficking into the inflammatory tumor environment and inhibit endothelial cell migration and proliferation affecting neoangiogenesis [351, 352]. Increased expression of CXCL9 and CXCL10 was positively correlated with the intensity of TILs infiltration and better survival [66, 305, 353, 354]. CXCL9 and CXCL10 could also be secreted by TAMs and similarly could positively affect patients' survival [305].

Both CCR9 receptor and its ligand, thymus-expressed chemokine (CCL25), are expressed in normal ovaries; however, their overexpression by serous, endometrioid, and to a lesser extent by mucinous ovarian cancer has been confirmed. CCR9 upregulation was also observed in EOC cell lines. Expression of a broad spectrum of MMPs, including MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-10, MMP-11, and MMP-13, was demonstrated to be modulated by the CCL25/CCR9-dependent pathway. Therefore, it is a reasonable assumption that CCL25/CCR9 pathway contributes to migration and invasion of ovarian cancer cells [355]. It was also shown that CCR6, receptor for macrophage inflammatory protein-3 (MIP-3α, CCL20), was overexpressed by liver metastases of EOC, suggesting that CCL20/CCR6 interactions promote creation of metastases to the liver [356].

The profile of chemokines differs between ovarian tumor and ascites, what may be attributable to immunological differences in both environments according to the role of two main chemokine stimulants – TNF-α and hypoxia. Solid ovarian tumors are highly hypoxic and oversecrete TNF-α, whereas ascites is mostly normoxic and characterized by lower TNF-α concentrations [357]. The study on CC chemokine proteins and their receptors in ovarian cancer ascites revealed the existence of a complex network regulating the cell–cell interactions and the composition of immune cells. Immune cells isolated from ascites were found to produce mRNA for CCL2, CCL3, CCL4, CCL5, CCL8, and CCL22 chemokines, as well as mRNA for the following chemokine receptors: CCR1, CCR2a, CCR2b, CCR3, CCR4, CCR5, and CCR8. Variable concentrations of chemokine molecules were also found in ascitic fluid. Macrophages CD14+ isolated from ascites consistently expressed CCR1 (receptor for macrophage inflammatory protein-1α – MIP-1α), RANTES (Regulated on Activation, Normal T-cell Expressed and Secreted, CCL5), monocyte chemoattractant protein-3 (MCP-3), myeloid progenitor inhibitory factor-1 (MPIF-1), CCR2 (receptor

for MCP-1), and CCR5 (receptor for RANTES, MIP-1α, and MIP-1β), whereas T CD4+ lymphocytes expressed CCR2 and CCR5. Correlation existed between CCL5 (RANTES) concentration in ascitic fluid and T-cell count [357].

22.4 Regulatory and Inflammatory Cells in Ovarian Cancer

Solid tumors including EOC contain many populations of infiltrating immune cells, and tumor TILs, TAMs, DCs, and MDSCs are all of the great relevance for both anticancer actions and tumor promotion. The presence and composition of TILs infiltrate could be a prognostic factor in EOC patients [358]. It was shown that the density of T CD3+ lymphocytes, especially T CD8+ cells, was correlated with favorable response to chemotherapy and better survival [66, 359]. A considerable part of T CD3+ cells belongs to TCRγδ population [359]. There are suggestions, based on the studies of T CD4+ cells and their TCR Vβ profiles, that TILs show evidence of clonal expansion, thus recognizing the common tumor-associated antigens [360]. However, there is no simple correlation between the expression of tumor-associated antigens and the intensity of TILs infiltrate inside the tumor [353]. It rather seems that the composition and activation status of TILs depends on the expression of chemokines and cytokines originating from both cancer and immune cells in tumor environment. It was shown that many regulatory cytokines present in the tumor and ascites, including IL-10, TGF-β, TNF-α, and VEGF, indicate immunosuppressive actions against TILs [361]. Contrary to that observation, tumors with significantly increased T-cell density were shown to overexpress chemokines CCL2, CCL5, CXCL9, and CCL22 and activatory cytokines IL-2 and IFN-γ, whereas concentrations of VEGF were low [361, 362]. The great importance of tumor environment on the TILS was further confirmed in other studies. During *in vitro* experiments, TILs were capable to secrete cytokines, showed the expression of activation marker (HLA-DR) and co-stimulatory molecules (CD28, CD80, CD86), and indicated cytotoxicity against cultured EOC cells [215, 363–366]. However, inside the tumor, TILs are functionally impaired as was indicated by downregulation of CD3ζ chain, which plays an important role in coupling antigen recognition to several signal-transduction pathways [367]. The similar conclusions can be drawn from the studies showing decreased proliferation and expression of activation antigens (CD25, CD69, HLA-DR) on TILs, as well as low secretion of stimulatory cytokines, like IL-2, IL-4, and IFN-γ [215, 367, 368]. The mechanisms of effector TILs inhibition also include tolerance-inducing plasmacytoid DCs, B7-H4+ macrophages, TAMs, and MDSCs [369–372].

On the contrary to the CD8+ T cells, high numbers of CD4+CD25+FoxP+ Tregs accumulating inside EOC tumors were considered to be a sign of poor prognosis [338]. Expression of indoleamine 2,3-dioxygenase (IDO) by both EOC cells and myeloid DCs can contribute to recruitment of Tregs and is associated with poor clinical outcomes in ovarian cancer [373–377]. Ovarian tumors with increased Tregs infiltrate were characterized by TGF-β upregulation and functional impairment of both T CD8+ and T CD4+25- cell activity which was shown by low secretion of IL-2, IFN-γ, and TNF-α [349, 378]. The spatial proximity of Tregs and T effector cells suggested inhibition of cell cytotoxicity by Tregs by a direct cell–cell contact [378]. Consistently with these observations, it was revealed that the T CD8+-to-Tregs ratio was the most appropriate predictor of patient survival, whereas the density of either T CD3+ or T CD4+ cells alone had a lower prognostic value [353]. One of the features subjected to discussion is a meaning of TILs localization in tumor epithelial *vs*. stromal part. The studies showed that while increased intraepithelial T CD8+ lymphocyte density was correlated with better prognosis, the intensity of stromal T CD8+ infiltrate did not indicate such correlation. This observation points out the fact that distinct regulatory mechanisms are probably engaged in control of TIL function in different intratumor localizations. The mechanism of possible importance for intraepithelial T CD8+ depletion and

deactivation is overexpression of PD-L1, a ligand for programmed death-1 (PD-1) inhibitory molecule, on EOC epithelial cells [379]. Analogical situation like inside the tumor has been noted in ascitic environment, where high concentrations of $CD3^+CD56^+$ NKT cells and TNF-α cytokine were correlated with better prognosis for the patients, whereas abundance of Tregs and raised VEGF were observed in patients with poor prognosis [361]. However, in recent studies of familial ovarian cancer, the high Treg density inside the tumor was found to correlate with better prognosis, despite the fact that it was observed mainly in the high-grade tumors [380]. It is consistent with clinical observation that patients with familial ovarian cancer and carriers of BRCA mutations have better outcome although their tumors are usually more aggressive. These observations suggest that different genetic determination of tumor growth may influence the immunoregulatory network in different ways, thus pointing out the necessity of identification of ovarian cancer types of certain genotypes and immune phenotypes in order to individualize therapy and enhance its efficacy.

In addition to Tregs, ovarian tumor environment contains another immune cell indicating potent regulatory properties, including TAMs, and DCs. Tumor-associated macrophages have been considered as promoters of both cancer growth and angiogenesis and correlated with poor prognosis in EOC patients [181, 381, 382]. High-grade tumors were characterized by more abundant $CD68^+$ and $CD163^+$ TAM populations, and a correlation between $CD68^+$ macrophages and Tregs was noted, suggesting the cooperation between both populations existing on the regulatory level [380]. Tregs can induce expression of $B7\text{-}H4^+$ regulatory molecule on TAMs, thus inducing TAM-mediated inhibition of T effectors in tumor [370, 371]. Accordingly, the negative correlation between $CD68^+$ macrophages and T $CD8^+$ cells was observed [383]. Some studies suggest that the number of $CD68^+$ macrophages may be different depending on the histological type (serous/mucinous) of the tumors [383]. The precursors for tumor TAMs are peripheral blood monocytes recruited preferentially to the stroma–tumor interface by monocyte chemotactic protein-1 (CCL2) [384, 385]. The recruited macrophages are further trapped by tumor-mediated downregulation of CCR2 receptors in the tumor environment, particularly in the hypoxic areas of the tumor, and shaped into tumorigenic TAM phenotype [386, 387]. Similarly, the TAMs are the most abundant mononuclear cell population in the ascites of EOC patients, where they contribute to suppression of T effector cells by secretion of IL-10 and TGF-β [244, 325]. The influence of EOC cells on macrophage functional phenotype was demonstrated by *in vitro* studies which revealed that coculture of macrophages with cancer cells upregulated mRNA for a broad spectrum of genes, including those responsible for expression of CCL2, CCL4, CCL22, CXCR4, CXCL12, TNF-α, TGF-β, MMP-7, CSF-1, and VEGF. The secretory function of macrophages cocultured with EOC cells changed compared to control cultures and indicated increase of IL-10, TNF-α, IL-1β, IL-6, and IL-8 with concurrent IL-12 decrease [388]. Moreover, compared to normal macrophages, TAMs isolated from EOC patients had significantly lower antibody-dependent cell-mediated cytotoxicity and phagocytic activity [325]. The paracrine regulatory signals are not only directed from EOC cells toward TAMs but also in the opposite direction, as *in vitro* studies demonstrated that TAM-dependent TNF-α secretion upregulated NF-κB and JNK pathways and EMMPRIN and MIF expression in cocultured EOC cells, thus increasing their invasive capacity [389]. Animal studies provided an observation that TAM-mediated inflammation facilitates EOC metastases by a VEGF-dependent mechanism [390]. TAMs are also capable to regulate the function of both ovarian cancer cells and host immune cells by expression of COX-2 and iNOS suppressor molecules [391].

Another cell population relevant for tumor spread are myeloid DCs, met abundantly in advanced human and experimental tumors and represented in the ascites, however, in lower density compared to TAMs. Patients with ovarian cancer show progressive depletion of circulating mDCs indicating that mDCs in the tumor environment originate from periph-

eral blood [222]. Myeloid DCs isolated from ovarian tumors exhibit expression of indoleamine 2,3-dioxygenase (IDO) and programmed cell death-1 ligand 1 (PD-L1, B7-H1) suppressor molecules. IDO is responsible for recruitment of Tregs and direct inhibition of T effectors [349]. Accumulation of PD-1$^+$B7-H1$^+$ DCs in the tumor was associated with suppression of TCD4$^+$ helper, T CD3$^+$CD8$^+$ cytotoxic/regulatory cell activity, and decreased infiltration of T cells [392]. Murine studies revealed that blockade of PD-L1 enhanced T-cell activation, increased secretion of IL-2 and IFN-γ, decreased secretion of IL-10, and augmented antitumor immunity [392, 393]. Murine studies found the presence of functionally immature CD11c$^+$ DCs expressing low levels of co-stimulatory CD86 and CD40 molecules in tumor and tumor-draining lymph nodes. It was shown that acceleration of tumor growth occurred parallelly to rapid increase of DC infiltration and was accompanied by loss of DC immunostimulatory activities and PD-L1 upregulation. Tumor-derived PGE_2 and TGF-β were found to be indispensable promoters of DC immunosuppressive actions. Consistent with these observations, depletion of DCs in tumor-bearing mice significantly retarded tumor progression [394]. Due to the gross abundance of mDCs in the tumor, suppression mechanism based on PD-1/B7-H1 interactions may be one of the most important promoters of EOC growth. As a confirmation of that assumption, it was found that 5-year survival was significantly shorter for patients whose tumors expressed high levels of B7-H1 [379]. Murine studies demonstrated that a population of immature mDCs which acquired a proangiogenic CD11c$^+$DEC205$^+$VE-cadherin$^+$ phenotype upon VEGF stimulation migrated to perivascular areas of the tumor and maintained its vasculogenesis. Depletion of this mDCs population abrogated tumor growth in that experimental model (Huarte et al. 2008; Coukos et al. 2005). This means that mDCs play a multidirectional role in ovarian cancer development, and their targeting by immunotherapy might be an effective option in EOC treatment.

Plasmacytoid DCs accumulate in tumor environment, preferentially in ascites [338, 347, 395]. Chemoattraction of pDCs into the tumor environment depends on CXCL12 expression and produces depletion of pDCs in peripheral blood of EOC patients, especially in advanced disease. Chemotherapeutic treatment partially restores pDCs in peripheral blood of patients with complete remission [222]. Similar to mDCs, ascitic pDCs have immature phenotype. Plasmacytoid DCs promote the generation of immunoregulatory IL-10$^+$ T CD8$^+$ suppressors, which independent from T CD4$^+$CD25$^+$FoxP3$^+$ Tregs downregulate IFN-γ secretion mediated by T effectors and prevent them from proliferation [347, 396]. They also secrete TNF-α and IL-8, thus being capable of promoting angiogenesis [338]. Population of pDCs homing into the tumor (tumor-associated pDCs) was found to have different phenotypes compared to ascitic pDCs. This population of cells was identified as CD11$^-$ CD4$^+$CD123$^+$ cells and expressed semi-mature phenotype with higher level of CD86 and CD40 expression, thus being capable of partial activation in tumor localization. Tumor-associated pDCs produced lower amounts of IFN-γ, TNF-α, IL-6, CCL3, and CCL5 upon TLR stimulation compared to ascitic pDCs and induced IL-10 secretion from naïve T CD4$^+$ cells. Function of tumor-associated pDCs was modulated by tumor-derived TNF-α and TGF-β [222]. The correlation was found between the concentration of tumor-associated pDCs and shorter progression-free survival, as well as early relapse [222].

Myeloid-derived suppressor cells (MDSC) characterized by CD11b$^+$/Gr-1$^+$ phenotype are a multifunctional population of cells which is engaged in both chronic inflammation and tumor progression [29, 121]. MDSCs are capable of suppressing DC maturation and host antitumor responses mediated by CD8$^+$ T cytotoxic cells, NK, and NKT cells [397–400]. Animal studies showed that targeted elimination of MDSCs resulted in significant decrease of IL-10 in ascites and tumor regression [401, 402]. The role of IL-10 for MDSC function is crucial, as MDSCs are both main producers and key autocrine responders for IL-10. Blockade of IL-10 function with antibodies disrupted the MDSC-mediated immunosuppression and resulted in

partial restoration of host antitumor activity and thus improved survival [401]. It was shown that attraction of MDSCs into the ascites environment in EOC patients depended on PGE_2, which shaped the CXCL12/CXCR4 interactions. PGE_2 was responsible for the expression of CXCR4 receptor on MDSCs and for production of CXCL12 ligand on ascites cancer cells. MDSC migrated toward ascites in a CXCR4-dependent manner and required COX-2 activity and PGE_2 autocrine stimulation for obtaining functionality [403]. This mechanism could be relevant for creating immunosuppressive environment augmenting intraperitoneal cancer propagation.

22.5 Cytokines in Diagnosis and Prognosis of Ovarian Cancer

22.5.1 Diagnosis

Ovarian cancer is often called a "silent killer" since approximately 75 % of patients are diagnosed in the advanced stage of the disease due to the absence of or nonspecific clinical symptoms at the beginning and the lack of screening methods for diagnosis. Up till now, the gold standard in the diagnosis of pelvic masses is still a bimanual gynecological examination supplemented by transvaginal sonography (with color Doppler) and serum markers (CA125, eventually combined with HE4 and calculated ROMA). In the last few decades, many serologic biomarkers based on tumor–host immunologic interactions (various cytokines and antibodies) have been evaluated in the diagnosis of ovarian cancer, but up till now, none of them have been applied to the general practice.

Gorelik et al. [187] used multianalyte LabMAP profiling technology for early detection of ovarian cancer. A panel of 24 serologic markers (CA 125, cytokines, chemokines, growth and angiogenic factors) was analyzed in the blood of women with early ovarian cancer and benign pelvic masses and healthy individuals. They constructed a classification tree consisting of CA 125, G-CSF, IL-6, EGF, and VEGF for comparison of benign tumors and cancer which resulted in a sensitivity of 84.1 % and specificity of 75.7 % (80.2 % of patients were correctly classified). For comparisons of early stages of ovarian cancer *vs.* healthy controls, the classification tree was composed of CA 125, EGF, VEGF, IL-6, and IL-8. It resulted in 91 % sensitivity and 96 % specificity (93 % of subjects correctly classified) and the area under the ROC curve was 0.966 [187]. The combination of serum IL-8, anti-IL-8 antibodies, and CA 125 had a sensitivity of 87.5 % and specificity of 98 % for ovarian cancer detection (compared to healthy controls); this three-marker composite panel resulted in 98 % specificity but only 42 % sensitivity in distinguishing between malignant and benign ovarian tumors [404, 405]. Lambeck et al. [405] reported the use of cytokine bead array for the simultaneous analysis of 14 serum cytokines in sera of women with ovarian cancer, benign ovarian tumors, and healthy controls. They found that serum CA 125, IL-6, IL-7, and IL-10 were elevated in ovarian cancer patients and had the highest diagnostic value in discriminating between malignant and benign lesions or healthy controls (AUC were 0.7–0.88), and the highest predictive value was achieved by combining IL-7 and CA 125. Bertenshaw et al. [406] analyzed serum concentrations of 204 analytes representing 104 antigens and 44 autoimmune and 56 infectious disease molecules measured using a set of proprietary multiplexed immunoassays in patients with ovarian cancer. The control group was not homogenic and consisted of women with benign ovarian conditions (71 %) and other gynecologic cancers (10 %) and healthy individuals (19 %). The highest discriminatory power for ovarian cancer was noted for CA 125 (AUC = 0.966), C-reactive protein (0.756), epidermal growth factor receptor (0.733), IL-10 (0.725), and IL-8 (AUC = 0.715). The use of serum IFN-γ, TNF-α, IL-2, IL-10, IL-6, IL-8, and TGF- β1 concentrations was also determined to discriminate between malignant and benign ovarian conditions in cases of tumors clinically and sonographically assessed as "suspected" [188]. The highest accuracy was for IL-10, IL-6, and IL-8 (AUC for IL-6 was 0.87, 0.84 for IL-10, and 0.8 for IL-8).

22.5.2 Prognosis

The role of TIL, TAL, and their specific subsets in prognosis of patients with ovarian cancers was discussed in the previous part of our article.

Many cytokines have also been investigated as prognostic factors in these patients – measured in serum and/or ascites or cyst fluid before and during treatment and follow-up. Understanding the biology of ovarian cancer is a rationale for special attention which was focused on pro-inflammatory and proangiogenic factors.

IL-6 is elevated in serum of patients with ovarian cancer, and it was shown that elevated serum IL-6 levels were correlated with poor prognosis and decrease of overall survival [407]. Increased IL-8 levels in serum, ovarian cyst fluid, and ascites from ovarian cancer patients were associated with poor prognosis and decreased patient survival [42, 328–331]. Serum IL-8 is also important for the follow-up of patients treated with paclitaxel – decreasing IL-8 levels pointed on decreasing tumor burden and the effectiveness of chemotherapy [330, 331]. Canadian studies reported that high IL-6 but not IL-8 levels in ascites were correlated with shorter progression-free survival (PFS); IL-6 was an independent marker of poor prognosis of ovarian cancer patients [179]. Moreover, they found that also high levels of IL-10, leptin, and osteoprotegerin in ascites were connected with shorter PFS [255]. The study analyzing serum and ascites levels of IL-1 α, β and IL-1 RA in ovarian cancer patients showed that IL-1 RA levels below 695.6 pg/ml in ascites were associated with significant improvement in PFS [408].

In a univariate analysis, the use of cytokine bead array for the simultaneous measurement of 14 serum cytokines in the sera of women with ovarian cancer revealed that serum levels of CA-125, IL-6, IL-7, IL-8, IL-10, MCP-1, and IP-10 above the median were associated with a shorter progression-free and lower overall survival [405]. Moreover, a combination of CA-125, IL-7, and IP-10 with serum levels higher than the median gave the best association with overall survival, with a hazard ratio of 5.77. Multivariate analysis of the results revealed that IL-7 and IP-10 were independent predictors of overall survival, and CA-125 and IP-10 were independent predictors of progression-free survival [405].

Several studies were conducted to assess the prognostic value of VEGF levels in serum and ascites of ovarian cancer patients. Serum preoperative VEGF levels >380 pg/ml were an independent predictor of poor prognosis and were connected with eightfold increased risk of cancer-related death [46]. Multivariate analysis of data from five studies (314 EOC patients) showed that high preoperative serum levels of VEGF were correlated with poor overall survival [409]. Ascites VEGF levels >1,900 pg/ml were associated with decreased survival of patients with ovarian cancer [361, 410].

Other clinical studies showed that such cytokines as interleukin-18 (IL-18) and Stromal cell-derived factor-1 (SDF-1) were correlated with poor prognosis in ovarian cancer patients [411, 412]. By contrast, the increased levels of intereukin-12 in both serum and ascites were correlated with better outcome and response to treatment of patients with advanced ovarian cancer [413]. TNF-α has also been studied as a prognostic factor, but reports on whether it is connected with poor or good prognosis are inconsistent [410, 414].

Studies on tissue samples of ovarian cancer by means of RT-PCR revealed that patients with high levels of IFN-γ expression showed significantly longer progression-free and overall survival [415].

22.6 Immunotherapy of Ovarian Cancer

Actually, the standard treatment of ovarian cancer consists of primary surgery regarding optimal cytoreduction (leaving no macroscopic residual disease) followed by chemotherapy (paclitaxel or docetaxel with carboplatin or cisplatin). However, the results of treatment, especially advanced ovarian cancers, are still unsatisfactory. Thus, novel therapeutic strategies are urgently needed. One of them is immunotherapy.

The potential use of immunotherapy for ovarian cancer is based on immunogenicity of ovarian

cancer cells (to evoke or enhance antitumor response) and the dynamic interactions between host immunologic system and cancer (to move the balance toward elimination of cancer cells).

Ovarian cancer cells differ from normal human cells. On their surface tumor antigens are expressed, which can be the targets for humoral or cellular response. Initially, tumor antigens were divided to tumor-specific antigens (TSA) present only on cancer cells and tumor-associated antigens (TAA) found also on noncancer cells. However, during subsequent investigations, antigens primarily thought as TSA have been found also on normal cells. Actually, the classification of tumor antigens is based on their molecular structure and origin. Thus, there are differentiation antigens, mutational antigens, cancer testis antigens, oncofetal antigens, and viral antigens [416]. At present, more than 1,000 human tumor antigens have been described (Cancer Immunome Database). The most important tumor antigens in ovarian cancer (targets for immunotherapy) are MUC-16, MFRs, HER-2/neu, MUC-1, OA3, TAG-72, NY-ESO-1, and sialyl-Tn [417–425]. Moreover, ovarian cancer cells express peptide/MHC complexes which can be recognized by $CD8^+$ T cells.

The main strategies for immunotherapy of ovarian cancer implement mAbs, cytokines, peptide vaccines, adoptive cell transfers, inverting immunological dysfunctions, and some other approaches.

22.6.1 Monoclonal Antibodies

22.6.1.1 Bevacizumab

Bevacizumab (Avastin) is a monoclonal recombinant, humanized anti-VEGF antibody used in the treatment of some types of solid cancers like colorectal cancer, non-small cell lung cancer, and renal cell cancer. Recently, bevacizumab has been also registered in the EU for the treatment of advanced epithelial ovarian , fallopian tube, or primary peritoneal cancer. Bevacizumab reaches its antiangiogenic effects by blocking the binding of VEGF to its receptors (VEGFR 1 and 2) and thus neutralizes VEGF biologic activity [426]. The role of VEGF in ovarian cancer has been described previously in this chapter. Blocking VEGF, bevacizumab inhibits and regresses neovascularization and so suppresses tumor growth and metastasizing, also in cisplatin refractory tumors [427, 428]. Moreover, it probably sensitizes tumors to chemotherapy by normalization of the network of tumor vessels leading to decrease of intratumoral pressure, increase of oxygenation, and drug perfusion [429].

The use of bevacizumab in the treatment of ovarian cancer was investigated since 2007 in phase II trials assessing Avastin in monotherapy [430, 431] or in combination with standard cytostatic chemotherapy [432–434]. The investigators showed promising results leading to conducting phase III trials.

The efficacy of bevacizumab in combination with standard chemotherapy (paclitaxel with carboplatin) for the first-line treatment of ovarian cancer was studied in two randomized phase III trials: ICON7 [435] and GOG-218 [436].

In ICON7 (International Collaboration on Ovarian Neoplasms 7) trial, 1,528 women with newly diagnosed epithelial ovarian, fallopian tube, or primary peritoneal cancer were randomized to two arms: chemotherapy alone (paclitaxel + carboplatin for 6 cycles every 3 weeks) or chemotherapy + bevacizumab (7.5 mg/kg) in 6 cycles (every 3 weeks) and 12 cycles of maintenance with bevacizumab (every 3 weeks) [435]. The latest updated results showed the significantly prolonged progression-free survival (PFS) in the bevacizumab group (24.1 *vs*. 22.4 months, $p<0.005$), but the overall survival (OS) remained the same in both arms [435]. Final analysis is expected – up till now, the results of quality of life (QoL) were published [437]. The authors stated that bevacizumab continuation treatment seemed to be associated with a small but clinically significant decrement in QoL compared with standard treatment for women with ovarian cancer, so the trade-off between the prolongation of progression-free survival and the quality of that period of time needs to be considered in clinical practice when making treatment decisions [437].

The next clinical phase III trial was GOG-218 [436]. An overall of 1,873 women with stage III or IV epithelial ovarian cancer or fallopian tube

or primary peritoneal cancer were randomized to three arms: chemotherapy alone (paclitaxel + carboplatin), 6 cycles; chemotherapy + bevacizumab (15 mg/kg), 6 cycles; and chemotherapy + bevacizumab (6 cycles) + additionally 16 cycles of maintenance with bevacizumab (other arms were given placebo). The updated results showed the improvement in PFS in patients receiving additional maintenance therapy with bevacizumab compared to chemotherapy alone (median prolongation was 3.8 months). Median PFS did not differ significantly between the group receiving bevacizumab plus chemotherapy followed by placebo maintenance and the group receiving standard chemotherapy. OS remained the same in all three groups of patients [436].

Other phase III clinical trials of bevacizumab especially for recurrent disease are still ongoing.

OCEANS is a randomized, multicenter, blinded, placebo-controlled phase III trial testing the efficacy and safety of bevacizumab (BV) with gemcitabine and carboplatin (GC) compared with GC in platinum-sensitive 484 women with recurrent ovarian, primary peritoneal, or fallopian tube cancer (ROC) [438]. Patients with platinum-sensitive ROC and measurable disease were randomly assigned to GC plus either BV or placebo (PL) for six to ten cycles. BV or PL was then continued until disease progression. Results showed that GC plus BV followed by BV until progression resulted in a statistically significant improvement in PFS when compared with GC plus PL in platinum-sensitive ovarian cancer patients (12.4 *vs*. 8.4 months, $p<0.0001$) [438]. The data about overall survival has not been published yet.

In patients with platinum-resistant recurrent ovarian, primary peritoneal, or fallopian tube cancer, there is also an ongoing phase III clinical trial – AURELIA (http://clinicaltrials.gov/show/NCT00976911). Patients are still recruited (target: approximately 500 patients) and randomized to two arms: chemotherapy (paclitaxel, topotecan, or liposomal doxorubicin) against chemotherapy plus bevacizumab (10 mg/kg every 2 weeks or 15 mg/kg every 3 weeks).

Bevacizumab is quite well tolerated and the side effects are related to its unique mechanism of action. In monotherapy studies, the most common adverse effects were hypertension and proteinuria (1.6–16 %) and additionally, hemorrhage, thromboembolism, complicated wound healing, and gastrointestinal tract perforations [430, 431]. Meta-analysis of 16 randomized controlled trials including 10,217 patients with solid tumors treated with bevacizumab revealed 2.9 % of fatal adverse events [439]. The most common fatal adverse events were bleeding (23.5 %), neutropenia (12.2 %), and gastrointestinal tract perforations (7.1 %). Bevacizumab increased the risk of fatal adverse events in patients treated with taxanes or platinum – its addition to the standard chemotherapy increased the relative risk to 1.33 [439].

22.6.1.2 Catumaxomab

Catumaxomab (Removab) is a trifunctional, monoclonal bispecific (anti-EpCAM x anti-CD3) antibody which has been approved in the EU for the intraperitoneal treatment of malignant ascites in patients with EpCAM-positive carcinomas where standard therapy is not available or no longer feasible (Removab 2013). Malignant ascites is usually a manifestation of advanced epithelial cancer and is connected with poor prognosis and is mainly caused by ovarian cancer (37 % of patients with malignant ascites), pancreatobiliary cancers (21 %), and gastric cancer (18 %) followed by esophageal cancer (4 %), colorectal cancer (4 %), and breast cancer (3 %) [440].

The trifunctional mode of action of catumaxomab includes: (1) first, antigen-binding site (the mouse IgG2a) binds to the tumor-associated antigen EpCAM; (2) second, antigen-binding site (rat IgG2b) binds to CD3, part of the T-cell receptor complex; and (3) third, Fc fragment binds to FcγR type I and III positive cells like macrophages, dendritic cells, and natural killer cells [441–444]. EpCAM is a good target for immunotherapy with antibodies since it is expressed only in epithelial cells and cancers of epithelial origin [445], has direct positive effect on cell cycle and proliferation, and upregulates proto-oncogene c-myc and cyclin A/E [446]. Catumaxomab, by blocking of EpCAM, decreases the metabolism and proliferation of cancer cells and by stimulating T cells and accessory immune cells leads to elimination of cancer cells by induction of apoptosis, release of

cytokines and perforins, and antibody-dependent cytotoxicity [441–444]. In fact, catumaxomab has shown antitumor activity *in vitro*, by elimination of EpCAM-positive tumor cells from ascites fluid samples which were associated with a significant increase in the secretion of IFN-γ, TNF-α, IL-2, and IL-6, indicating immune cell activation and self-supporting T-cell proliferation [441, 442]. Clinical efficacy, side effects, and immunological impact of catumaxomab were studied in a pivotal phase II/III trial in patients with symptomatic recurrent malignant ascites resistant to standard chemotherapy requiring therapeutic paracenteses [447–450]. In this trial, 258 patients were enrolled and randomized to two arms: intraperitoneal catumaxomab and paracentesis (170 patients) and paracentesis alone (88 patients). From the whole enrolled group, 129 patients suffered from ovarian cancer, and 129 patients from nonovarian cancer, mostly gastric cancer ($n=66$). Results of immunomonitoring of the trial revealed that catumaxomab eliminated EpCAM-positive tumor cells and putative cancer stem cells, activated peritoneal T cells, and reduced VEGF levels [449]. Clinical outcome showed a significant prolongation of puncture-free period in the overall catumaxomab and paracentesis group and both subgroups of cancers *vs*. paracentesis alone (52 *vs*. 11 days in ovarian and 37 *vs*. 14 days in nonovarian subgroup, $p<0.0001$). The median overall survival was not prolonged significantly in the overall catumaxomab group (72 *vs*. 68 days, $p=0.08$) except the subgroup restricted to gastric cancer patients (71 *vs*. 44 days, $p<0.05$) [448]. However, in a post hoc analysis, after removing 13 patients from the catumaxomab + paracentesis group who did not receive the catumaxomab dose, improvement in overall survival rates (1 year: 12 % *vs*. 3.4 %, $p<0.05$) in the catumaxomab group [451] was observed. Moreover, the addition of catumaxomab resulted in the reduction of signs of ascites and its symptoms like anorexia, nausea, dyspnea, abdominal pain, and swelling [448]. Side effects of catumaxomab were as expected and mostly consisted of cytokine-release-like symptoms (pyrexia (60 %), nausea, vomiting, chills, tachycardia, hypotension), hematological side effects (anemia, leukocytosis), and others such as abdominal pain, fatigue, diarrhea, and ileus (6.4 %) [447, 448].

22.6.1.3 Oregovomab and Abagovomab

Oregovomab (OvaRex) is a mAb-binding main TAA of ovarian cancer – CA125 (MUC-16) forming immunogenic complexes for T and B lymphocytes. Immunological monitoring of patients treated with oregovomab revealed that proliferation and activation of T cells and the induction of anti-CA-125 antibodies correlate with longer survival [452, 453]. The treatment was well tolerated with mild and transient side effects such as nausea [452]. In fact, results of a phase II randomized trial conducted in patients with recurrent ovarian cancer showed that 58 % of patients generated the response to oregovomab (anti-CA-125 antibodies and specific T cells) but in only 23 % of cases stabilization of the disease was observed [454]. Berek et al. in 2004 [117] enrolled 145 women with advanced ovarian cancer to a randomized placebo-controlled study to estimate the safety and efficacy of oregovomab in a maintenance therapy. The results after a 5-year follow-up revealed that patients from the oregovomab and placebo groups had similar progression-free survival (57 *vs*. 48.6 months, $p=0.28$) [455]. Final analysis from this study was reported in 2009 [456]. A total of 371 patients with advanced ovarian cancer after standard chemotherapy were recruited at more than 60 centers; 251 patients received oregovomab in a maintenance monoimmunotherapy and 120 patients were given placebo. There were no difference in the clinical outcomes between both groups. The authors stated that although oregovomab had demonstrated bioactivity, the strategy of monoimmunotherapy was not effective as maintenance therapy after frontline treatment of a favorable subset of patients with advanced ovarian cancer [456].

Thus, some investigators tried to use anti-idiotype antibodies to increase immunogenicity.

Abagovomab (formerly ACA-125) is a mouse anti-idiotype monoclonal antibody whose variable epitope mirrors CA125. In the

initial phase I/II studies and in a preliminary report of the randomized phase III MIMOSA trial, abagovomab induced a specific anti-idiotypic antibody (Ab3) response in 68.1–100 % of treated patients and also cellular immunity in some of them; moreover, it was well tolerated [457, 458]. However, the final results of phase III MIMOSA trial conducted in 888 patients with advanced ovarian cancer in complete clinical remission after primary surgery and platinum- and taxane-based chemotherapy showed that abagovomab administered as repeated monthly injections is safe and induces a measurable immune response, but administration as maintenance therapy for patients with ovarian cancer in first remission does not prolong PFS or OS [459].

22.6.1.4 Trastuzumab and Pertuzumab

The epithelial growth factor receptor (EGFR) is a family of tyrosine kinase receptors: ErbB1/HER1 (commonly referred to as EGFR), ErbB2/HER2 (commonly referred to as HER2), ErbB3/HER3, and ErbB4/HER4. Their activation mediates a variety of cellular responses, including cancer cell proliferation, survival, and invasion. They are overexpressed in many solid tumors and promising targets for cancer immunotherapy [460].

Trastuzumab (Herceptin) is humanized mAb specific to the extracellular domain of HER2. It is registered for the treatment of breast cancer. In a phase II clinical trial, only 11 % of patients with recurrent ovarian cancer overexpressed HER-2, and the response for monotherapy with trastuzumab was very low (7 %) with a median progression-free interval of 2 months [461].

Pertuzumab (Omnitarg) is another anti-HER-2 mAb with a different binding site than trastuzumab and not requiring HER-2 overexpression to exert cytotoxic effects [462, 463]. In a phase II clinical trial of pertuzumab performed on patients with advanced ovarian cancer refractory to chemotherapy, the response rate and stabilization of disease were only about 5 %, and the patients reported some serious side effects [452]. Another phase II randomized trial evaluating the use of gemcitabine with or without pertuzumab in the group of platinum-refractory patients showed moderate advantage of combined protocol over gemcitabine monotherapy [464].

22.6.1.5 Farletuzumab

Farletuzumab is a humanized immunoglobulin G mAb targeting human folate receptor α (FRα). Folate receptor α is a tumor-associated antigen that is overexpressed in many cancers including ovarian cancer but largely absent on normal tissue [465]. Farletuzumab has shown safety and efficacy (prolongation of PFS) in phase I and II trials in the treatment of ovarian cancer patients [466, 467]. Armstrong et al. in 2013 published the results of the study on clinical activity of farletuzumab, alone and combined with chemotherapy, in women with first-relapse, platinum-sensitive ovarian, fallopian tube and primary peritoneal cancers. It was well-tolerated as a single agent and also when administered with standard chemotherapy. Farletuzumab with carboplatin and taxane enhanced the response rate and duration of response in platinum-sensitive ovarian cancer patients with first relapse [468].

22.6.2 Cytokines

In vitro studies showed that interferons exert cytotoxic activity against ovarian cancer cells [469, 470]. Several phase I, II, and III trials were conducted regarding intraperitoneal or subcutaneous administration of IFN-α and IFN-γ in monotherapy or with platinum during first-line therapy or afterwards and showed no benefits of IFN with high incidence of side effects [471–478].

Interleukin-2 (IL-2) activates T cells and was tested as an anticancer agent. High doses of IL-2 were connected with severe toxicity (hypotension, kidney necrosis) and only low doses were tested in clinical trials [479, 480]. Pilot studies and phase II trials in patients with platinum refractory ovarian cancer showed no satisfactory effects or stabilization of the disease or partial response in about 40 % patients, but the number of cases was very limited and firm conclusions could not be drawn [479–482].

The other cytokines like TNF, IL-1, and IL-12 were also tested, but besides anticancer activity, they exert pro-inflammatory and proangiogenic effects and may stimulate neoangiogenesis and metastases.

22.6.3 Dendritic Cells

Dendritic cells (DCs) are bone marrow-derived leukocytes playing a crucial role in the initiation of T-cell-mediated immunity – they present antigens to T cells [483, 484]. The idea for immunotherapy was to isolate DCs from the cancer patient, sensitize them with tumor cells or tumor antigens, and infuse them back to the patient. DCs were obtained from peripheral blood monocytes or directly from mDCs or from tumor or ascites TAMs. In fact, DCs fused with patient-derived ovarian cancer cells induced cytotoxic T lymphocyte against autologous cancer cells [485, 486].

Clinical trials performed on patients with advanced ovarian cancer, using HER-2/MUC-1 pulsed DCs, showed induction of immunological response but no satisfactory clinical benefits [487–489]. However, there are some promising data from case studies. The case study on a vaccination regimen created with autologous dendritic cells engineered with mRNA-encoded α-FR indicated 50 % regression of para-aortic lymph node metastases and decrease of CA-125 serum levels 16 months after DC vaccination [490]. Bernall et al. in 2012 published the results of a treatment with hematopoietic stem cells collected of a patient with advanced ovarian cancer (with progression besides multiple courses of chemotherapy) induced into dendritic cell differentiation and fused with liposomal constructs of autologous ovarian cancer antigens. The liposomal preparations of DCs were injected monthly. Following DC treatment, the metastatic lesions progressively decreased in size to the point of being undetectable by serial CT scans. After 7 years, the patient remains to be free of disease.

The other strategies of immunotherapy like adoptive T-cell transfers, inverting immunological dysfunctions, Treg depletion, activating toll-like receptors, oncolytic viruses, and many others are tested but are still far for clinical use in patients suffering from ovarian cancer.

Up till now from the whole spectrum of immunotherapeutic approaches, only mAbs bevacizumab and catumaxomab are registered for the treatment of patients with ovarian cancer (catumaxomab is restricted to certain cases of malignant ascites).

22.7 Concluding Remarks

The growing knowledge concerning the immunopathologic background of ovarian cancer has expanded the spectrum of new diagnostic and therapeutic possibilities. However, the progress in clinical management is still unsatisfactory, despite some improvements in patients' survival brought by the use of bevacizumab and catumaxomab. We believe that the marriage of basic investigations on the field of both immunology and genetics, including epigenetics, will open the new horizons and bring effective treatment options for ovarian cancer patients.

References

1. Khong HT, Restifo NP. Natural selection of tumor variants in the generation of "tumor escape" phenotypes. Nat Immunol. 2002;3(11):999–1005.
2. Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. Immunity. 2004;21(2):137–48.
3. Whiteside TL. Immune responses to malignancies. J Allergy Clin Immunol. 2010;125(2):S272–83.
4. Wilczyński JR, Duechler M. How do tumors actively escape from host immunosurveillance? Arch Immunol Ther Exp (Warsz). 2010;58(6):435–48.
5. McMahon G. VEGF receptor signaling in tumor angiogenesis. Oncologist. 2000;5 Suppl 1:3–10.
6. Cao Y. Tumor angiogenesis and therapy. Biomed Pharmacother. 2005;59:S340–3.
7. Ferrara N. Pathways mediating VEGF-independent tumor angiogenesis. Cytokine Growth Factor Rev. 2010;21(1):21–6.
8. Yoshiji H, Kuriyama S, Yoshii J, Ikenaka Y, Noguchi R, Hicklin DJ, et al. Synergistic effect of basic fibroblast growth factor and vascular endothelial growth factor in murine hepatocellular carcinoma. Hepatology. 2002;35(4):834–42.
9. O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, et al. Endostatin: an endogenous inhibitor

of angiogenesis and tumor growth. Cell. 1997; 88(2):277–85.
10. Nishida N, Yano H, Nishida T, Kamura T, Kojiro M. Angiogenesis in cancer. Vasc Health Risk Manag. 2006;2(3):213.
11. Meyer M, Clauss M, Lepple-Wienhues A, Waltenberger J, Augustin HG, Ziche M, et al. A novel vascular endothelial growth factor encoded by Orf virus, VEGF-E, mediates angiogenesis via signalling through VEGFR-2 (KDR) but not VEGFR-1 (Flt-1) receptor tyrosine kinases. EMBO J. 1999;18(2):363–74.
12. Sullivan LA, Brekken RA. The VEGF family in cancer and antibody-based strategies for their inhibition. MAbs. 2010;2(2):165–75.
13. Majewski S, Marczak M, Szmurlo A, Jablonska S, Bollag W. Interleukin-12 inhibits angiogenesis induced by human tumor cell lines in vivo. J Invest Dermatol. 1996;106(5):1114–8.
14. Greenhough A, Smartt HJ, Moore AE, Roberts HR, Williams AC, Paraskeva C, et al. The COX-2/PGE2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. Carcinogenesis. 2009;30(3):377–86.
15. Cao D, Hou M, Guan Y-s, Jiang M, Yang Y, Gou H-f. Expression of HIF-1alpha and VEGF in colorectal cancer: association with clinical outcomes and prognostic implications. BMC Cancer. 2009; 9(1):432.
16. Fang J, Ding M, Yang L, Liu L-Z, Jiang B-H. PI3K/PTEN/AKT signaling regulates prostate tumor angiogenesis. Cell Signal. 2007;19(12):2487–97.
17. Moeller BJ, Cao Y, Vujaskovic Z, Li CY, Haroon ZA, Dewhirst MW. The relationship between hypoxia and angiogenesis. Semin Radiat Oncol. 2004;14(3):215–21.
18. Chlenski A, Liu S, Cohn SL. The regulation of angiogenesis in neuroblastoma. Cancer Lett. 2003; 197(1):47–52.
19. Bamberger E, Perrett C. Angiogenesis in epithelian ovarian cancer. Mol Pathol. 2002;55(6):348.
20. Gabrilovich DI, Chen HL, Girgis KR, Cunningham HT, Meny GM, Nadaf S, et al. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. Nat Med. 1996;2(10):1096–103.
21. Oyama T, Ran S, Ishida T, Nadaf S, Kerr L, Carbone DP, et al. Vascular endothelial growth factor affects dendritic cell maturation through the inhibition of nuclear factor-kappa B activation in hemopoietic progenitor cells. J Immunol. 1998;160(3):1224–32.
22. Ostrand-Rosenberg S. Immune surveillance: a balance between protumor and antitumor immunity. Curr Opin Genet Dev. 2008;18(1):11–8.
23. Sica A, Allavena P, Mantovani A. Cancer related inflammation: the macrophage connection. Cancer Lett. 2008;267(2):204–15.
24. Biswas SK, Gangi L, Paul S, Schioppa T, Saccani A, Sironi M, et al. A distinct and unique transcriptional program expressed by tumor-associated macrophages (defective NF-κB and enhanced IRF-3/STAT1 activation). Blood. 2006;107(5):2112–22.
25. Raghunand N, Gatenby R, Gillies R. Microenvironmental and cellular consequences of altered blood flow in tumours. Br J Radiol. 2003;76(1):S11–22.
26. Grimshaw MJ, Naylor S, Balkwill FR. Endothelin-2 is a hypoxia-induced autocrine survival factor for breast tumor cells 1 supported, in part, by Oxford BioMedica United Kingdom Ltd.(to MJG). 1. Mol Cancer Ther. 2002;1(14):1273–81.
27. Cramer T, Yamanishi Y, Clausen BE, Förster I, Pawlinski R, Mackman N, et al. HIF-1a is essential for myeloid cell-mediated inflammation. Cell. 2003;112:645–57.
28. Duechler M, Wilczynski JR. Hypoxia inducible factor-1 in cancer immune suppression. Curr Immunol Rev. 2010;6(3):260–71.
29. Bennaceur K, Chapman JA, Touraine J-l, Portoukalian J. RETRACTED: immunosuppressive networks in the tumour environment and their effect in dendritic cells. Biochi Biophys Acta (BBA)-Rev Cancer. 2009;1795(1):16–24.
30. Kalluri R, Zeisberg M. Fibroblasts in cancer. Nat Rev Cancer. 2006;6(5):392–401.
31. Östman A, Heldin CH. PDGF receptors as targets in tumor treatment. Adv Cancer Res. 2007;97:247–74.
32. Kammertoens T, Schüler T, Blankenstein T. Immunotherapy: target the stroma to hit the tumor. Trends Mol Med. 2005;11(5):225–31.
33. Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell. 2005;121(3):335–48.
34. Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, et al. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature. 2007;449(7162):557–63.
35. Blankenstein T. The role of tumor stroma in the interaction between tumor and immune system. Curr Opin Immunol. 2005;17(2):180–6.
36. Ramakrishnan S, Subramanian I, Yokoyama Y, Geller M. Angiogenesis in normal and neoplastic ovaries. Angiogenesis. 2005;8(2):169–82.
37. Chechlinska M, Kaminska J, Markowska J, Kramar A, Steffen J. Peritoneal fluid cytokines and the differential diagnosis of benign and malignant ovarian tumors and residual/recurrent disease examination. Int J Biol Markers. 2006;22(3):172–80.
38. Schumacher JJ, Dings RP, Cosin J, Subramanian IV, Auersperg N, Ramakrishnan S. Modulation of angiogenic phenotype alters tumorigenicity in rat ovarian epithelial cells. Cancer Res. 2007;67(8): 3683–90.
39. Abu-Jawdeh GM, Faix JD, Niloff J, Tognazzi K, Manseau E, Dvorak HF, et al. Strong expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in ovarian borderline and malignant neoplasms. Lab Invest. 1996; 74(6):1105–15.

40. Wong Te Fong L, Gammell S, Bamberger E. Quantification of VEGF-A and PD-ECGF/TP in normal, benign and malignant ovarian lesions. Rev Oncol. 2002;4 suppl 1:122.
41. Wong Te Fong LF, Kini M, Morris R. Angiogenesis in primary epithelial ovarian carcinomas. Anticancer Res. 2001;21:1662–8.
42. Fasciani A, D'Ambrogio G, Bocci G, Luisi S, Artini P, Genazzani A. Vascular endothelial growth factor and interleukin-8 in ovarian cystic pathology. Fertil Steril. 2001;75(6):1218–21.
43. Roszkowski P, Wronkowski Z, Szamborski J, Romejko M. Evaluation of selected prognostic factors in ovarian cancer. Eur J Gynaecol Oncol. 1992;14:140–5.
44. Zang R, Harter P, Chi D, Sehouli J, Jiang R, Trope C, et al. Predictors of survival in patients with recurrent ovarian cancer undergoing secondary cytoreductive surgery based on the pooled analysis of an international collaborative cohort. Br J Cancer. 2011; 105(7):890–6.
45. Ghosh S, Maity P. Isolation and purification of vascular endothelial growth factor (VEGF) from ascitic fluid of ovarian cancer patients. Pathol Oncol Res. 2004;10(2):104–8.
46. Cooper BC, Ritchie JM, Broghammer CL, Coffin J, Sorosky JI, Buller RE, et al. Preoperative serum vascular endothelial growth factor levels significance in ovarian cancer. Clin Cancer Res. 2002;8(10): 3193–7.
47. Tempfer C, Obermair A, Hefler L, Haeusler G, Gitsch G, Kainz C. Vascular endothelial growth factor serum concentrations in ovarian cancer. Obstet Gynecol. 1998;92(3):360–3.
48. Hefler LA, Mustea A, Könsgen D, Concin N, Tanner B, Strick R, et al. Vascular endothelial growth factor gene polymorphisms are associated with prognosis in ovarian cancer. Clin Cancer Res. 2007;13(3): 898–901.
49. Kryczek I, Lange A, Mottram P, Alvarez X, Cheng P, Hogan M, et al. CXCL12 and vascular endothelial growth factor synergistically induce neoangiogenesis in human ovarian cancers. Cancer Res. 2005;65(2):465–72.
50. Cao R, Björndahl MA, Religa P, Clasper S, Garvin S, Galter D, et al. PDGF-BB induces intratumoral lymphangiogenesis and promotes lymphatic metastasis. Cancer Cell. 2004;6(4):333–45.
51. Liu LZ, Hu XW, Xia C, He J, Zhou Q, Shi X, et al. Reactive reactive oxygen species regulate epidermal growth factor induced vascular endothelial growth factor and hypoxia-inducible factor-1a expression through activation of AKT and P70S6K1 in human ovarian cancer cells. Free Radic Biol Med. 2006;41:1521–33.
52. Matei D, Kelich S, Cao L, Menning N, Emerson RE, Rao J, et al. PDGF BB induces VEGF secretion in ovarian cancer. Cancer Biol Ther. 2007;6(12):1951–9.
53. Liao S, Liu J, Lin P, Shi T, Jain RK, Xu L. TGFbeta blockade controls ascites by preventing abnormalization of lymphatic vessels in orthotopic human ovarian carcinoma models. Cancer Res. 2011;17:1415–24.
54. Kulbe H, Thompson R, Wilson JL, Robinson S, Hagemann T, Fatah R, et al. The inflammatory cytokine tumor necrosis factor-α generates an autocrine tumor-promoting network in epithelial ovarian cancer cells. Cancer Res. 2007;67(2):585–92.
55. Coward J, Kulbe H, Chakravarty P, Leader D, Vassileva V, Leinster DA, et al. Interleukin-6 as a therapeutic target in human ovarian cancer. Clin Cancer Res. 2011;17(18):6083–96.
56. Yokoyama Y, Xin B, Shigeto T, Mizunuma H. Combination of ciglitazone, a peroxisome proliferator-activated receptor gamma ligand, and cisplatin enhances the inhibition of growth of human ovarian cancers. J Cancer Res Clin Oncol. 2011;137(8):1219–28.
57. Belotti D, Paganoni P, Manenti L, Garofalo A, Marchini S, Taraboletti G, et al. Matrix Metalloproteinases (MMP9 and MMP2) induce the release of Vascular Endothelial Growth Factor (VEGF) by ovarian carcinoma cells implications for ascites formation. Cancer Res. 2003;63(17):5224–9.
58. Gupta RA, Tejada LV, Tong BJ, Das SK, Morrow JD, Dey SK, et al. Cyclooxygenase-1 is overexpressed and promotes angiogenic growth factor production in ovarian cancer. Cancer Res. 2003;63(5):906–11.
59. Yang G, Cai KQ, Thompson-Lanza JA, Bast RC, Liu J. Inhibition of breast and ovarian tumor growth through multiple signaling pathways by using retrovirus-mediated small interfering RNA against Her-2/neu gene expression. J Biol Chem. 2004;279(6):4339–45.
60. Mesiano S, Ferrara N, Jaffe RB. Role of vascular endothelial growth factor in ovarian cancer: inhibition of ascites formation by immunoneutralization. Am J Pathol. 1998;153(4):1249–56.
61. Olson TA, Mohanraj D, Carson LF, Ramakrishnan S. Vascular permeability factor gene expression in normal and neoplastic human ovaries. Cancer Res. 1994;54(1):276–80.
62. Boocock CA, Charnock-Jones DS, Sharkey AM, McLaren J, Barker PJ, Wright KA, et al. Expression of vascular endothelial growth factor and its receptors fit and KDR in ovarian carcinoma. J Natl Cancer Inst. 1995;87(7):506–16.
63. Paley PJ, Staskus KA, Gebhard K, Mohanraj D, Twiggs LB, Carson LF, et al. Vascular endothelial growth factor expression in early stage ovarian carcinoma. Cancer. 1997;80(1):98–106.
64. Sowter H, Corps A, Evans A, Clark D, Charnock-Jones D, Smith S. Expression and localization of the vascular endothelial growth factor family in ovarian epithelial tumors. Lab Invest. 1997;77(6):607–14.
65. Fujimoto J, Sakaguchi H, Hirose R, Ichigo S, Tamaya T. Biologic implications of the expression of vascular endothelial growth factor subtypes in ovarian carcinoma. Cancer. 1998;83(12):2528–33.

66. Zhang L, Yang N, Park J-W, Katsaros D, Fracchioli S, Cao G, et al. Tumor-derived vascular endothelial growth factor up-regulates angiopoietin-2 in host endothelium and destabilizes host vasculature, supporting angiogenesis in ovarian cancer. Cancer Res. 2003;63(12):3403–12.
67. Mattern J, Stammler G, Koomagi R, Wallwiener D, Kaufmann M, Volm M. Association of vascular endothelial growth factor expression with tumor cell proliferation in ovarian carcinoma. Anticancer Res. 1996;17(1B):621–4.
68. Chen H, Ye D, Xie X, Chen B, Lu W. VEGF, VEGFRs expressions and activated STATs in ovarian epithelial carcinoma. Gynecol Oncol. 2004;94(3): 630–5.
69. Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, et al. VEGFR1-positive haematopoietic bone marrow progenitors initiate the premetastatic niche. Nature. 2005;438(7069):820–7.
70. Naora H, Montell DJ. Ovarian cancer metastasis: integrating insights from disparate model organisms. Nat Rev Cancer. 2005;5(5):355–66.
71. Ziogas AC, Gavalas NG, Tsiatas M, Tsitsilonis O, Politi E, Terpos E, et al. VEGF directly suppresses activation of T cells from ovarian cancer patients and healthy individuals via VEGF receptor type 2. Int J Cancer. 2012;130(4):857–64.
72. Weis S, Cui J, Barnes L, Cheresh D. Endothelial barrier disruption by VEGF-mediated Src activity potentiates tumor cell extravasation and metastasis. J Cell Biol. 2004;167(2):223–9.
73. Wang J-M, Ko C-Y, Chen L-C, Wang W-L, Chang W-C. Functional role of NF-IL6β and its sumoylation and acetylation modifications in promoter activation of cyclooxygenase 2 gene. Nucl Acids Res. 2006;34(1):217–31.
74. Belotti D, Calcagno C, Garofalo A, Caronia D, Riccardi E, Giavazzi R, et al. Vascular endothelial growth factor stimulates organ-specific host matrix metalloproteinase-9 expression and ovarian cancer invasion. Mol Cancer Res. 2008;6(4):525–34.
75. Zhang L, Yang N, Conejo Garcia J-R, Mohamed A, Benencia F, Rubin SC, et al. Generation of a syngeneic mouse model to study the effects of vascular endothelial growth factor in ovarian carcinoma. Am J Pathol. 2002;161(6):2295–309.
76. Sawano A, Iwai S, Sakurai Y, Ito M, Shitara K, Nakahata T, et al. Flt-1, vascular endothelial growth factor receptor 1, is a novel cell surface marker for the lineage of monocyte-macrophages in humans. Blood. 2001;97(3):785–91.
77. Mu J, Abe Y, Tsutsui T, Yamamoto N, Tai XG, Niwa O, et al. Inhibition of growth and metastasis of ovarian carcinoma by administering a drug capable of interfering with vascular endothelial growth factor activity. Cancer Sci. 1996;87(9):963–71.
78. Byrne AT, Ross L, Holash J, Nakanishi M, Hu L, Hofmann JI, et al. Vascular endothelial growth factor-trap decreases tumor burden, inhibits ascites, and causes dramatic vascular remodeling in an ovarian cancer model. Clin Cancer Res. 2003; 9(15):5721–8.
79. Pourgholami MH, Cai ZY, Lu Y, Wang L, Morris DL. Albendazole: a potent inhibitor of vascular endothelial growth factor and malignant ascites formation in OVCAR-3 tumor-bearing nude mice. Clin Cancer Res. 2006;12(6):1928–35.
80. Senger DR, Perruzzi CA, Feder J, Dvorak HF. A highly conserved vascular permeability factor secreted by a variety of human and rodent tumor cell lines. Cancer Res. 1986;46(11):5629–32.
81. Yeo K-T, Wang HH, Nagy JA, Sioussat TM, Ledbetter SR, Hoogewerf AJ, et al. Vascular permeability factor (vascular endothelial growth factor) in guinea pig and human tumor and inflammatory effusions. Cancer Res. 1993;53(12):2912–8.
82. Luo JC, Yamaguchi S, Shinkai A, Shitara K, Shibuya M. Significant expression of vascular endothelial growth factor/vascular permeability factor in mouse ascites tumors. Cancer Res. 1998;58(12):2652–60.
83. Hampl M, Tanaka T, Albert PS, Lee J, Ferrari N, Fine HA. Therapeutic effects of viral vector-mediated antiangiogenic gene transfer in malignant ascites. Hum Gene Ther. 2001;12(14):1713–29.
84. Stadlmann SAA, Pollheimer J, Gastl G, Offner FA, Margreiter R, et al. Ovarian carcinoma cells and IL1beta-activated human peritoneal mesothelial cells are possible sources of vascular endothelial growth factor in inflammatory and malignant peritoneal effusions. Gynecol Oncol. 2005;97:784–9.
85. Siddiqui GK, Wong TFL, Rolfe KJ. The expression of VEGF-A in metastatic epithelian ovarian cancer is of prognostic significance. Br J Obstet Gynaecol. 2001;108:549.
86. Xu L, Fidler IJ. Interleukin 8: an autocrine growth factor for human ovarian cancer. Oncol Res Feature Preclin Clin Cancer Therapeut. 2001;12(2):97–106.
87. Hasumi Y, Mizukami H, Urabe M, Kohno T, Takeuchi K, Kume A, et al. Soluble FLT-1 expression suppresses carcinomatous ascites in nude mice bearing ovarian cancer. Cancer Res. 2002;62(7):2019–23.
88. Liao S, Liu J, Lin P, Shi T, Jain RK, Xu L. TGF-β blockade controls ascites by preventing abnormalization of lymphatic vessels in orthotopic human ovarian carcinoma models. Clin Cancer Res. 2011;17(6):1415–24.
89. Huang K-J, Sui L-H. The relevance and role of vascular endothelial growth factor C, matrix metalloproteinase-2 and E-cadherin in epithelial ovarian cancer. Med Oncol. 2012;29(1):318–23.
90. Nakanishi Y, Kodama J, Yoshinouchi M, Tokumo K, Kamimura S, Okuda H, et al. The expression of vascular endothelial growth factor and transforming growth factor-[beta] associates with angiogenesis in epithelial ovarian cancer. Int J Gynecol Pathol. 1997;16(3):256–62.
91. Orre M, Rogers PA. VEGF, VEGFR-1, VEGFR-2, microvessel density and endothelial cell proliferation in tumours of the ovary. Int J Cancer. 1999;84(2): 101–8.

92. Shen G, Ghazizadeh M, Kawanami O, Shimizu H, Jin E, Araki T, et al. Prognostic significance of vascular endothelial growth factor expression in human ovarian carcinoma. Br J Cancer. 2000;83(2):196.
93. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420(6917):860–7.
94. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001;357(9255):539–45.
95. Lin W-W, Karin M. A cytokine-mediated link between innate immunity, inflammation, and cancer. J Clin Invest. 2007;117(5):1175–83.
96. Hussain SP, Hofseth LJ, Harris CC. Radical causes of cancer. Nat Rev Cancer. 2003;3(4):276–85.
97. Lu H, Ouyang W, Huang C. Inflammation, a key event in cancer development. Mol Cancer Res. 2006;4(4):221–33.
98. Jaiswal M, LaRusso NF, Burgart LJ, Gores GJ. Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. Cancer Res. 2000;60(1):184–90.
99. Ben-Baruch A. Inflammation-associated immune suppression in cancer: the roles played by cytokines, chemokines and additional mediators. Semin Cancer Biol. 2006;16:38–52. Elsevier.
100. Smyth MJ, Cretney E, Kershaw MH, Hayakawa Y. Cytokines in cancer immunity and immunotherapy. Immunol Rev. 2004;202(1):275–93.
101. Luo JL, Maeda S, Hsu LC, Yagita H, Karin M. Inhibition of NF-κB in cancer cells converts inflammation-induced tumor growth mediated by TNF-α to TRAIL-mediated tumor regression. Cancer Cell. 2004;6:297–305.
102. Medzhitov R. Toll-like receptors and innate immunity. Nat Rev Immunol. 2001;1(2):135–45.
103. Jego G, Bataille R, Geffroy-Luseau A, Descamps G, Pellat-Deceunynck C. Pathogen-associated molecular patterns are growth and survival factors for human myeloma cells through Toll-like receptors. Leukemia. 2006;20(6):1130–7.
104. Sun JWF, Hsu FC, Bälter K, Zheng SL, Johansson JE, et al. Interactions of sequence variants in interleukin-1 receptor-associated kinase 4 and the toll-cell receptor 6-1-10 gene cluster increase prostate cancer risk. Cancer Epidemiol Biomarkers Prev. 2006;15:480–5.
105. Harmey JH, Bucana CD, Lu W, Byrne AM, McDonnell S, Lynch C, et al. Lipopolysaccharide-induced metastatic growth is associated with increased angiogenesis, vascular permeability and tumor cell invasion. Int J Cancer. 2002;101:415–22.
106. Elgert KD, Alleva DG, Mullins DW. Tumor-induced immune dysfunction: the macrophage connection. J Leukoc Biol. 1998;64(3):275–90.
107. Mocellin S, Rossi CR, Pilati P, Nitti D. Tumor necrosis factor, cancer and anticancer therapy. Cytokine Growth Factor Rev. 2005;16(1):35–53.
108. Arnott CH, Scott KA, Moore RJ, Robinson SC, Thompson RG, Balkwill FR. Expression of both TNF-α receptor subtypes is essential for optimal skin tumour development. Oncogene. 2003;23(10):1902–10.
109. Balkwill F. Tumor necrosis factor or tumor promoting factor? Cytokine Growth Factor Rev. 2002;13(2):135–41.
110. Takeda K, Smyth MJ, Cretney E, Hayakawa Y, Kayagaki N, Yagita H, et al. Critical role for tumor necrosis factor–related apoptosis-inducing ligand in immune surveillance against tumor development. J Exp Med. 2002;195(2):161–9.
111. Xu J, Zhou J-Y, Wei W-Z, Wu GS. Activation of the Akt survival pathway contributes to TRAIL resistance in cancer cells. PLoS One. 2010;5(4):e10226.
112. Wang S, El-Deiry WS. TRAIL and apoptosis induction by TNF-family death receptors. Oncogene. 2003;22(53):8628–33.
113. Haura EB, Turkson J, Jove R. Mechanisms of disease: insights into the emerging role of signal transducers and activators of transcription in cancer. Nat Clin Pract Oncol. 2005;2(6):315–24.
114. Berger FG. The interleukin-6 gene: a susceptibility factor that may contribute to racial and ethnic disparities in breast cancer mortality. Breast Cancer Res Treat. 2004;88(3):281–5.
115. Schneider MR, Hoeflich A, Fischer JR, Wolf E, Sordat B, Lahm H. Interleukin-6 stimulates clonogenic growth of primary and metastatic human colon carcinoma cells. Cancer Lett. 2000;151(1):31–8.
116. Chung YC, Chang YF. Serum interleukin-6 levels reflect the disease status of colorectal cancer. J Surg Oncol. 2003;83(4):222–6.
117. Becker C, Fantini MC, Schramm C, Lehr HA, Wirtz S, Nikolaev A, et al. TGF-β suppresses tumor progression in colon cancer by inhibition of IL-6 *trans-signaling*. Immunity. 2004;21(4):491–501.
118. Fujiwaki R, Iida K, Kanasaki H, Ozaki T, Hata K, Miyazaki K. Cyclooxygenase-2 expression in endometrial cancer: correlation with microvessel count and expression of vascular endothelial growth factor and thymidine phosphorylase. Hum Pathol. 2002;33(2):213–9.
119. Gallo O, Masini E, Bianchi B, Bruschini L, Paglierani M, Franchi A. Prognostic significance of cyclooxygenase-2 pathway and angiogenesis in head and neck squamous cell carcinoma. Hum Pathol. 2002;33(7):708–14.
120. Zhang H, Sun X-F. Overexpression of cyclooxygenase-2 correlates with advanced stages of colorectal cancer. Am J Gastroenterol. 2002;97(4):1037–41.
121. Bennaceur K, Chapman JA, Touraine JL, Portoukalian J. Immunosuppressive networks in the tumour environment and their effect in dendritic cells. Biochim Biophys Acta. 2009;1795:16–24.
122. Parham C, Chirika M, Timans J, Vaisberg E, Travis M, Cheung J, et al. A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rb1 and a novel cytokine receptor subunit, IL-23R. J Immunol. 2002;168:5699–708.
123. Langowski JL, Kastelein RA, Oft M. Swords into plowshares: IL-23 repurposes tumor immune surveillance. Trends Immunol. 2007;28:207–12.

124. Huang S, Ullrich SE, Bar-Eli M. Regulation of tumor growth and metastasis by interleukin-10: the melanoma experience. J Interferon Cytokine Res. 1999;19(7):697–703.
125. Kundu N, Fulton AM. Interleukin-10 inhibits tumor metastasis, downregulates MHC class I, and enhances NK lysis. Cell Immunol. 1997;180(1):55–61.
126. Erdman SE, Rao VP, Olipitz W, Taylor CL, Jackson EA, Levkovich T, et al. Unifying roles for regulatory T cells and inflammation in cancer. Int J Cancer. 2010;126(7):1651–65.
127. Sredni B, Weil M, Khomenok G, Lebenthal I, Teitz S, Mardor Y, et al. Ammonium trichloro (dioxoethylene-o, o') tellurate (AS101) sensitizes tumors to chemotherapy by inhibiting the tumor interleukin 10 autocrine loop. Cancer Res. 2004;64(5):1843–52.
128. Urosevic M, Dummer R. Human leukocyte antigen–G and cancer immunoediting. Cancer Res. 2008;68(3):627–30.
129. Link AA, Kino T, Worth JA, McGuire JL, Crane ML, Chrousos GP, et al. Ligand-activation of the adenosine A2a receptors inhibits IL-12 production by human monocytes. J Immunol. 2000;164(1):436–42.
130. Zhang JG, Hepburn L, Cruz G, Borman RA, Clark KL. The role of adenosine A_{2A} and A_{2B} receptors in the regulation of TNF-α production by human monocytes. Biochem Pharmacol. 2005;69(6):883–9.
131. Spaner DE. Amplifying cancer vaccine responses by modifying pathogenic gene programs in tumor cells. J Leukoc Biol. 2004;76(2):338–51.
132. Moutsopoulos NM, Wen J, Wahl SM. TGF-β and tumors—an ill-fated alliance. Curr Opin Immunol. 2008;20(2):234–40.
133. Seo N, Hayakawa S, Tokura Y. Mechanisms of immune privilege for tumor cells by regulatory cytokines produced by innate and acquired immune cells. Semin Cancer Biol. 2002;12(4):291–300.
134. Yu P, Rowley DA, Fu Y-X, Schreiber H. The role of stroma in immune recognition and destruction of well-established solid tumors. Curr Opin Immunol. 2006;18(2):226–31.
135. Smyth MJ, Crowe NY, Godfrey DI. NK cells and NKT cells collaborate in host protection from methylcholanthrene-induced fibrosarcoma. Int Immunol. 2001;13(4):459–63.
136. Mainou-Fowler T, Taylor P, Miller S, Dickinson AM, Proctor SJ. Intracellular cytokine profiles by peripheral blood CD3+ T-cells in patients with classical Hodgkin lymphoma. Leuk Lymphoma. 2003;44(8):1325–31.
137. Agarwal A, Rani M, Saha G, Valarmathi T, Bahadur S, Mohanti B, et al. Disregulated expression of the Th2 cytokine gene in patients with intraoral squamous cell carcinoma. Immunol Investig. 2003;32(1–2):17–30.
138. Dummer W, Bastian BC, Ernst N, Schänzle C, Schwaaf A, Bröcker EB. Interleukin-10 production in malignant melanoma: Preferential detection of IL-10-secreting tumor cells in metastatic lesions. Int J Cancer. 1996;66(5):607–10.
139. Becker C, Fantini MC, Neurath MF. TGF-beta as a T cell regulator in colitis and colon cancer. Cytokine Growth Factor Rev. 2006;17(1):97–106.
140. Jarnicki AG, Lysaght J, Todryk S, Mills KH. Suppression of antitumor immunity by IL-10 and TGF-beta-producing T cells infiltrating the growing tumor: influence of tumor environment on the induction of CD4+ and CD8+ regulatory T cells. J Immunol. 2006;177:896–904.
141. Ness RB, Cottreau C. Possible role of ovarian epithelial inflammation in ovarian cancer. J Natl Cancer Inst. 1999;91(17):1459–67.
142. Risch HA, Marrett LD, Howe GR. Parity, contraception, infertility, and the risk of epithelial ovarian cancer. Am J Epidemiol. 1994;140(7):585–97.
143. Booth M, Beral V, Smith P. Risk factors for ovarian cancer: a case-control study. Br J Cancer. 1989; 60(4):592.
144. Whittmore AS, Harris R, Itnyre J. Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies II. Invasive epithelial ovarian cancers in white women. Am J Epidemiol. 1992;136(10):1184–203.
145. Graham J, Graham R. Ovarian cancer and asbestos. Environ Res. 1967;1(2):115–28.
146. Cook LS, Kamb ML, Weiss NS. Perineal powder exposure and the risk of ovarian cancer. Am J Epidemiol. 1997;145(5):459–65.
147. Risch HA, Howe GR. Pelvic inflammatory disease and the risk of epithelial ovarian cancer. Cancer Epidemiol Biomark Prev. 1995;4(5):447–51.
148. Green A, Purdie D, Bain C, Siskind V, Russell P, Quinn M, et al. Tubal sterilisation, hysterectomy and decreased risk of ovarian cancer. Survey of Women's Health Study Group. Int J Cancer. 1997;71:948–51.
149. Altinoz M, Korkmaz R. NF-kappaB, macrophage migration inhibitory factor and cyclooxygenase-inhibitions as likely mechanisms behind the acetaminophen-and NSAID-prevention of the ovarian cancer. Neoplasma. 2003;51(4):239–47.
150. Murdoch WJ, McDonnel AC. Roles of the ovarian surface epithelium in ovulation and carcinogenesis. Reproduction. 2002;123(6):743–50.
151. Liu Z, Shimada M, Richards JS. The involvement of the Toll-like receptor family in ovulation. J Assist Reprod Genet. 2008;25(6):223–8.
152. Jabbour HN, Sales KJ, Catalano RD, Norman JE. Focus on vascular function in female reproduction. Inflammatory pathways in female reproductive health and disease. Reproduction. 2009;138:903–19.
153. Fleming JS, Beaugié CR, Haviv I, Chenevix-Trench G, Tan OL. Incessant ovulation, inflammation and epithelial ovarian carcinogenesis: revisiting old hypotheses. Mol Cell Endocrinol. 2006;247(1): 4–21.
154. Cramer DW, Welch WR. Determinants of ovarian cancer risk. II. Inferences regarding pathogenesis. J Natl Cancer Inst. 1983;71(4):717–21.
155. Friedlander M, Dembo A. Prognostic factors in ovarian cancer. Semin Oncol. 1991;18(3):205–12.

156. Brinton LA, Gridley G, Persson I, Baron J, Bergqvist A. Cancer risk after a hospital discharge diagnosis of endometriosis. Am J Obstet Gynecol. 1997; 176(3):572–9.
157. Kyama CM, Debrock S, Mwenda JM, D'Hooghe TM. Potential involvement of the immune system in the development of endometriosis. Reprod Biol Endocrinol. 2003;1(1):123.
158. Styer AK, Sullivan BT, Puder M, Arsenault D, Petrozza JC, Serikawa T, et al. Ablation of leptin signaling disrupts the establishment, development, and maintenance of endometriosis-like lesions in a murine model. Endocrinology. 2008;149(2):506–14.
159. Machado DE, Berardo PT, Palmero CY, Nasciutti LE. Higher expression of vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 (Flk-1) and metalloproteinase-9 (MMP-9) in a rat model of peritoneal endometriosis is similar to cancer diseases. Development. 2010;7:8.
160. Tariverdian N, Theoharides TC, Siedentopf F, Gutiérrez G, Jeschke U, Rabinovich GA, et al. Neuroendocrine–immune disequilibrium and endometriosis: an interdisciplinary approach. Semin Immunopathol. 2007;29(2):193–210.
161. Kurman RJ, Shih I-M. Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer—shifting the paradigm. Hum Pathol. 2011;42(7): 918–31.
162. Demopoulos RI, Aronov R, Mesia A. Clues to the pathogenesis of fallopian tube carcinoma: a morphological and immunohistochemical case control study. Int J Gynecol Pathol. 2001;20(2):128–32.
163. Cooper MD, Rapp J, Jeffery-Wiseman C, Barnes RC, Stephens DS. Chlamydia trachomatis infection of human fallopian tube organ cultures. J Gen Microbiol. 1990;136(6):1109–15.
164. Maisey K, Nardocci G, Imarai M, Cardenas H, Rios M, Croxatto HB, et al. Expression of proinflammatory cytokines and receptors by human fallopian tubes in organ culture following challenge with Neisseria gonorrhoeae. Infect Immun. 2003;71(1):527–32.
165. Strandell A, Thorburn J, Wallin A. The presence of cytokines and growth factors in hydrosalpingeal fluid. J Assist Reprod Genet. 2004;21(7):241–7.
166. Macciò A, Madeddu C. Inflammation and ovarian cancer. Cytokine. 2012;58(2):133–47.
167. Kim J, Coffey DM, Creighton CJ, Yu Z, Hawkins SM, Matzuk MM. High-grade serous ovarian cancer arises from fallopian tube in a mouse model. Proc Natl Acad Sci. 2012;109(10):3921–6.
168. Landen CN, Birrer MJ, Sood AK. Early events in the pathogenesis of epithelial ovarian cancer. J Clin Oncol. 2008;26(6):995–1005.
169. Ali-Fehmi R, Semaan A, Sethi S, Arabi H, Bandyopadhyay S, Hussein YR, et al. Molecular typing of epithelial ovarian carcinomas using inflammatory markers. Cancer. 2011;117(2):301–9.
170. Mor G, Yin G, Chefetz I, Yang Y, Alvero A. Ovarian cancer stem cells and inflammation. Cancer Biol Ther. 2011;11(8):708.
171. Kim KH, Xie Y, Tytler EM, Woessner R, Mor G, Alvero AB. KSP inhibitor ARRY-520 as a substitute for Paclitaxel in Type I ovarian cancer cells. J Transl Med. 2009;7(1):63.
172. Kelly MG, Alvero AB, Chen R, Silasi D-A, Abrahams VM, Chan S, et al. TLR-4 signaling promotes tumor growth and paclitaxel chemoresistance in ovarian cancer. Cancer Res. 2006;66(7):3859–68.
173. Zhou M, McFarland-Mancini MM, Funk HM, Husseinzadeh N, Mounajjed T, Drew AF. Toll-like receptor expression in normal ovary and ovarian tumors. Cancer Immunol Immunother. 2009;58(9):1375–85.
174. Muccioli M, Sprague L, Nandigam H, Pate M, Benencia F. Toll-like receptors as novel therapeutic targets for ovarian cancer. ISRN Oncol. 2012;2012:642141.
175. Berger R, Fiegl H, Goebel G, Obexer P, Ausserlechner M, Doppler W, et al. Toll-like receptor 9 expression in breast and ovarian cancer is associated with poorly differentiated tumors. Cancer Sci. 2010;101(4):1059–66.
176. Shan W, Liu J. A hidden path to breaking the spell of ovarian cancer. Cell Cycle. 2009;8(19):3107–11.
177. Coppé J-P, Patil CK, Rodier F, Sun Y, Muñoz DP, Goldstein J, et al. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol. 2008;6(12):e301.
178. Yang G, Rosen DG, Zhang Z, Bast RC, Mills GB, Colacino JA, et al. The chemokine growth-regulated oncogene 1 (Gro-1) links RAS signaling to the senescence of stromal fibroblasts and ovarian tumorigenesis. Proc Natl Acad Sci. 2006;103(44):16472–7.
179. Lane D, Matte I, Rancourt C, Piché A. Prognostic significance of IL-6 and IL-8 ascites levels in ovarian cancer patients. BMC Cancer. 2011;11(1):210.
180. Kulbe H, Chakravarty P, Leinster DA, Charles KA, Kwong J, Thompson RG, et al. A dynamic inflammatory cytokine network in the human ovarian cancer microenvironment. Cancer Res. 2012;72(1):66–75.
181. Nash M, Ferrandina G, Gordinier M, Loercher A, Freedman R. The role of cytokines in both the normal and malignant ovary. Endocrinol Relat Cancer. 1999;6(1):93–107.
182. Asschert JG, Vellenga E, Hollema H, van der Zee AG, de Vries ED. Expression of macrophage colony-stimulating factor (M-CSF), interleukin-6 (IL-6), interleukin-1 beta (IL-1 beta), interleukin-11 (IL-11) and tumor necrosis factor-alpha (TNFalpha) in p53-characterised human ovarian carcinomas. Eur J Cancer. 1997;33:2246–51.
183. Hefler LA, Grimm C, Ackermann S, Malur S, Radjabi-Rahat AR, Leodolter S, et al. An interleukin-6 gene promoter polymorphism influences the biological phenotype of ovarian cancer. Cancer Res. 2003;63(12):3066–8.
184. Garg R, Wollan M, Galic V, Garcia R, Goff BA, Gray HJ, et al. Common polymorphism in interleukin 6 influences survival of women with ovarian and peritoneal carcinoma. Gynecol Oncol. 2006;103(3): 793–6.

185. Clendenen TV, Lundin E, Zeleniuch-Jacquotte A, Koenig KL, Berrino F, Lukanova A, et al. Circulating inflammation markers and risk of epithelial ovarian cancer. Cancer Epidemiol Biomark Prev. 2011; 20:799–810. cebp. 1180.2010.
186. Nowak M, Glowacka E, Szpakowski M, Szyllo K, Malinowski A, Kulig A, et al. Proinflammatory and immunosuppressive serum, ascites and cyst fluid cytokines in patients with early and advanced ovarian cancer and benign ovarian tumors. Neuro Endocrinol Lett. 2010;31(3):375–83.
187. Gorelik E, Landsittel DP, Marrangoni AM, Modugno F, Velikokhatnaya L, Winans MT, et al. Multiplexed immunobead-based cytokine profiling for early detection of ovarian cancer. Cancer Epidemiol Biomark Prev. 2005;14(4):981–7.
188. Nowak M, Klink M, Glowacka E, Sulowska Z, Kulig A, Szpakowski M, et al. Production of cytokines during interaction of peripheral blood mononuclear cells with autologous ovarian cancer cells or benign ovarian tumour cells. Scand J Immunol. 2010;71(2):91–8.
189. Wang X, Wang E, Kavanagh JJ, Freedman RS. Ovarian cancer, the coagulation pathway, and inflammation. J Transl Med. 2005;3(1):25.
190. Lo C-W, Chen M-W, Hsiao M, Wang S, Chen C-A, Hsiao S-M, et al. IL-6 trans-signaling in formation and progression of malignant ascites in ovarian cancer. Cancer Res. 2011;71(2):424–34.
191. Rath KS, Funk HM, Bowling MC, Richards WE, Drew AF. Expression of soluble interleukin-6 receptor in malignant ovarian tissue. Am J Obstet Gynecol. 2010;203(3):230. e1–e8.
192. Rabinovich A, Medina L, Piura B, Segal S, Huleihel M. Regulation of ovarian carcinoma SKOV-3 cell proliferation and secretion of MMPs by autocrine IL-6. Anticancer Res. 2007;27(1A):267–72.
193. Min H, Wei-hong Z. Constitutive activation of signal transducer and activator of transcription 3 in epithelial ovarian carcinoma. J Obstet Gynaecol Res. 2009;35(5):918–25.
194. Zhang X, Liu P, Zhang B, Wang A, Yang M. Role of STAT3 decoy oligodeoxynucleotides on cell invasion and chemosensitivity in human epithelial ovarian cancer cells. Cancer Genet Cytogenet. 2010; 197(1):46–53.
195. Colomiere M, Ward A, Riley C, Trenerry M, Cameron-Smith D, Findlay J, et al. Cross talk of signals between EGFR and IL-6R through JAK2/ STAT3 mediate epithelial–mesenchymal transition in ovarian carcinomas. Br J Cancer. 2008;100(1): 134–44.
196. Macciò A, Lai P, Santona MC, Pagliara L, Melis GB, Mantovani G. High serum levels of soluble IL-2 receptor, cytokines, and C reactive protein correlate with impairment of T cell response in patients with advanced epithelial ovarian cancer. Gynecol Oncol. 1998;69(3):248–52.
197. Giuntoli RL, Webb TJ, Zoso A, ROGERS O, Diaz-Montes TP, Bristow RE, et al. Ovarian cancer-associated ascites demonstrates altered immune environment: implications for antitumor immunity. Anticancer Res. 2009;29(8):2875–84.
198. Klink M, Kielbik M, Nowak M, Bednarska K, Sulowska Z. JAK3, STAT3 and CD3-zeta signaling proteins status in regard to the lymphocytes function in patients with ovarian cancer. Immunol Invest. 2012;41(4):382–98.
199. Mantovani G, Macciò A, Melis G, Mura L, Massa E, Mudu MC. Restoration of functional defects in peripheral blood mononuclear cells isolated from cancer patients by thiol antioxidants alpha-lipoic acid and N-acetyl cysteine. Int J Cancer. 2000;86(6):842–7.
200. Mantovani G, Macciò A, Madeddu C, Mura L, Gramignano G, Lusso MR, et al. Antioxidant agents are effective in inducing lymphocyte progression through cell cycle in advanced cancer patients: assessment of the most important laboratory indexes of cachexia and oxidative stress. J Mol Med. 2003;81(10):664–73.
201. Jeannin P, Duluc D, Delneste Y. IL-6 and leukemia-inhibitory factor are involved in the generation of tumor-associated macrophage: regulation by IFN-γ. Immunotherapy. 2011;3(4s):23–6.
202. Argilés JM, López-Soriano FJ. The role of cytokines in cancer cachexia. Med Res Rev. 1999;19:223–48.
203. Delano MJ, Moldawer LL. The origins of cachexia in acute and chronic inflammatory diseases*. Nutr Clin Prac. 2006;21(1):68–81.
204. Macciò A, Madeddu C, Massa D, Astara G, Farci D, Melis GB, et al. Interleukin-6 and leptin as markers of energy metabolic changes in advanced ovarian cancer patients. J Cell Mol Med. 2009;13(9b):3951–9.
205. Mantovani G, Macciò A, Massa E, Madeddu C. Managing cancer-related anorexia/cachexia. Drugs. 2001;61(4):499–514.
206. Macciò A, Madeddu C, Massa D, Mudu MC, Lusso MR, Gramignano G, et al. Hemoglobin levels correlate with interleukin-6 levels in patients with advanced untreated epithelial ovarian cancer: role of inflammation in cancer-related anemia. Blood. 2005;106(1):362–7.
207. Van Der Zee AG, De Cuyper EM, Limburg PC, De Bruijn HW, Hollema H, Bijzet J, et al. Higher levels of interleukin-6 in cystic fluids from patients with malignant versus benign ovarian tumors correlate with decreased hemoglobin levels and increased platelet counts. Cancer-Philadelphia. 1995;75:1004.
208. Lutgendorf SK, Weinrib AZ, Penedo F, Russell D, DeGeest K, Costanzo ES, et al. Interleukin-6, cortisol, and depressive symptoms in ovarian cancer patients. J Clin Oncol. 2008;26(29):4820–7.
209. Matsumura N, Huang Z, Mori S, Baba T, Fujii S, Konishi I, et al. Epigenetic suppression of the TGF-beta pathway revealed by transcriptome profiling in ovarian cancer. Genome Res. 2011;21(1):74–82.
210. Wang N, Zhang H, Yao Q, Wang Y, Dai S, Yang X. TGFBI promoter hypermethylation correlating with paclitaxel chemoresistance in ovarian cancer. J Exp Clin Cancer Res. 2012;31:6.

211. Wang D, Kanuma T, Mizunuma H, Takama F, Ibuki Y, Wake N, et al. Analysis of specific gene mutations in the transforming growth factor-β signal transduction pathway in human ovarian cancer. Cancer Res. 2000;60(16):4507–12.
212. Yin J, Lu K, Lin J, Wu L, Hildebrandt MA, Chang DW, et al. Genetic variants in TGF-β pathway are associated with ovarian cancer risk. PLoS One. 2011;6(9):e25559.
213. Toutirais O, Chartier P, Dubois D, Bouet F, Lévêque J, Catros-Quemener V, et al. Constitutive expression of TGF-beta1, interleukin-6 and interleukin-8 by tumor cells as a major component of immune escape in human ovarian carcinoma. Eur Cytokine Netw. 2003;14:246–55.
214. Do TV, Kubba LA, Du H, Sturgis CD, Woodruff TK. Transforming growth factor-ß1, transforming growth factor ß2, and transforming growth factor-ß3 enhance ovarian cancer metastatic potential by inducing a Smad3-dependent epithelial-to-mesenchymal transition. Mol Cancer Res. 2008;6: 695–705.
215. Santin AD, Bellone S, Ravaggi A, Roman J, Smith CV, Pecorelli S, et al. Increased levels of interleukin-10 and transforming growth factor-β in the plasma and ascitic fluid of patients with advanced ovarian cancer. BJOG. 2001;108(8):804–8.
216. Gordinier ME, Zhang H-Z, Patenia R, Levy LB, Atkinson EN, Nash MA, et al. Quantitative analysis of transforming growth factor β1 and 2 in ovarian carcinoma. Clin Cancer Res. 1999;5(9):2498–505.
217. Bartlett J, Langdon S, Scott W, Love S, Miller E, Katsaros D, et al. Transforming growth factor-[beta] isoform expression in human ovarian tumours. Eur J Cancer. 1997;33(14):2397–403.
218. Hirashima Y, Kobayashi H, Suzuki M, Tanaka Y, Kanayama N, Terao T. Transforming growth factor-β1 produced by ovarian cancer cell line HRA stimulates attachment and invasion through an up-regulation of plasminogen activator inhibitor type-1 in human peritoneal mesothelial cells. J Biol Chem. 2003;278(29):26793–802.
219. Vánky F, Nagy N, Hising C, Sjövall K, Klein E. Human ex vivo carcinoma cells produce transforming growth factor β and thereby can inhibit lymphocyte functions in vitro. Cancer Immunol Immunother. 1997;43(6):317–23.
220. Gavalas NG, Karadimou A, Dimopoulos MA, Bamias A. Immune response in ovarian cancer: how is the immune system involved in prognosis and therapy: potential for treatment utilization. Clin Dev Immunol. 2011;2010:791603.
221. Pepper MS. Transforming growth factor-beta: vasculogenesis, angiogenesis, and vessel wall integrity. Cytokine Growth Factor Rev. 1997;8(1):21–43.
222. Labidi-Galy SI, Sisirak V, Meeus P, Gobert M, Treilleux I, Bajard A, et al. Quantitative and functional alterations of plasmacytoid dendritic cells contribute to immune tolerance in ovarian cancer. Cancer Res. 2011;71(16):5423–34.
223. Daraï E, Detchev R, Hugol D, Quang NT. Serum and cyst fluid levels of interleukin (IL)-6, IL-8 and tumour necrosis factor-alpha in women with endometriomas and benign and malignant cystic ovarian tumours. Hum Reprod. 2003;18(8):1681–5.
224. DeJaco P, Asselain B, Orlandi C, Fridman WH, Teillaud JL. Evaluation of circulating tumor necrosis factor-alpha in patients with gynecological malignancies. Int J Cancer. 1991;48:375–8.
225. Takeyama H, Wakamiya N, O'Hara C, Arthur K, Niloff J, Kufe D, et al. Tumor necrosis factor expression by human ovarian carcinoma in vivo. Cancer Res. 1991;51(16):4476–80.
226. Dobrzycka B, Terlikowski SJ, Garbowicz M, Niklińska W, Bernaczyk PS, Nikliński J, et al. Tumor necrosis factor-alpha and its receptors in epithelial ovarian cancer. Folia Histochem Cytobiol. 2010;47(4):609–13.
227. Wu S, Boyer C, Whitaker R, Berchuck A, Wiener J, Weinberg J, et al. Tumor necrosis factor α as an autocrine and paracrine growth factor for ovarian cancer: monokine induction of tumor cell proliferation and tumor necrosis factor α expression. Cancer Res. 1993;53(8):1939–44.
228. Naylor M, Stamp GW, Foulkes WD, Eccles D, Balkwill FR. Tumor necrosis factor and its receptors in human ovarian cancer. Potential role in disease progression. J Clin Invest. 1993;91(5):2194.
229. Goldstein I, Ben-Horin S, Koltakov A, Chermoshnuk H, Polevoy V, Berkun Y, et al. α1ß1 integrin+ and regulatory Foxp3+ T cells constitute two functionally distinct human CD4+ T cell subsets oppositely modulated by TNFa blockade. J Immunol. 2007;178:201–10.
230. Malik S, Griffin D, Fiers W, Balkwill F. Paradoxical effects of tumour necrosis factor in experimental ovarian cancer. Int J Cancer. 1989;44(5):918–25.
231. Kulbe H, Hagemann T, Szlosarek PW, Balkwill FR, Wilson JL. The inflammatory cytokine tumor necrosis factor-a regulates chemokine receptor expression on ovarian cancer cells. Cancer Res. 2005;65:10355–65.
232. Lancaster JM, Sayer R, Blanchette C, Calingaert B, Whitaker R, Schildkraut J, et al. High expression of tumor necrosis factor-related apoptosis-inducing ligand is associated with favorable ovarian cancer survival. Clin Cancer Res. 2003;9(2):762–6.
233. Schummer M, Ng WV, Bumgarner RE, Nelson PS, Schummer B, Bednarski DW, et al. Comparative hybridization of an array of 21 500 ovarian cDNAs for the discovery of genes overexpressed in ovarian carcinomas. Gene. 1999;238(2):375–85.
234. Cuello M, Ettenberg SA, Nau MM, Lipkowitz S. Synergistic induction of apoptosis by the combination of trail and chemotherapy in chemoresistant ovarian cancer cells. Gynecol Oncol. 2001;81(3):380–90.
235. Yousef GM, Polymeris M-E, Yacoub GM, Scorilas A, Soosaipillai A, Popalis C, et al. Parallel overexpression of seven kallikrein genes in ovarian cancer. Cancer Res. 2003;63(9):2223–7.

236. Fang J, Wei W, Xia L, Song S. Study of the effect of TF/FVIIa complex on the expression of u-PAR mRNA in human ovarian cancer. Zhonghua xue ye xue za zhi Zhonghua xueyexue zazhi. 2004;25(3): 143–6.
237. Cheng T, Liu D, Griffin JH, Fernández JA, Castellino F, Rosen ED, et al. Activated protein C blocks p53-mediated apoptosis in ischemic human brain endothelium and is neuroprotective. Nat Med. 2003;9(3):338–42.
238. Conway EM, Rosenberg RD. Tumor necrosis factor suppresses transcription of the thrombomodulin gene in endothelial cells. Mol Cell Biol. 1988;8(12):5588–92.
239. Yousef GM, Polymeris M-E, Grass L, Soosaipillai A, Chan P-C, Scorilas A, et al. Human kallikrein 5 a potential novel serum biomarker for breast and ovarian cancer. Cancer Res. 2003;63(14):3958–65.
240. Werling R, Zacharski L, Kisiel W, Bajaj S, Memoli V, Rousseau S. Distribution of tissue factor pathway inhibitor in normal and malignant human tissues. Thromb Haemost. 1993;69(4):366–9.
241. Hembrough TA, Swartz GM, Papathanassiu A, et al. Tissue factor/factor VIIi inhibitors block angiogenesis and tumor growth through a non-hemostatic mechanism. Cancer Res. 2003;63:2997–3000.
242. Naschitz JE, Yeshurun D, Lev LM. Thromboembolism in cancer. Chang Trends Cancer. 1993;71(4):1384–90.
243. Schmeidler-Sapiro KT, Ratnoff OD, Gordon EM. Mitogenic effects of coagulation factor XII and factor XIIa on HepG2 cells. Proc Natl Acad Sci. 1991;88(10):4382–5.
244. Loercher AE, Nash MA, Kavanagh JJ, Platsoucas CD, Freedman RS. Identification of an IL-10-producing HLA-DR-negative monocyte subset in the malignant ascites of patients with ovarian carcinoma that inhibits cytokine protein expression and proliferation of autologous T cells. J Immunol. 1999;163(11):6251–60.
245. Kim J, Modlin RL, Moy RL, Dubinett S, McHugh T, Nickoloff BJ, et al. IL-10 production in cutaneous basal and squamous cell carcinomas. A mechanism for evading the local T cell immune response. J Immunol. 1995;155(4):2240–7.
246. Rabinovich A, Medina L, Piura B, Huleihel M. Expression of IL-10 in human normal and cancerous ovarian tissues and cells. Eur Cytokine Netw. 2010;21(2):122–8.
247. Berger S, Siegert A, Denkert C, Köbel M, Hauptmann S. Interleukin-10 in serous ovarian carcinoma cell lines. Cancer Immunol Immunother. 2001;50(6):328–33.
248. Mocellin S, Wang E, Marincola FM. Cytokines and immune response in the tumor microenvironment. J Immunother. 2001;24(5):392–407.
249. Vicari AP, Trinchieri G. Interleukin-10 in viral diseases and cancer: exiting the labyrinth? Immunol Rev. 2004;202(1):223–36.
250. Cervenak L, Morbidelli L, Donati D, Donnini S, Kambayashi T, Wilson JL, et al. Abolished angiogenicity and tumorigenicity of Burkitt lymphoma by interleukin-10. Blood. 2000;96(7):2568–73.
251. Stearns ME, Rhim J, Wang M. Interleukin 10 (IL-10) inhibition of primary human prostate cell-induced angiogenesis IL-10 stimulation of tissue inhibitor of metalloproteinase-1 and inhibition of Matrix Metalloproteinase (MMP)-2/MMP-9 secretion. Clin Cancer Res. 1999;5(1):189–96.
252. Mustea A, KÖnsgen D, Braicu E, Pirvulescu C, Sun P, Sofroni DO, et al. Expression of IL-10 in patients with ovarian carcinoma. Anticancer Res. 2006; 26(2C):1715–8.
253. Gotlieb WH, Abrams JS, Watson JM, Velu TJ, Berek JS, Martínez-Maza O. Presence of interleukin 10 (IL-10) in the ascites of patients with ovarian and other intra-abdominal cancers. Cytokine. 1992;4(5): 385–90.
254. C-z L, Zhang L, Chang X-h, Cheng Y-x, Cheng H-y, Ye X, et al. Overexpression and immunosuppressive functions of transforming growth factor 1, vascular endothelial growth factor and interleukin-10 in epithelial ovarian cancer. Chin J Cancer Res. 2012;24(2):130–7.
255. Matte I, Lane D, Laplante C, Rancourt C, Piché A. Profiling of cytokines in human epithelial ovarian cancer ascites. Am J Cancer Res. 2012;2(5):566.
256. Kohno T, Mizukami H, Suzuki M, Saga Y, Takei Y, Shimpo M, et al. Interleukin-10-mediated inhibition of angiogenesis and tumor growth in mice bearing VEGF-producing ovarian cancer. Cancer Res. 2003;63(16):5091–4.
257. Daikoku T, Tranguch S, Trofimova IN, Dinulescu DM, Jacks T, Nikitin AY, et al. Cyclooxygenase-1 is overexpressed in multiple genetically engineered mouse models of epithelial ovarian cancer. Cancer Res. 2006;66(5):2527–31.
258. Rodríguez-Burford C, Barnes MN, Oelschlager DK, Myers RB, Talley LI, Partridge EE, et al. Effects of Nonsteroidal Anti-Inflammatory Agents (NSAIDs) on ovarian carcinoma cell lines preclinical evaluation of NSAIDs as chemopreventive agents. Clin Cancer Res. 2002;8(1):202–9.
259. Symowicz J, Adley BP, Woo MM, Auersperg N, Hudson LG, Stack MS. Cyclooxygenase-2 functions as a downstream mediator of lysophosphatidic acid to promote aggressive behavior in ovarian carcinoma cells. Cancer Res. 2005;65(6):2234–42.
260. Prizment AE, Folsom AR, Anderson KE. Nonsteroidal anti-inflammatory drugs and risk for ovarian and endometrial cancers in the Iowa Women's Health Study. Cancer Epidemiol Biomark Prev. 2010;19(2):435–42.
261. Smith WL, Garavito RM, DeWitt DL. Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. J Biol Chem. 1996;271:33157–60.
262. Li J-Y, Wang X-Z, Chen F-L, Yu J-P, Luo H-S. Nimesulide inhibits proliferation via induction of apoptosis and cell cycle arrest in human gastric adenocarcinoma cell line. World J Gastroenterol. 2003;9(5):915–20.

263. Li W, Zhang H-h, Xu R-j, Zhuo G-c, Hu Y-q, Li J. Effects of a selective cyclooxygenase-2 inhibitor, nimesulide, on the growth of ovarian carcinoma in vivo. Med Oncol. 2008;25(2):172–7.
264. Vane J, Bakhle Y, Botting R. Cyclooxygenases 1 and 2. Ann Rev Pharmacol Toxicol. 1998;38(1):97–120.
265. Rask K, Zhu Y, Wang W, Hedin L, Sundfeldt K. Ovarian epithelial cancer: a role for PGE 2 -synthesis and signalling in malignant transformation and progression. Mol Cancer. 2006;5:62–74.
266. Auersperg N, Wong AS, Choi K-C, Kang SK, Leung PC. Ovarian surface epithelium: biology, endocrinology, and pathology 1. Endocr Rev. 2001;22(2):255–88.
267. Sundfeldt K, Ivarsson K, Carlsson M, Enerbäck S, Janson PO, Brännström M, et al. The expression of CCAAT/enhancer binding protein (C/EBP) in the human ovary in vivo: specific increase in C/EBPbeta during epithelial tumour progression. Br J Cancer. 1999;79:1240–8.
268. Casado E, Gomez-Navarro J, Yamamoto M, Adachi Y, Coolidge CJ, Arafat WO, et al. Strategies to accomplish targeted expression of transgenes in ovarian cancer for molecular therapeutic applications. Clin Cancer Res. 2001;7(8):2496–504.
269. Roland IH, Yang WL, Yang DH, Daly MB, Ozols RF, Hamilton TC, et al. Loss of surface and cyst epithelial basement membranes and preneoplastic morphologic changes in prophylactic oophorectomies. Cancer. 2003;98(12):2607–23.
270. Daikoku T, Wang D, Tranguch S, Morrow JD, Orsulic S, DuBois RN, et al. Cyclooxygenase-1 is a potential target for prevention and treatment of ovarian epithelial cancer. Cancer Res. 2005;65(9):3735–44.
271. Ferrandina G, Ranelletti FO, Martinelli E, Paglia A, Zannoni GF, Scambia G. Cyclo-oxygenase-2 (Cox-2) expression and resistance to platinum versus platinum/paclitaxel containing chemotherapy in advanced ovarian cancer. BMC Cancer. 2006;6(1):182.
272. Ferrandina G, Lauriola L, Zannoni G, Fagotti A, Fanfani F, Legge F, et al. Increased cyclooxygenase-2 (COX-2) expression is associated with chemotherapy resistance and outcome in ovarian cancer patients. Ann Oncol. 2002;13(8):1205–11.
273. Raspollini MR, Amunni G, Villanucci A, Boddi V, Baroni G, Taddei A, et al. COX-2 status in relation to tumor microvessel density and VEGF expression: analysis in ovarian carcinoma patients with low versus high survival rates. Oncol Rep. 2004;11(2): 309–14.
274. Denkert C, Köbel M, Pest S, Koch I, Berger S, Schwabe M, et al. Expression of cyclooxygenase 2 is an independent prognostic factor in human ovarian carcinoma. Am J Pathol. 2002;160(3):893–903.
275. Erkinheimo T-L, Lassus H, Finne P, van Rees BP, Leminen A, Ylikorkala O, et al. Elevated cyclooxygenase-2 expression is associated with altered expression of p53 and SMAD4, amplification of HER-2/neu, and poor outcome in serous ovarian carcinoma. Clin Cancer Res. 2004;10(2):538–45.
276. Khunnarong J, Tangjitgamol S, Manusirivithaya S, Suekwattana P, Leelahakorn S. Expression of cyclooxygenase-1 in epithelial ovarian cancer: a clinicopathological study. Asian Pac J Cancer Prev. 2008;9:757–62.
277. Liu M, Matsumura N, Mandai M, Li K, Yagi H, Baba T, et al. Classification using hierarchical clustering of tumor-infiltrating immune cells identifies poor prognostic ovarian cancers with high levels of COX expression. Mod Pathol. 2008;22(3):373–84.
278. Li W, Graeber MB. The molecular profile of microglia under the influence of glioma. Neuro-Oncol. 2012;14(8):958–78.
279. Li W, Wang J, Jiang H-R, Xu X-L, Zhang J, Liu M-L, et al. Combined effects of cyclooxygenase-1 and cyclooxygenase-2 selective inhibitors on ovarian carcinoma in vivo. Int J Mol Sci. 2011;12(1): 668–81.
280. Sarraf P, Mueller E, Jones D, King FJ, DeAngelo DJ, Partridge JB, et al. Differentiation and reversal of malignant changes in colon cancer through PPAR&ggr. Nat Med. 1998;4(9):1046–52.
281. Sakamoto A, Yokoyama Y, Umemoto M, Futagami M, Sakamoto T, Bing X, et al. Clinical implication of expression of cyclooxygenase-2 and peroxisome proliferator activated-receptor γ in epithelial ovarian tumours. Br J Cancer. 2004;91(4):633–8.
282. Xin B, Yokoyama Y, Shigeto T, Futagami M, Mizunuma H. Inhibitory effect of meloxicam, a selective cyclooxygenase-2 inhibitor, and ciglitazone, a peroxisome proliferator-activated receptor gamma ligand, on the growth of human ovarian cancers. Cancer. 2007;110(4):791–800.
283. Daikoku T, Tranguch S, Chakrabarty A, Wang D, Khabele D, Orsulic S, et al. Extracellular signal-regulated kinase is a target of cyclooxygenase-1-peroxisome proliferator-activated receptor-δ signaling in epithelial ovarian cancer. Cancer Res. 2007;67(11):5285–92.
284. Beck EP, Russo P, Gliozzo B, Jaeger W, Papa V, Wildt L, et al. Identification of insulin and insulin-like growth factor I (IGF I) receptors in ovarian cancer tissue. Gynecol Oncol. 1994;53(2):196–201.
285. Foekens JA, van Putten WLJ, Portengen H, Rodenburg CJ, Reubi JC, Berns PM, et al. Prognostic value of pS2 protein and receptors for epidermal growth factor (EGF-R), insulin-like growth factor-I (IGF-IR) and somatostatin (SS-R) in patients with breast and ovarian cancer. J Steroid Biochem Mol Biol. 1990;37:815–21.
286. Foekens JA, van Putten WLJ, Portengen H, Rodenburg CJ, Reubi JC, Berns PM, et al. Prognostic value of receptors for epidermal growth factor (EGF-r), insulin-like growth factor-I (IGF-I-r) and somatostatin (SSTr) and of pS2 protein, in patients with breast and ovarian cancer. Eur J Cancer. 1990;26: 154–8.
287. Van Dam P, Vergote I, Lowe D, Watson J, Van Damme P, Van der Auwera J, et al. Expression of c-erbB-2, c-myc, and c-ras oncoproteins, insulin-like

growth factor receptor I, and epidermal growth factor receptor in ovarian carcinoma. J Clin Pathol. 1994;47(10):914–9.
288. Weigang B, Nap M, Bittl A, Jaeger W. Immunohistochemical localization of insulin-like growth factor 1 receptors in benign and malignant tissues of the female genital tract. Tumor Biol. 1994;15(4):236–46.
289. Flyvbjerg A, Mogensen O, Mogensen B, Nielsen OS. Elevated serum Insulin-Like Growth Factor-Binding Protein 2 (IGFBP-2) and decreased IGFBP-3 in epithelial ovarian cancer: correlation with cancer antigen 125 and tumor-associated trypsin inhibitor 1. J Clin Endocrinol Metab. 1997;82(7):2308–13.
290. Chakrabarty S, Kondratick L. Research paper insulin-like growth factor binding protein-2 stimulates proliferation and activates multiple cascades of the mitogen-activated protein kinase pathways in NIH-OVCAR3 human epithelial ovarian cancer cells. Cancer Biol Ther. 2006;5(2):189–97.
291. Cao Z, Liu L-Z, Dixon DA, Zheng JZ, Chandran B, Jiang B-H. Insulin-like growth factor-I induces cyclooxygenase-2 expression via PI3K, MAPK and PKC signaling pathways in human ovarian cancer cells. Cell Signal. 2007;19(7):1542–53.
292. Xu Y, Gaudette DC, Boynton JD, Frankel A, Fang X-J, Sharma A, et al. Characterization of an ovarian cancer activating factor in ascites from ovarian cancer patients. Clin Cancer Res. 1995;1(10):1223–32.
293. Fang X, Schummer M, Mao M, Yu S, Tabassam FH, Swaby R, et al. Lysophosphatidic acid is a bioactive mediator in ovarian cancer. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of. Lipids. 2002;1582(1):257–64.
294. Fang X, Gaudette D, Furui T, Mao M, Estrella V, Eder A, et al. Lysophospholipid growth factors in the initiation, progression, metastases, and management of ovarian cancer. Ann N Y Acad Sci. 2000;905(1):188–208.
295. Westermann AM, Havik E, Postma FR, Beijnen JH, Dalesio O, Moolenaar WH, et al. Malignant effusions contain lysophosphatidic acid (LPA)-like activity. Ann Oncol. 1998;9(4):437–42.
296. Fishman DA, Liu Y, Ellerbroek SM, Stack MS. Lysophosphatidic acid promotes matrix metalloproteinase (MMP) activation and MMP-dependent invasion in ovarian cancer cells. Cancer Res. 2001;61(7):3194–9.
297. Heinonen P, Metsä-Ketelä T. Prostaglandin and thromboxane production in ovarian cancer tissue. Gynecol Obstetr Invest. 1984;18(5):225–9.
298. Munkarah A, Morris R, Baumann P, Deppe G, Malone J, Diamond M, et al. Effects of prostaglandin E2 on proliferation and apoptosis of epithelial ovarian cancer cells. J Soc Gynecol Invest. 2002;9(3):168–73.
299. Spinella F, Rosanò L, Di Castro V, Natali PG, Bagnato A. Endothelin-1-induced prostaglandin E2-EP2, EP4 signaling regulates vascular endothelial growth factor production and ovarian carcinoma cell invasion. J Biol Chem. 2004;279(45):46700–5.
300. Spinella F, Rosanò L, Di Castro V, Nicotra MR, Natali PG, Bagnato A. Inhibition of cyclooxygenase-1 and -2 expression by targeting the endothelin a receptor in human ovarian carcinoma cells. Clin Cancer Res. 2004;9:4670–9.
301. Lau M-T, Wong AS, Leung PC. Gonadotropins induce tumor cell migration and invasion by increasing cyclooxygenases expression and prostaglandin E2 production in human ovarian cancer cells. Endocrinology. 2010;151(7):2985–93.
302. Charles KA, Kulbe KH, Soper R, Escorcio-Correia M, Lawrence T, Schultheis A, et al. The tumor-promoting actions of TNF-ainvolve TNFR1 and IL-17 in ovarian cancer in mice and humans. J Clin Invest. 2009;119:311–23.
303. Miyahara Y, Odunsi K, Chen W, Peng G, Matsuzaki J, Wang R-F. Generation and regulation of human CD4+ IL-17-producing T cells in ovarian cancer. Proc Natl Acad Sci. 2008;105(40):15505–10.
304. Su X, Ye J, Hsueh EC, Zhang Y, Hoft DF, Peng G. Tumor microenvironments direct the recruitment and expansion of human Th17 cells. J Immunol. 2010;184(3):1630–41.
305. Kryczek I, Banerjee M, Cheng P, Vatan L, Szeliga W, Wei S, et al. Phenotype, distribution, generation, and functional and clinical relevance of Th17 cells in the human tumor environments. Blood. 2009;114(6):1141–9.
306. Bloom BR, Bennett B. Mechanism of a reaction in vitro associated with delayed-type hypersensitivity. Science. 1966;153(3731):80–2.
307. Calandra T, Echtenacher B, Le Roy D, Pugin J, Metz CN, Hültner L, et al. Protection from septic shock by neutralization of macrophage migration inhibitory factor. Nat Med. 2000;6(2):164–70.
308. Heimberger AB, Hlatky R, Suki D, Yang D, Weinberg J, Gilbert M, et al. Prognostic effect of epidermal growth factor receptor and EGFRvIII in glioblastoma multiforme patients. Clin Cancer Res. 2005;11(4):1462–66.
309. Hudson JD, Shoaibi MA, Maestro R, Carnero A, Hannon GJ, Beach DH. A proinflammatory cytokine inhibits p53 tumor suppressor activity. J Exp Med. 1999;190(10):1375–82.
310. Mitchell RA, Liao H, Chesney J, Fingerle-Rowson G, Baugh J, David J, et al. Macrophage migration inhibitory factor (MIF) sustains macrophage proinflammatory function by inhibiting p53: regulatory role in the innate immune response. Proc Natl Acad Sci. 2002;99(1):345–50.
311. Hagemann T, Robinson SC, Thompson RG, Charles K, Kulbe H, Balkwill FR. Ovarian cancer cell–derived migration inhibitory factor enhances tumor growth, progression, and angiogenesis. Mol Cancer Ther. 2007;6(7):1993–2002.
312. Krockenberger M, Dombrowski Y, Weidler C, Ossadnik M, Hönig A, Häusler S, et al. Macrophage migration inhibitory factor contributes to the immune escape of ovarian cancer by down-regulating NKG2D. J Immunol. 2008;180(11):7338–48.

313. Chambers SK. Role of CSF-1 in progression of epithelial ovarian cancer. Future Oncol. 2009;5(9):1429–40.
314. Cecchini M, Hofstetter W, Halasy J, Wetterwald A, Felix R. Role of CSF-1 in bone and bone marrow development. Mol Reprod Dev. 1997;46(1):75–84.
315. Chambers SK, Kacinski BM, Ivins CM, Carcangiu ML. Overexpression of epithelial macrophage colony-stimulating factor (CSF-1) and CSF-1 receptor: a poor prognostic factor in epithelial ovarian cancer, contrasted with a protective effect of stromal CSF-1. Clin Cancer Res. 1997;3(6):999–1007.
316. Kacinski BM, Carter D, Mittal K, Yee LD, Scata KA, Donofrio L, et al. Ovarian adenocarcinomas express fms-complementary transcripts and fms antigen, often with coexpression of CSF-1. Am J Pathol. 1990;137(1):135.
317. Kacinski BM, Richard Stanley E, Carter D, Chambers JT, Chambers SK, Kohorn EI, et al. Circulating levels of CSF-1 (M-CSF) a lymphohematopoietic cytokine may be a useful marker of disease status in patients with malignant ovarian neoplasms. Int J Radiat Oncol Biol Phys. 1989;17(1):159–64.
318. Price FV, Chambers SK, Chambers JT, Carcangiu ML, Schwartz PE, Kohorn EI, et al. Colony-stimulating factor-1 in primary ascites of ovarian cancer is a significant predictor of survival. Am J Obstet Gynecol. 1993;168(2):520–7.
319. Scholl S, Bascou C, Mosseri V, Olivares R, Magdelenat H, Dorval T, et al. Circulating levels of colony-stimulating factor 1 as a prognostic indicator in 82 patients with epithelial ovarian cancer. Br J Cancer. 1994;69(2):342.
320. Chambers SK, Wang Y, Gertz RE, Kacinski BM. Macrophage colony-stimulating factor mediates invasion of ovarian cancer cells through urokinase. Cancer Res. 1995;55(7):1578–85.
321. Chambers S, Ivins C, Carcangiu M. Urokinase-type plasminogen activator in epithelial ovarian cancer: a poor prognostic factor, associated with advanced stage. Int J Gynecol Cancer. 1998;8(3):242–50.
322. Kuhn W, Schmalfeldt B, Reuning U, Pache L, Berger U, Ulm K, et al. Prognostic significance of urokinase (uPA) and its inhibitor PAI-1 for survival in advanced ovarian carcinoma stage FIGO IIIc. Br J Cancer. 1999;79(11–12):1746.
323. van der Burg ME, Henzen-Logmans SC, Berns EM, Van Putten WL, Klijn JG, Foekens JA. Expression of urokinase-type plasminogen activator (uPA) and its inhibitor PAI-1 in benign, borderline, malignant primary and metastatic ovarian tumors. Int J Cancer. 1996;69(6):475–9.
324. Toy EP, Azodi M, Folk NL, Zito CM, Zeiss CJ, Chambers SK. Enhanced ovarian cancer tumorigenesis and metastasis by the macrophage colony-stimulating factor. Neoplasia. 2009;11(2):136–44.
325. Gordon IO, Freedman RS. Defective antitumor function of monocyte-derived macrophages from epithelial ovarian cancer patients. Clin Cancer Res. 2006;12(5):1515–24.
326. Walz A, Peveri P, Aschauer H, Baggiolini M. Purification and amino acid sequencing of NAF, a novel neutrophil-activating factor produced by monocytes. Biochem Biophys Res Commun. 1987;149(2):755–61.
327. Murdoch C, Monk PN, Finn A. Cxc chemokine receptor expression on human endothelial cells. Cytokine. 1999;11(9):704–12.
328. Ivarsson K, Runesson E, Sundfeldt K, Haeger M, Hedin L, Janson PO, et al. The chemotactic cytokine interleukin-8—a cyst fluid marker for malignant epithelial ovarian cancer? Gynecol Oncol. 1998;71(3):420–3.
329. Herrera CA, Xu L, Bucana CD, Silva EG, Hess KR, Gershenson DM, et al. Expression of metastasis-related genes in human epithelial ovarian tumors. Int J Oncol. 2002;20(1):5–14.
330. Mayerhofer K, Bodner K, Bodner-Adler B, Schindl M, Kaider A, Hefler L, et al. Interleukin-8 serum level shift in patients with ovarian carcinoma undergoing paclitaxel-containing chemotherapy. Cancer. 2001;91(2):388–93.
331. Uslu R, Sanli U, Dikmen Y, Karabulut B, Ozsaran A, Sezgin C, et al. Predictive value of serum interleukin-8 levels in ovarian cancer patients treated with paclitaxel-containing regimens. Int J Gynecol Cancer. 2005;15(2):240–5.
332. Merritt WM, Lin YG, Spannuth WA, Fletcher MS, Kamat AA, Han LY, et al. Effect of interleukin-8 gene silencing with liposome-encapsulated small interfering RNA on ovarian cancer cell growth. J Natl Cancer Inst. 2008;100(5):359–72.
333. Liu J, Yang G, Thompson-Lanza JA, Glassman A, Hayes K, Patterson A, et al. A genetically defined model for human ovarian cancer. Cancer Res. 2004;64(5):1655–63.
334. Xu L, Xie K, Mukaida N, Matsushima K, Fidler IJ. Hypoxia-induced elevation in interleukin-8 expression by human ovarian carcinoma cells. Cancer Res. 1999;59(22):5822–9.
335. Xu L, Pathak PS, Fukumura D. Hypoxia-induced activation of p38 mitogen-activated protein kinase and phosphatidylinositol 3′-kinase signaling pathways contributes to expression of interleukin 8 in human ovarian carcinoma cells. Clin Cancer Res. 2004;10(2):701–7.
336. Huang S, Robinson JB, DeGuzman A, Bucana CD, Fidler IJ. Blockade of nuclear factor-κB signaling inhibits angiogenesis and tumorigenicity of human ovarian cancer cells by suppressing expression of vascular endothelial growth factor and interleukin 8. Cancer Res. 2000;60(19):5334–9.
337. Agarwal A, Tressel SL, Kaimal R, Balla M, Lam FH, Covic L, et al. Identification of a metalloprotease-chemokine signaling system in the ovarian cancer microenvironment: implications for antiangiogenic therapy. Cancer Res. 2010;70(14):5880–90.
338. Curiel TJ, Cheng P, Mottram P, Alvarez X, Moons L, Evdemon-Hogan M, et al. Dendritic cell subsets differentially regulate angiogenesis in human ovarian cancer. Cancer Res. 2004;64(16):5535–8.

339. Yang G, Rosen DG, Liu G, Yang F, Guo X, Xiao X, et al. CXCR2 promotes ovarian cancer growth through dysregulated cell cycle, diminished apoptosis, and enhanced angiogenesis. Clin Cancer Res. 2010;16(15):3875–86.
340. Abdollahi T, Robertson NM, Abdollahi A, Litwack G. Identification of interleukin 8 as an inhibitor of tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis in the ovarian carcinoma cell line OVCAR3. Cancer Res. 2003;63(15):4521–6.
341. Duan Z, Feller AJ, Penson RT, Chabner BA, Seiden MV. Discovery of differentially expressed genes associated with paclitaxel resistance using cDNA array technology: analysis of interleukin (IL) 6, IL-8, and monocyte chemotactic protein 1 in the paclitaxel-resistant phenotype. Clin Cancer Res. 1999;5(11):3445–53.
342. Scotton CJ, Wilson JL, Milliken D, Stamp G, Balkwill FR. Epithelial cancer cell migration a role for chemokine receptors? Cancer Res. 2001;61(13):4961–5.
343. Kajiyama H, Shibata K, Terauchi M, Ino K, Nawa A, Kikkawa F. Involvement of SDF-1a/CXCR4 axis in the enhanced peritoneal metastasis of epithelial ovarian carcinoma. Int J Cancer. 2008;122:91–9.
344. Miyanishi N, Suzuki Y, Simizu S, Kuwabara Y, Banno K, Umezawa K. Involvement of autocrine CXCL12/CXCR4 system in the regulation of ovarian carcinoma cell invasion. Biochem Biophys Res Commun. 2010;403(1):154–9.
345. Jiang Y-p, Wu X-h, Shi B, Wu W-x, Yin G-r. Expression of chemokine CXCL12 and its receptor CXCR4 in human epithelial ovarian cancer: an independent prognostic factor for tumor progression. Gynecol Oncol. 2006;103(1):226–33.
346. Jiang Y-p, Wu X-h, Xing H-y, X-y D. Role of CXCL12 in metastasis of human ovarian cancer. Chin Med J. 2007;120(14):1251.
347. Zou W, Machelon V, Coulomb-L'Hermin A, Borvak J, Nome F, Isaeva T, et al. Stromal-derived factor-1 in human tumors recruits and alters the function of plasmacytoid precursor dendritic cells. Nat Med. 2001;7(12):1339–46.
348. Righi E, Kashiwagi S, Yuan J, Santosuosso M, Leblanc P, Ingraham R, et al. CXCL12/CXCR4 blockade induces multimodal antitumor effects that prolong survival in an immunocompetent mouse model of ovarian cancer. Cancer Res. 2011;71(16):5522–34.
349. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med. 2004;10(9):942–9.
350. Guo L, Cui Z-M, Zhang J, Huang Y. Chemokine axes CXCL14/CXCR4 and CXCL16/CXCR6 correlate with lymph node metastasis in epithelial ovarian carcinoma. Chin Med J. 2011;30:336–43.
351. Rainczuk A, Rao J, Gathercole J, Stephens AN. The emerging role of CXC chemokines in epithelial ovarian cancer. Reproduction. 2012;144(3):303–17.
352. Clark-Lewis I, Mattioli I, Gong J-H, Loetscher P. Structure-function relationship between the human chemokine receptor CXCR3 and its ligands. J Biol Chem. 2003;278(1):289–95.
353. Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F, et al. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proc Natl Acad Sci U S A. 2005;102(51):18538–43.
354. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med. 2003;348(3):203–13.
355. Johnson EL, Singh R, Singh S, Johnson-Holiday CM, Grizzle WE, Partridge EE, et al. CCL25-CCR9 interaction modulates ovarian cancer cell migration, metalloproteinase expression, and invasion. World J Surg Oncol. 2010;8:62.
356. Dellacasagrande J, Schreurs O, Hofgaard P, Omholt H, Steinsvoll S, Schenck K, et al. Liver metastasis of cancer facilitated by chemokine receptor CCR6. Scand J Immunol. 2003;57(6):534–44.
357. Milliken D, Scotton C, Raju S, Balkwill F, Wilson J. Analysis of chemokines and chemokine receptor expression in ovarian cancer ascites. Clin Cancer Res. 2002;8(4):1108–14.
358. Nelson BH. The impact of T-cell immunity on ovarian cancer outcomes. Immunol Rev. 2008;222(1):101–16.
359. Raspollini M, Castiglione F, Degl'Innocenti DR, Amunni G, Villanucci A, Garbini F, et al. Tumour-infiltrating gamma/delta T-lymphocytes are correlated with a brief disease-free interval in advanced ovarian serous carcinoma. Ann Oncol. 2005;16(4):590–6.
360. Halapi E, Yamamoto Y, Juhlin C, Jeddi-Tehrani M, Grunewald J, Andersson R, et al. Restricted T cell receptor V-beta and J-beta usage in T cells from interleukin-2-cultured lymphocytes of ovarian and renal carcinomas. Cancer immunology, immunotherapy: CII, Cancer Immunol Immunother. 1993;36(3):191–7.
361. Bamias A, Koutsoukou V, Terpos E, Tsiatas ML, Liakos C, Tsitsilonis O, et al. Correlation of NKT-like CD3+CD56+ cells and CD4+CD25+(hi) regulatory T cells with VEGF and TNF alpha in ascites from advanced ovarian cancer: association with platinum resistance and prognosis in patients receiving first-line platinum based chemotherapy. Gynecol Oncol. 2008;108:421–7.
362. Negus R, Stamp G, Hadley J, Balkwill FR. Quantitative assessment of the leukocyte infiltrate in ovarian cancer and its relationship to the expression of CC chemokines. Am J Pathol. 1997;150(5):1723.
363. Santin A, Bellone S, Palmieri M, Bossini B, Cane S, Bignotti E, et al. Restoration of tumor specific human leukocyte antigens class I-restricted cytotoxicity by dendritic cell stimulation of tumor infiltrating lymphocytes in patients with advanced ovarian cancer. Int J Gynecol Cancer. 2004;14(1):64–75.

364. Freedman RS, Deavers M, Liu J, Wang E. Peritoneal inflammation–a microenvironment for epithelial ovarian cancer (EOC). J Transl Med. 2004;2(1):23.
365. Kooi S, Freedman RS, Rodriguez-Villanueva J, Platsoucas CD. Cytokine production by T-cell lines derived from tumor-infiltrating lymphocytes from patients with ovarian carcinoma: tumor-specific immune responses and inhibition of antigen-independent cytokine production by ovarian tumor cells. Lymphokine Cytokine Res. 1993;12(6):429–37.
366. Melichar B, Nash M, Lenzi R, Platsoucas C, Freedman R. Expression of costimulatory molecules CD80 and CD86 and their receptors CD28, CTLA-4 on malignant ascites CD3+ tumour-infiltrating lymphocytes (TIL) from patients with ovarian and other types of peritoneal carcinomatosis. Clin Exp Immunol. 2000;119(1):19–27.
367. Lockhart DC, Chan AK, Mak S, Joo H-G, Daust HA, Carritte A, et al. Loss of T-cell receptor-CD3ζ and T-cell function in tumor-infiltrating lymphocytes but not in tumor-associated lymphocytes in ovarian carcinoma. Surgery. 2001;129(6):749–56.
368. Chen C, Wu M, Chao K, Ho H, Sheu B, Huang S. T lymphocytes and cytokine production in ascitic fluid of ovarian malignancies. J Formos Med Assoc Taiwan Yi Zhi. 1999;98(1):24–30.
369. Serafini P, De Santo C, Marigo I, Cingarlini S, Dolcetti L, Gallina G, et al. Derangement of immune responses by myeloid suppressor cells. Cancer Immunol Immunother. 2004;53(2):64–72.
370. Kryczek I, Wei S, Zhu G, Myers L, Mottram P, Cheng P, et al. Relationship between B7-H4, regulatory T cells, and patient outcome in human ovarian carcinoma. Cancer Res. 2007;67(18):8900–5.
371. Kryczek I, Zou L, Rodriguez P, Zhu G, Wei S, Mottram P, et al. B7-H4 expression identifies a novel suppressive macrophage population in human ovarian carcinoma. J Exp Med. 2006;203(4):871–81.
372. Piver MS, Mettlin CJ, Tsukada Y, Nasca P, Greenwald P, McPhee ME. Familial ovarian cancer registry. Obstet Gynecol. 1984;64(2):195–9.
373. Cannon MJ, Goyne H, Stone PJ, Chiriva-Internati M. Dendritic cell vaccination against ovarian cancer-tipping the Treg/TH17 balance to therapeutic advantage? Expert Opin Biol Ther. 2011;11(4):441–5.
374. Sharma MD, Hou D-Y, Liu Y, Koni PA, Metz R, Chandler P, et al. Indoleamine 2, 3-dioxygenase controls conversion of Foxp3+ Tregs to TH17-like cells in tumor-draining lymph nodes. Blood. 2009;113(24):6102–11.
375. Chung DJ, Rossi M, Romano E, Ghith J, Yuan J, Munn DH, et al. Indoleamine 2, 3-dioxygenase–expressing mature human monocyte-derived dendritic cells expand potent autologous regulatory T cells. Blood. 2009;114(3):555–63.
376. Okamoto A, Nikaido T, Ochiai K, Takakura S, Saito M, Aoki Y, et al. Indoleamine 2, 3-dioxygenase serves as a marker of poor prognosis in gene expression profiles of serous ovarian cancer cells. Clin Cancer Res. 2005;11(16):6030–9.
377. Inaba T, Ino K, Kajiyama H, Yamamoto E, Shibata K, Nawa A, et al. Role of the immunosuppressive enzyme indoleamine 2, 3-dioxygenase in the progression of ovarian carcinoma. Gynecol Oncol. 2009;115(2):185–92.
378. Woo EY, Chu CS, Goletz TJ, Schlienger K, Yeh H, Coukos G, et al. Regulatory CD4+ CD25+ T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. Cancer Res. 2001;61(12):4766–72.
379. Hamanishi J, Mandai M, Iwasaki M, Okazaki T, Tanaka Y, Yamaguchi K, et al. Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. Proc Natl Acad Sci. 2007;104(9):3360–5.
380. Mhawech-Fauceglia P, Wang D, Ali L, Lele S, Huba MA, Liu S, et al. Intraepithelial T cells and tumor-associated macrophages in ovarian cancer patients. Cancer Immun. 2013;13.
381. Shah CA, Allison KH, Garcia RL, Gray HJ, Goff BA, Swisher EM. Intratumoral T cells, tumor-associated macrophages, and regulatory T cells: association with p53 mutations, circulating tumor DNA and survival in women with ovarian cancer. Gynecol Oncol. 2008;109(2):215–9.
382. Lewis J, Lee J, Underwood J, Harris A, Lewis C. Macrophage responses to hypoxia: relevance to disease mechanisms. J Leukoc Biol. 1999;66(6):889–900.
383. Thanaa El-A H, Alla AEK, Laban MA, Fahmy RM. Immunophenotyping of tumor-infiltrating mononuclear cells in ovarian carcinoma. Pathol Oncol Res. 2004;10(2):80–4.
384. Negus R, Stamp G, Relf M, Burke F, Malik S, Bernasconi S, et al. The detection and localization of monocyte chemoattractant protein-1 (MCP-1) in human ovarian cancer. J Clin Invest. 1995;95(5):2391.
385. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer. 2004;4(1):71–8.
386. Turner L, Scotton C, Negus R, Balkwill F. Hypoxia inhibits macrophage migration. Eur J Immunol. 1999;29(7):2280–7.
387. Sica A, Saccani A, Bottazzi B, Bernasconi S, Allavena P, Gaetano B, et al. Defective expression of the monocyte chemotactic protein-1 receptor CCR2 in macrophages associated with human ovarian carcinoma. J Immunol. 2000;164(2):733–8.
388. Hagemann T, Wilson J, Burke F, Kulbe H, Li NF, Plüddemann A, et al. Ovarian cancer cells polarize macrophages toward a tumor-associated phenotype. J Immunol. 2006;176(8):5023–32.
389. Hagemann T, Wilson J, Kulbe H, Li NF, Leinster DA, Charles K, et al. Macrophages induce invasiveness of epithelial cancer cells via NF-nB and JNK. J Immunol. 2005;175:1197–205.
390. Robinson-Smith TM, Isaacsohn I, Mercer CA, Zhou M, Van Rooijen N, Husseinzadeh N, et al. Macrophages mediate inflammation-enhanced metastasis of ovarian tumors in mice. Cancer Res. 2007;67(12):5708–16.

391. Klimp AH, Hollema H, Kempinga C, van der Zee AG, de Vries EG, Daemen T. Expression of cyclooxygenase-2 and inducible nitric oxide synthase in human ovarian tumors and tumor-associated macrophages. Cancer Res. 2001;61(19):7305–9.
392. Krempski J, Karyampudi L, Behrens MD, Erskine CL, Hartmann L, Dong H, et al. Tumor-infiltrating programmed death receptor-1+ dendritic cells mediate immune suppression in ovarian cancer. J Immunol. 2011;186(12):6905–13.
393. Curiel TJ, Wei S, Dong H, Alvarez X, Cheng P, Mottram P, et al. Blockade of B7-H1 improves myeloid dendritic cell–mediated antitumor immunity. Nat Med. 2003;9(5):562–7.
394. Scarlett UK, Rutkowski MR, Rauwerdink AM, Fields J, Escovar-Fadul X, Baird J, et al. Ovarian cancer progression is controlled by phenotypic changes in dendritic cells. J Exp Med. 2012;209(3):495–506.
395. Wertel I, Polak G, Bednarek W, Barczyński B, Roliński J, Kotarski J. Dendritic cell subsets in the peritoneal fluid and peripheral blood of women suffering from ovarian cancer. Cytom Part B Clin Cytom. 2008;74(4):251–8.
396. Wei S, Kryczek I, Zou L, Daniel B, Cheng P, Mottram P, et al. Plasmacytoid dendritic cells induce CD8+ regulatory T cells in human ovarian carcinoma. Cancer Res. 2005;65(12):5020–6.
397. Serafini P, Borrello I, Bronte V. Myeloid suppressor cells in cancer: recruitment, phenotype, properties, and mechanisms of immune suppression. Semin Cancer Biol. 2006;16(1):53–65.
398. Bunt SK, Yang L, Sinha P, Clements VK, Leips J, Ostrand-Rosenberg S. Reduced inflammation in the tumor microenvironment delays the accumulation of myeloid-derived suppressor cells and limits tumor progression. Cancer Res. 2007;67(20):10019–26.
399. Sinha P, Clements VK, Ostrand-Rosenberg S. Interleukin-13–regulated M2 macrophages in combination with myeloid suppressor cells block immune surveillance against metastasis. Cancer Res. 2005;65(24):11743–51.
400. Yanagisawa K, Exley MA, Jiang X, Ohkochi N, Taniguchi M, Seino K. Hyporesponsiveness to natural killer T-cell ligand alpha-galactosylceramide in cancer-bearing state mediated by CD11b+ Gr-1+ cells producing nitric oxide. Cancer Res. 2006;66:11441–6.
401. Hart KM, Byrne KT, Molloy MJ, Usherwood EM, Berwin B. IL-10 immunomodulation of myeloid cells regulates a murine model of ovarian cancer. Front Immunol. 2011;2.
402. Peter S, Bak G, Hart K, Berwin B. Ovarian tumor-induced T cell suppression is alleviated by vascular leukocyte depletion. Transl Oncol. 2009;2(4):291–9.
403. Obermajer N, Muthuswamy R, Odunsi K, Edwards RP, Kalinski P. PGE2-induced CXCL12 production and CXCR4 expression controls the accumulation of human MDSCs in ovarian cancer environment. Cancer Res. 2011;71(24):7463–70.
404. Lokshin AE, Winans M, Landsittel D, Marrangoni AM, Velikokhatnaya L, Modugno F, et al. Circulating IL-8 and anti-IL-8 autoantibody in patients with ovarian cancer. Gynecol Oncol. 2006;102(2):244–51.
405. Lambeck AJ, Crijns AP, Leffers N, Sluiter WJ, Klaske A, Braid M, et al. Serum cytokine profiling as a diagnostic and prognostic tool in ovarian cancer: a potential role for interleukin 7. Clin Cancer Res. 2007;13(8):2385–91.
406. Bertenshaw GP, Yip P, Seshaiah P, Zhao J, Chen T-H, Wiggins WS, et al. Multianalyte profiling of serum antigens and autoimmune and infectious disease molecules to identify biomarkers dysregulated in epithelial ovarian cancer. Cancer Epidemiol Biomark Prev. 2008;17(10):2872–81.
407. Scambia G, Testa U, Panici PB, Foti E, Martucci R, Gadducci A, et al. Prognostic significance of interleukin 6 serum levels in patients with ovarian cancer. Br J Cancer. 1995;71(2):354.
408. Mustea A, Pirvulescu C, Könsgen D, Braicu E, Yuan S, Sun P, et al. Decreased IL-1 RA concentration in ascites is associated with a significant improvement in overall survival in ovarian cancer. Cytokine. 2008;42(1):77–84.
409. Hefler LA, Zeillinger R, Grimm C, Sood AK, Cheng WF, Gadducci A, et al. Preoperative serum vascular endothelial growth factor as a prognostic parameter in ovarian cancer. Gynecol Oncol. 2006;103(2):512–7.
410. Bamias A, Tsiatas M, Kafantari E, Liakou C, Rodolakis A, Voulgaris Z, et al. Significant differences of lymphocytes isolated from ascites of patients with ovarian cancer compared to blood and tumor lymphocytes. Association of $CD3^+$ $CD56^+$ cells with platinum resistance. Gynecol Oncol. 2007;106(1):75–81.
411. Jaszczynska-Nowinka K, Markowska A. New cytokine: stromal derived factor-1. Eur J Gynaecol Oncol. 2008;30(2):124–7.
412. Dehaghani AS, Shahriary K, Kashef MA, Naeimi S, Fattahi MJ, Mojtahedi Z, et al. Interleukin-18 gene promoter and serum level in women with ovarian cancer. Mol Biol Rep. 2009;36(8):2393–7.
413. Bozkurt N, Yuce K, Basaran M, Gariboglu S, Kose F, Ayhan A. Correlation of serum and ascitic IL-12 levels with second-look laparotomy results and disease progression in advanced epithelial ovarian cancer patients. Int J Gynecol Cancer. 2006;16(1):83–6.
414. Kusuda T, Shigemasa K, Arihiro K, Fujii T, Nagai N, Ohama K. Relative expression levels of Th1 and Th2 cytokine mRNA are independent prognostic factors in patients with ovarian cancer. Oncol Rep. 2005;13(6):1153–8.
415. Marth C, Fiegl H, Zeimet AG, Müller-Holzner E, Deibl M, Doppler W, et al. Interferon-γ expression is an independent prognostic factor in ovarian cancer. Am J Obstet Gynecol. 2004;191(5):1598–605.
416. Chu CS, Kim SH, June CH, Coukos G. Immunotherapy opportunities in ovarian cancer. Expert review of anticancer therapy, Expert Rev Anticancer Ther. 2008;8(2):243–57.

417. Chang K, Pastan I. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. Proc Natl Acad Sci. 1996;93(1):136–40.
418. Coliva A, Zacchetti A, Luison E, Tomassetti A, Seregni E, Bombardieri E, et al. 90Y Labeling of monoclonal antibody MOv18 and preclinical validation for radioimmunotherapy of human ovarian carcinomas. Cancer Immunol Immunother. 2005;54(12):1200–13.
419. Disis ML, Gooley TA, Rinn K, Davis D, Piepkorn M, Cheever MA, et al. Generation of T-cell immunity to the HER-2/neu protein after active immunization with HER-2/neu peptide–based vaccines. J Clin Oncol. 2002;20(11):2624–32.
420. Disis ML, Goodell V, Schiffman K, Knutson KL. Humoral epitope-spreading following immunization with a HER-2/neu peptide based vaccine in cancer patients. J Clin Immunol. 2004;24(5): 571–8.
421. Kenemans P. CA 125 and OA 3 as target antigens for immunodiagnosis and immunotherapy in ovarian cancer. Eur J Obstet Gynecol Reprod Biol. 1990;36(3):221–8.
422. Odunsi K, Jungbluth AA, Stockert E, Qian F, Gnjatic S, Tammela J, et al. NY-ESO-1 and LAGE-1 cancer-testis antigens are potential targets for immunotherapy in epithelial ovarian cancer. Cancer Res. 2003;63(18):6076–83.
423. Resenblum MG, Verschraegen CF, Murray JL, Kudelka AP, Gano J, Cheung L, et al. Phase I study of 90Y-labelled B72.3 intraperitoneal administration in patients with ovarian cancer: effect of dose and EDTA coadministration on pharmacokinetics and toxicity. Clin Cancer Res. 1999;5:953–61.
424. Sandmaier BM, Oparin DV, Holmberg LA, Reddish MA, MacLean GD, Longenecker BM. Evidence of a cellular immune response against sialyl-Tn in breast and ovarian cancer patients after high-dose chemotherapy, stem cell rescue, and immunization with Theratope STn-KLH cancer vaccine. J Immunother. 1999;22(1):54–66.
425. Vlad AM, Kettel JC, Alajez NM, Carlos CA, Finn OJ. MUC1 immunobiology: from discovery to clinical applications. Adv Immunol. 2004;82:249–94.
426. Wang T, Niu G, Kortylewski M, Burdelya L, Shain K, Zhang S, et al. Regulation of the innate and adaptive immune responses by Stat-3 signaling in tumor cells. Nat Med. 2004;10(1):48–54.
427. Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS, et al. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. Nature. 1993;362(6423):841–4.
428. Mabuchi S, Kawase C, Altomare DA, Morishige K, Hayashi M, Sawada K, et al. Vascular endothelial growth factor is a promising therapeutic target for the treatment of clear cell carcinoma of the ovary. Mol Cancer Ther. 2010;9(8):2411–22.
429. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science. 2005;307(5706):58–62.
430. Burger RA, Sill MW, Monk BJ, Greer BE, Sorosky JI. Phase II trial of bevacizumab in persistent or recurrent epithelial ovarian cancer or primary peritoneal cancer: a Gynecologic Oncology Group Study. J Clin Oncol. 2007;25(33):5165–71.
431. Cannistra SA, Matulonis UA, Penson RT, Hambleton J, Dupont J, Mackey H, et al. Phase II study of bevacizumab in patients with platinum-resistant ovarian cancer or peritoneal serous cancer. J Clin Oncol. 2007;25(33):5180–6.
432. Chura JC, Van Iseghem K, Downs Jr LS, Carson LF, Judson PL. Bevacizumab plus cyclophosphamide in heavily pretreated patients with recurrent ovarian cancer. Gynecol Oncol. 2007;107(2):326–30.
433. Garcia AA, Hirte H, Fleming G, Yang D, Tsao-Wei DD, Roman L, et al. Phase II clinical trial of bevacizumab and low-dose metronomic oral cyclophosphamide in recurrent ovarian cancer: a trial of the California, Chicago, and Princess Margaret Hospital phase II consortia. J Clin Oncol. 2008;26(1):76–82.
434. McGonigle KF, Muntz HG, Vuky J, Paley PJ, Veljovich DS, Greer BE, et al. Combined weekly topotecan and biweekly bevacizumab in women with platinum-resistant ovarian, peritoneal, or fallopian tube cancer. Cancer. 2011;117(16):3731–40.
435. Perren TJ, Swart AM, Pfisterer J, Ledermann JA, Pujade-Lauraine E, Kristensen G, et al. A phase 3 trial of bevacizumab in ovarian cancer. N Engl J Med. 2011;365(26):2484–96.
436. Burger RA, Brady MF, Bookman MA, Fleming GF, Monk BJ, Huang H, et al. Incorporation of bevacizumab in the primary treatment of ovarian cancer. N Engl J Med. 2011;365(26):2473–83.
437. Stark D, Nankivell M, Pujade-Lauraine E, Kristensen G, Elit L, Stockler M, et al. Standard chemotherapy with or without bevacizumab in advanced ovarian cancer: quality-of-life outcomes from the International Collaboration on Ovarian Neoplasms (ICON7) phase 3 randomised trial. Lancet Oncol. 2013;14(3):236–43.
438. Aghajanian C, Blank SV, Goff BA, Judson PL, Teneriello MG, Husain A, et al. OCEANS: a randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer. J Clin Oncol. 2012;30(17):2039–45.
439. Ranpura V, Hapani S, Wu S. Treatment-related mortality with bevacizumab in cancer patients: a meta-analysis. JAMA. 2011;305(5):487–94.
440. Ayantunde A, Parsons S. Pattern and prognostic factors in patients with malignant ascites: a retrospective study. Ann Oncol. 2007;18(5):945–9.
441. Schmitt M, Schmitt A, Reinhardt P, Thess B, Manfras B, Lindhofer H, et al. Opsonization with a trifunctional bispecific (alphaCD3 x alphaEpCAM) antibody results in efficient lysis in vitro and in vivo of EpCAM positive tumor cells by cytotoxic T lymphocytes. Int J Oncol. 2004;25:841–8.
442. Ruf P, Gires O, Jäger M, Fellinger K, Atz J, Lindhofer H. Characterisation of the new

EpCAM-specific antibody HO-3: implications for trifunctional antibody immunotherapy of cancer. Br J Cancer. 2007;97(3):315–21.
443. Zeidler R, Mysliwietz J, Csanady M, Walz A, Ziegler I, Schmitt B, et al. The Fc-region of a new class of intact bispecific antibody mediates activation of accessory cells and NK cells and induces direct phagocytosis of tumour cells. Br J Cancer. 2000;83(2):261.
444. Zeidler R, Reisbach G, Wollenberg B, Lang S, Chaubal S, Schmitt B, et al. Simultaneous activation of T cells and accessory cells by a new class of intact bispecific antibody results in efficient tumor cell killing. J Immunol. 1999;163(3):1246–52.
445. Balzar M, Winter M, De Boer C, Litvinov S. The biology of the 17–1A antigen (Ep-CAM). J Mol Med. 1999;77(10):699–712.
446. Litvinov SV, Balzar M, Winter MJ, Bakker HA, Briaire-de Bruijn IH, Prins F, et al. Epithelial cell adhesion molecule (Ep-CAM) modulates cell–cell interactions mediated by classic cadherins. J Cell Biol. 1997;139(5):1337–48.
447. Bokemeyer C, Heiss M, Gamperl H, Linke R, Schulze E, Friccius-Quecke H, et al. Safety of catumaxomab: cytokine-release-related symptoms as a possible predictive factor for efficacy in a pivotal phase II/III trial in malignant ascites. ASCO Ann Meet Proc. 2009;27:3036.
448. Heiss MM, Murawa P, Koralewski P, Kutarska E, Kolesnik OO, Ivanchenko VV, et al. The trifunctional antibody catumaxomab for the treatment of malignant ascites due to epithelial cancer: results of a prospective randomized phase II/III trial. Int J Cancer. 2010;127(9):2209–21.
449. Jäger M, Schoberth A, Ruf P, Hess J, Hennig M, Schmalfeldt B, et al. Immunomonitoring results of a phase II/III study of malignant ascites patients treated with the trifunctional antibody catumaxomab (anti-EpCAM× anti-CD3). Cancer Res. 2012;72(1):24–32.
450. Ott MG, Marmé F, Moldenhauer G, Lindhofer H, Hennig M, Spannagl R, et al. Humoral response to catumaxomab correlates with clinical outcome: results of the pivotal phase II/III study in patients with malignant ascites. Int J Cancer. 2012;130(9):2195–203.
451. Schmidt-Rimpler C SE, Seimetz D. Clinical development of the trifunctional antibody catumaxomab in malignant ascites. International symposium on targeted anticancer therapies. International symposium on targeted anticancer therapies. 7–9 Mar 2011;Paris, abstract no. 39.
452. Gordon MS, Matei D, Aghajanian C, Matulonis UA, Brewer M, Fleming GF, et al. Clinical activity of pertuzumab (rhuMAb 2C4), a HER dimerization inhibitor, in advanced ovarian cancer: potential predictive relationship with tumor HER2 activation status. J Clin Oncol. 2006;24(26):4324–32.
453. Noujaim AA, Schultes BC, Baum RP, Madiyalakan R. Induction of CA125-specific B and T cell responses in patients injected with MAb-B43.13-evidence for antibody-mediated antigen-processing and presentation of CA125 in vivo. Cancer Biother Radiopharm. 2001;16(3):187–203.
454. Ehlen T, Hoskins P, Miller D, Whiteside T, Nicodemus C, Schultes B, et al. A pilot phase 2 study of oregovomab murine monoclonal antibody to CA125 as an immunotherapeutic agent for recurrent ovarian cancer. Int J Gynecol Cancer. 2005;15(6):1023–34.
455. Berek JS, Taylor PT, Nicodemus CF. CA125 velocity at relapse is a highly significant predictor of survival post relapse: results of a 5-year follow-up survey to a randomized placebo-controlled study of maintenance oregovomab immunotherapy in advanced ovarian cancer. J Immunother. 2008;31(2):207–14.
456. Berek J, Taylor P, McGuire W, Smith LM, Schultes B, Nicodemus CF. Oregovomab maintenance monoimmunotherapy does not improve outcomes in advanced ovarian cancer. J Clin Oncol. 2009;27(3):418–25.
457. Reinartz S, Köhler S, Schlebusch H, Krista K, Giffels P, Renke K, et al. Vaccination of patients with advanced ovarian carcinoma with the anti-idiotype ACA125 immunological response and survival (phase Ib/II). Clin Cancer Res. 2004;10(5):1580–7.
458. Sabbatini P, Berek J, Casada A. Abagovomab maintenance therapy in patients with epithelial ovarian cancer after complete response (CR) post-first-line chemotherapy (FLCT): preliminary results of the randomized, double-blind, placebo-controlled, multicenter MIMOSA trial. ASCO Meeting Abstracts; 2010.
459. Sabbatini P, Harter P, Scambia G, Sehouli J, Meier W, Wimberger P, et al. Abagovomab as maintenance therapy in patients with epithelial ovarian cancer: a phase III trial of the AGO OVAR, COGI, GINECO, and GEICO—the MIMOSA study. J Clin Oncol. 2013;31(12):1554–61.
460. Mendelsohn J, Baselga J. Status of epidermal growth factor receptor antagonists in the biology and treatment of cancer. J Clin Oncol. 2003;21(14):2787–99.
461. Bookman MA, Darcy KM, Clarke-Pearson D, Boothby RA, Horowitz IR. Evaluation of monoclonal humanized anti-HER2 antibody, trastuzumab, in patients with recurrent or refractory ovarian or primary peritoneal carcinoma with overexpression of HER2: a phase II trial of the Gynecologic Oncology Group. J Clin Oncol. 2003;21(2):283–90.
462. Agus DB, Akita RW, Fox WD, Lewis GD, Higgins B, Pisacane PI, et al. Targeting ligand-activated ErbB2 signaling inhibits breast and prostate tumor growth. Cancer Cell. 2002;2(2):127–37.

463. Franklin MC, Carey KD, Vajdos FF, Leahy DJ, de Vos AM, Sliwkowski MX. Insights into ErbB signaling from the structure of the ErbB2-pertuzumab complex. Cancer Cell. 2004;5(4):317–28.
464. Amler L, Makhija S, Januario T, Matulonis U, Strauss A, Dizon D, et al. HER pathway gene expression analysis in a phase II study of pertuzumab+ gemcitabine vs. gemcitabine+ placebo in patients with platinum-resistant epithelial ovarian cancer. J Clin Oncol. 2008;26:5552.
465. Parker N, Turk MJ, Westrick E, Lewis JD, Low PS, Leamon CP. Folate receptor expression in carcinomas and normal tissues determined by a quantitative radioligand binding assay. Anal Biochem. 2005;338(2):284–93.
466. Markman M, Blessing J, Rubin SC, Connor J, Hanjani P, Waggoner S. Phase II trial of weekly paclitaxel (80 mg/m^2) in platinum and paclitaxel-resistant ovarian and primary peritoneal cancers: an Gynecologic Oncology Group study. Gynecol Oncol. 2006;101(3):436–40.
467. Pujade-Lauraine E, Wagner U, Aavall-Lundqvist E, Gebski V, Heywood M, Vasey PA, et al. Pegylated liposomal doxorubicin and carboplatin compared with paclitaxel and carboplatin for patients with platinum-sensitive ovarian cancer in late relapse. J Clin Oncol. 2010;28(20):3323–9.
468. Armstrong DK, White AJ, Weil SC, Phillips M, Coleman RL. Farletuzumab (a monoclonal antibody against folate receptor alpha) in relapsed platinum-sensitive ovarian cancer. Gynecol Oncol. 2013;129(3):452–8.
469. Epstein LB, Shen JT, Abele JS, Reese CC. Sensitivity of human ovarian carcinoma cells to interferon and other antitumor agents as assessed by an in vitro semi-solid agar technique. Ann NY Acad Sci. 1980;350(1):228–44.
470. Einhorn N, Cantell K, Einhorn S, Strander H. Human leukocyte interferon therapy for advanced ovarian carcinoma. Am J Clin Oncol. 1982;5(2):167–72.
471. Alberts DS, Hannigan EV, Liu P-Y, Jiang C, Wilczynski S, Copeland L, et al. Randomized trial of adjuvant intraperitoneal alpha-interferon in stage III ovarian cancer patients who have no evidence of disease after primary surgery and chemotherapy: an intergroup study. Gynecol Oncol. 2006;100(1):133–8.
472. Berek JS, Hacker NF, Lichtenstein A, Jung T, Spina C, Knox RM, et al. Intraperitoneal recombinant α-interferon for "salvage" immunotherapy in stage III epithelial ovarian cancer: a Gynecologic Oncology Group Study. Cancer Res. 1985;45(9):4447–53.
473. Berek JS, Markman M, Blessing JA, Kucera PR, Nelson BE, Anderson B, et al. Intraperitoneal α-interferon alternating with cisplatin in residual ovarian carcinoma: a phase II Gynecologic Oncology Group study. Gynecol Oncol. 1999;74(1):48–52.
474. Bruzzone M, Rubagotti A, Gadducci A, Catsafados E, Foglia G, Brunetti I, et al. Intraperitoneal carboplatin with or without interferon-α in advanced ovarian cancer patients with minimal residual disease at second look: a prospective randomized trial of 111 patients. Gynecol Oncol. 1997;65(3):499–505.
475. Hall G, Brown J, Coleman R, Stead M, Metcalf K, Peel K, et al. Maintenance treatment with interferon for advanced ovarian cancer: results of the Northern and Yorkshire gynaecology group randomised phase III study. Br J Cancer. 2004;91(4):621–6.
476. Marth C, Windbichler G, Hausmaninger H, Petru E, Estermann K, Pelzer A, et al. Interferon-gamma in combination with carboplatin and paclitaxel as a safe and effective first-line treatment option for advanced ovarian cancer: results of a phase I/II study. Int J Gynecol Cancer. 2006;16(4):1522–8.
477. Willemse P, De Vries E, Mulder N, Aalders J, Bouma J, Sleijfer D. Intraperitoneal human recombinant interferon alpha-2b in minimal residual ovarian cancer. Eur J Cancer Clin Oncol. 1990;26(3):353–8.
478. Windbichler G, Hausmaninger H, Stummvoll W, Graf A, Kainz C, Lahodny J, et al. Interferon-gamma in the first-line therapy of ovarian cancer: a randomized phase III trial. Br J Cancer. 2000;82(6):1138.
479. Parkinson DR. Interleukin-2 in cancer therapy. Semin Oncol. 1988;15:10.
480. Yee C, Wallen H, Hunder N, Thompson JA, Byrd D, Reilly JZ, et al. Recent advances in the use of antigen-specific T cells for the treatment of cancer. Updat Cancer Ther. 2006;1(3):333–42.
481. Edwards R, Gooding W, D'Angelo G. A phase II trial of intraperitoneal interleukin-2 demonstrates extended survival in taxane platinum refractory ovarian cancer. Proc Am Soc Clin Oncol. 2010;59(2):293–301.
482. Freedman RS, Edwards CL, Kavanagh JJ, Kudelka AP, Katz RL, Carrasco CH, et al. Intraperitoneal adoptive immunotherapy of ovarian carcinoma with tumor-infiltrating lymphocytes and low-dose recombinant interleukin-2: a pilot trial. J Immunother. 1994;16(3):198–210.
483. Steinman RM. Dendritic cells and the control of immunity: enhancing the efficiency of antigen presentation. Mt Sinai J Med New York. 2001;68(3):160–6.
484. Steinman RM. The dendritic cell system and its role in immunogenicity. Ann Rev Immunol. 1991;9(1):271–96.
485. Gong J, Nikrui N, Chen D, Koido S, Wu Z, Tanaka Y, et al. Fusions of human ovarian carcinoma cells with autologous or allogeneic dendritic cells induce antitumor immunity. J Immunol. 2000;165(3):1705–11.
486. Koido S, Ohana M, Liu C, Nikrui N, Durfee J, Lerner A, et al. Dendritic cells fused with human

cancer cells: morphology, antigen expression, and T cell stimulation. Clin Immunol. 2004;113(3):261–9.
487. Hernando JJPT, Kübler K, Offergeld R, Schlebusch H, Bauknecht T. Vaccination with autologous tumour antigen-pulsed dendritic cells in advanced gynecological malignancies: clinical and immunological evaluation of a phase I trial. Cancer Immunol Immunother. 2002;51:45–52.
488. Brossart P, Wirths S, Stuhler G, Reichardt VL, Kanz L, Brugger W. Induction of cytotoxic T-lymphocyte responses in vivo after vaccinations with peptide-pulsed dendritic cells. Blood. 2000;96(9): 3102–8.
489. Loveland BE, Zhao A, White S, Gan H, Hamilton K, Xing P-X, et al. Mannan-MUC1–pulsed dendritic cell immunotherapy: a phase I trial in patients with adenocarcinoma. Clin Cancer Res. 2006;12(3): 869–77.
490. Hernando JJ, Park T-W, Fischer H-P, Zivanovic O, Braun M, Pölcher M, et al. Vaccination with dendritic cells transfected with mRNA-encoded folate-receptor-α for relapsed metastatic ovarian cancer. Lancet Oncol. 2007;8(5):451–4.
491. Yamada SD, Baldwin RL, Karlan BY. Ovarian carcinoma cell cultures are resistant to TGF-beta1-mediated growth inhibition despite expression of functional receptors. Gynecol Oncol. 1999;75(1): 72–7.
492. Huarte E, Cubillos-Ruiz JR, Nesbeth YC, Scarlett UK, Martinez DG, Buckanovich RJ, et al. Depletion of dendritic cells delays ovarian cancer progression by boosting antitumor immunity. Cancer Res. 2008;68(18):7684–91.
493. Coukos G, Conejo-Garcia JR, Roden RB, Wu TC. Immunotherapy for gynaecological malignancies. Expert Opin Biol Ther. 2005;5(9):1193–210.

Immunology and Immunotherapy of Breast Cancer

23

John Stagg and Sherene Loi

Contents

J. Stagg, PhD
Centre de Recherche du Centre Hospitalier de l'Université de Montréal, 900 St-Denis Street Montreal, Montreal, QC H2X 0A9, Canada
e-mail: john.stagg@umontreal.ca

S. Loi, MBBS (Hons), FRACP, PhD (✉)
Division of Cancer Medicine, Group Leader, Translational Breast Cancer Genomics Lab, Cancer Therapeutics Program, Division of Research, Peter MacCallum Cancer Centre, East Melbourne, VIC 3002, Australia
e-mail: sherene.loi@petermac.org

23.1 Introduction

Breast cancer has not traditionally been considered an "immunogenic" tumor type. While immunosurveillance mechanisms do not seem to have an effect on the growth of primary breast tumors (transplant patients receiving immunosuppressants do not have an increased incidence of breast cancer), immunosuppression does seem to adversely affect metastases growth [1].

Immune cells can exhibit pro- or antitumor effects and affect therapeutic resistance. Avoiding "immune destruction" is now considered an emerging hallmark of cancer [2]. It is now generally accepted that lymphocytic infiltrates, especially consisting of CD8⁺ T cells, are associated with a better cancer prognosis [3]. The analysis of location, density, and type of tumor-infiltrating immune cells – termed the "immune contexture" – offers the hope of prognostic information and the identification of patients most likely to respond to immunotherapies [4]. As well as this, standard therapies in breast cancer, for example, anthracyclines and trastuzumab, have been shown to modulate immunity as part of their

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_23, © Springer-Verlag Berlin Heidelberg 2015

mechanism of action, though not conventionally considered "immunotherapy." In this review, the role of different immune cell populations in the progression of breast cancer and how these could be harnessed or inhibited in the treatment of breast cancer would be discussed.

23.2 CD8$^+$ T Cells

Over the past recent years, it has become increasingly evident that tumor-infiltrating lymphocytes (TIL) can control the clinical progression of epithelial cancers [5]. In breast cancer, two large series in newly diagnosed or early-stage breast cancer reported a significant correlation between increased lymphocytic infiltration and better clinical outcomes. Denkert et al. [6] analyzed tumor-infiltrating lymphocytes (TILs: using hematoxylin/eosin sections and immunohistochemistry [IHC]) of pretherapeutic core biopsies from over 1,000 breast cancer patients. Mahmoud et al. [7] analyzed tumor-infiltrating CD8$^+$ T cells (by IHC) in 1,334 breast cancer patients. In both studies, high lymphocytic infiltrates or CD8$^+$ T cell counts were associated with improved patient outcome, independent of standard prognostic and predictive factors. In 2009 high risk, newly diagnosed breast cancer patients, TILs were also evaluated using the same method as Denkert et al. but this time on full-face H&E sections taken from surgical removal of the primary tumor [8]. In this study it was found that the prognostic benefit of TILs was largely restricted to the triple-negative breast cancer subtype (TNBC: negative for expression of estrogen receptor [ER], progesterone receptor [PR], and Human epidermal growth factor receptor 2 [HER2]). While TNBC patients usually have a worse, women with TNBC and high TILs had a numerically better disease-free outcome than the women with ER$^+$/HER2$^-$ negative tumors who usually have the best prognoses. These data suggest that some patients have preexisting antitumor immunity at diagnosis and this is associated with improved long-term clinical outcomes. The reasons why some breast cancers are associated with TILs and others are not are unclear.

Also in support of a role for tumor-infiltrating CD8$^+$ T cells in controlling breast cancer progression, unsupervised gene expression profiling of breast cancer-associated stroma has revealed an immune gene signature enriched for CD8$^+$ T cell genes associated with good prognosis [9]. Interestingly, Mahmoud et al. [7] reported a positive correlation between CD8$^+$ T cells and high histological grade, younger patient age, and negative estrogen receptor (ER) status. Two other studies found a similar correlation between intratumoral CD8$^+$ T cell counts and negative ER status [10, 11]. The underlying mechanisms for this observation are unclear (and discussed below).

23.3 CD4$^+$ and FOXP3$^+$ T Cells

In contrast to intratumoral CD8$^+$ T cells, analysis of CD4$^+$ T cells on clinical outcomes in breast cancer has resulted in inconsistent results. In general, the presence of CD4$^+$ T cells in tumors has been associated with worse prognosis. For instance, IHC analysis of tissue microarrays derived from 179 treatment-naïve breast tumors revealed that high levels of CD4$^+$ T cells and macrophages were correlated with reduced overall survival (OS). In contrast, high levels of CD8$^+$ T cells combined with low levels of CD4$^+$ T cells were correlated with improved OS [12].

The association between CD4$^+$ T cell infiltrates and poor prognosis is generally attributed to CD4$^+$ FOXP3$^+$ T regulatory cells (Tregs). Tregs were first described as a population of T cells capable of suppressing immune responses in mice and were originally defined by the surface markers CD4 and CD25 [13]. Further investigation revealed that Tregs express and functionally depend on the transcription factor FOXP3 [14–16]. Given its essential role in Treg development, FOXP3 has become a popular marker of Tregs in cancer studies. Intriguingly, studies of the prognostic value of FOXP3$^+$ T cells in cancer patients have led to discrepant findings. Tissue microarray analysis of FOXP3$^+$ cells in 1,445 cases of primary invasive breast cancer revealed that the number of FOXP3$^+$ cells was associated with a worse outcome, but was not an independent

prognostic factor in multivariate analysis [17]. On the other hand, Liu et al. [18] reported that intratumoral FOXP3$^+$ cells was an independent predictor of poor prognosis in 1,270 breast cancer cases. Other studies have assessed the impact of FOXP3$^+$ cells on neoadjuvant breast cancer chemotherapy. One study reported that high infiltration of FOXP3$^+$ cells after neoadjuvant chemotherapy was an unfavorable and independent predictor of relapse-free survival (RFS) and overall survival (OS) [19]. Likewise, Ladoire et al. [20] reported that a high CD8/FOXP3 ratio was an independent predictive factor of RFS and OS in HER2-overexpressing breast cancer following neoadjuvant chemotherapy. In contrast, Oda et al. [21] reported that a high level of FOXP3$^+$ cells before neoadjuvant chemotherapy was an independent predictor of pathological complete responses. Others have also reported a positive correlation between high levels of FOXP3$^+$ T cells and good prognosis [22, 23]. Taken together, the role of FOXP3$^+$ cells in breast tumors still remains unclear and warrants further investigation.

23.4 CD4$^+$ Follicular Helper T Cells

Interestingly, a recent publication reported a favorable role for CD4$^+$ T cells [24]. For the first time, CD4$^+$ follicular helper T (Tfh) cells were found in tumor-infiltrating T cell populations, and their presence was associated with better prognosis. Accordingly, human breast tumors with high TILs were found to have higher frequency of CXCL13-producing Tfh cells. Notably, Tfh cells were found associated with tertiary lymphoid structures. Tertiary lymphoid structures (TLS) had been previously identified in lung and colorectal cancers, and their presence linked with better prognosis. It is now clear that Tfh cells are an important constituent of TLS in human breast tumors.

23.5 B Cells in BC

Intratumoral B cells have been associated with a favorable prognosis in breast cancer patients. An early gene expression study of 200 consecutive lymph node-negative cases reported a B cell metagene primarily formed by immunoglobulin heavy- and light-chain genes that was associated with metastasis-free survival in highly proliferating breast tumors [25]. Interestingly, immunoglobulin κ C (IGKC) as a single marker has also been shown to have similar predictive and prognostic value compared to the entire B cell metagene [26]. Building up of these findings, Mahmoud et al. [27] recently analyzed the density and localization of B cells in 1,470 breast cancer cases using IHC. Consistent with previous studies, increased B cell infiltration was associated with improved survival. Notably, B cell infiltration correlated with hormone receptor negativity and basal phenotype.

23.6 Macrophages and MDSC in BC

Macrophage infiltrates in breast cancer often correlate with a worse clinical outcome. Tumor-associated macrophages have been associated with suppressed antitumor CD8$^+$ T cells, increased angiogenesis, and increased metastasis. The presence of CD68$^+$ macrophages is generally inversely correlated with CD8$^+$ T infiltrates in human breast tumors [12]. Interestingly, tumor-associated macrophages were found significantly increased after chemotherapy with paclitaxel and in patients who had received neoadjuvant chemotherapy compared to surgery alone. Using transgenic mouse models of breast cancer, Coussens and colleagues also demonstrated that tumor-associated macrophages can directly promote breast cancer metastasis, via IL-4 producing CD4$^+$ T cells [28]. Other myeloid cells composed of incompletely differentiated cells, termed myeloid-derived suppressor cells (MDSC), have also been shown to enhance breast tumor growth in mice [29]. Human equivalents of MDSC have been identified [30]. Recently, Sceneay et al. [31] demonstrated that tumor hypoxia, via MDSC recruitment, can alter the lung microenvironment to make them more permissive for metastasis, thereby driving the formation of a pre-metastatic niche. Furthermore, increase in circulating MDSC in human cancer has been associated with stage 4 disease [32].

23.7 Immune Infiltrates, Gene Signatures, and BC Subtypes

The correlation between lymphocytic infiltrates and clinical outcomes in breast cancer varies across molecular subtypes. In 2011, DeNardo et al. [12] performed a meta-analysis of *CD68* and *CD8* gene expression in 4,000 breast cancer cases and reported that a CD68high/CD8low immune gene signature correlated with reduced OS for basal or $HER2^+$ breast cancer subtypes, but not for luminal breast cancers. Similarly, a metagene of STAT1 signaling – a surrogate of interferon response – was associated with better outcome in triple-negative breast cancer (TNBC) and $HER2^+$ breast cancers, but not in luminal cases [33, 34]. Another independent group identified an immune response prognostic gene module in ER^- , but not ER^+ breast cancers [11].

Among ER^- breast cancers, accumulating evidence suggests that basal or TNBC breast cancers are particularly associated with lymphocytic infiltration. In 2012, Liu et al. [35] performed a large-scale IHC analysis of CD8 expression on 3,400 breast cancer cases representing different subtypes and reported that only core basal TNBC demonstrated a significant correlation between intratumoral CD8 staining and favorable prognosis.

A B cell metagene has also been associated with good outcome in TNBC. In a gene expression analysis of 579 TNBC, Rody et al. [36] revealed that a ratio of high B cell and low IL-8 metagenes was identified in 32 % of TNBC patients with good prognosis. Taken together, these studies suggest that clinical outcomes in ER^- breast cancers, especially TNBC, are particularly influenced by tumor immune responses.

Why immune infiltration appears more relevant for breast cancers which are ER negative or overexpress HER2 as opposed to other subtypes is unknown. We speculate that it could be due to the poorly differentiated nature and high genomic instability of these subtypes. In TNBC, the intrinsic nature of the tumor cells may help explain its propensity for inflammatory responses. For instance, signaling pathways able to downregulate ER and HER2 expression may be associated with increased pro-inflammatory activity. This is supported by the identification of lactoferrin as a repressor of hormone receptors and HER2 expression in breast cancer cells [37]. Lactoferrin concomitantly induces the production of cytokines and chemokines, including MIP-1, MIP-2, IL-10, and IL-4. Increased immune infiltration in TNBC may also be associated with increased genetic instability due to p53 loss of function and BRCA1/2 disruption, which are common features of this molecular subtype. In line with this model, a recent study in high-grade serous ovarian cancer revealed an important positive correlation between BRCA1 disruptions and high levels of TILs [38].

Among TAA, cancer-testis (CT) antigens represent potential antitumor antigens since they are not shared with normal somatic cells. Interestingly, analysis of CT antigens in human breast cancer revealed higher expression in ER^- *vs.* ER^+ breast cancers and co-expression with basal cell markers [38]. Similar findings were reported by Curigliano et al. [39]. Consistent with these two studies, IHC analysis of eight CT antigens revealed significantly more frequent expression in ER^- *vs.* ER^+ human breast cancers [40]. In a study of genes differently expressed in TNBC, Karn et al. [41] further reported that among genes showing no correlation with known markers of TNBC, the most prominent group of genes encoded for CT antigens. Taken together, these studies suggest that CT antigen expression is a common feature of TNBC.

23.8 Effect of Chemotherapy on Tumor Immunity

In addition to being involved in the natural progression of cancer, immunity can affect the activity of various anticancer agents. Recent evidence suggests that some chemotherapeutic drugs, such as anthracyclines and oxaliplatin, rely on the induction of anticancer immune responses. In mouse models of cancer, chemotherapy with anthracyclines or oxaliplatin requires priming of IFN-γ-producing $CD8^+$ T cells for optimal treatment response. In cancer patients, high levels of interferon (IFN)-gamma and $CD8^+$ T cells are

predictive of a good clinical response to anthracyclines. The immune-stimulating property of anthracyclines and oxaliplatin was shown to require preapoptotic translocation of calreticulin (CRT) on the tumor cell surface, post-apoptotic release of the chromatin-binding protein high-mobility group B1 (HMGB1), and extracellular release of adenosine triphosphate (ATP). CRT, HMGB1, and ATP act in concert to promote tumor antigen presentation by dendritic cells (DCs) via activation of CD91, TLR-4, and purinergic P2X7 receptors, respectively. It was recently demonstrated that chemotherapy-induced autophagy is essential for the release of ATP and subsequent anticancer immunity. Accordingly, autophagy-deficient tumor cells are unable to release ATP in response to anthracyclines or oxaliplatin and fail to elicit $CD8^+$ anticancer T cells. This suggests that patients with autophagy-deficient tumor cells might benefit from therapeutic strategies designed to compensate this process in order to trigger immunogenic signaling.

Extracellular ATP appears as a central activator of chemotherapy-induced antitumor immunity. However, tumors can overexpress ecto-nucleotidases, which catabolize the hydrolysis of extracellular ATP into adenosine. Expression of these ecto-nucleotidases, such as CD39 and CD73, has two major consequences: decreasing the concentration of pro-inflammatory ATP and increasing the concentration of immunosuppressive adenosine. Notably, while the catabolism of ATP into adenosine monophosphate (AMP) is reversible, catalyzed by membrane-bound kinases, the conversion of AMP into adenosine by CD73 is irreversible. This places CD73 at a crucial checkpoint in the conversion of immune-activating ATP into immunosuppressive adenosine. Several groups have now demonstrated the importance of CD73 in the suppression of anticancer immunity. In breast cancer, CD73 is overexpressed in response to loss of ER expression [42]. Studies undertaken by the authors and confirmed by others revealed that targeted blockade of CD73 can effectively reduce tumorigenesis and metastasis of breast cancer in mice [43–47]. Furthermore, the authors recently demonstrated that CD73 expression is significantly associated with worse prognosis in TNBC patients and that the CD73-adenosinergic pathway promotes chemoresistance to anthracycline in mice [48]. Targeting CD73 therefore represents a novel approach with the potential to enhance the efficacy of chemotherapy for treatment of breast cancer.

23.9 Targeted Therapies (Including Trastuzumab and Tyrosine Kinase Inhibitors)

Antitumor immunity also plays a major role in the efficacy of targeted therapies. Tumor-targeted monoclonal antibodies (mAbs), such as trastuzumab, rely in part on immune-mediated killing [48]. While innate immune responses, in particular via Antibody-dependent cell-mediated cytotoxicity (ADCC), appear to be important for trastuzumab activity [49], recent studies suggest that trastuzumab also stimulates adaptive antitumor immunity. Two studies in mice showed that trastuzumab-like therapy requires adaptive CD8-dependent immune responses to mediate optimal activity [50, 51]. These studies support a model whereby trastuzumab activates MyD88-dependent Toll-like receptors – most likely via the release of high-mobility group box 1 (HMGB1) following ADCC – stimulates the release of type I interferons (IFNs), and primes adaptive IFN-γ-producing $CD8^+$ T cells. These studies raise the possibility that combination strategies may be used to capitalize on the adaptive tumor-specific immunity generated by anti-ErbB2 mAbs. Consistent with this notion, Stagg et al. [51] demonstrated that anti-PD-1 and anti-CD137 mAbs can each synergize with anti-ErbB2 mAb therapy. This data advocates that enhancement of T cell antitumor immunity should be evaluated in the clinical setting in combination with trastuzumab.

Data also exists suggesting that preexisting immunity could be important for trastuzumab efficacy. Evaluation of TILs in baseline primary tumor specimens before treatment found that those patients with high levels of TILs derived more benefit from trastuzumab added to their cytotoxic chemotherapy [52]. This data suggested

that though trastuzumab has been thought to act primarily through inhibition of cell signaling, it may also serve to relieve tumor-mediated immunosuppression through yet undefined mechanisms. This data also supports that some breast cancer subtypes may be more amenable to immunotherapeutic approaches.

23.10 Immunotherapy of Breast Cancer

23.10.1 MUC-1 Vaccines

A promising candidate antigen for breast cancer vaccination is the MUC-1 antigen. Both normal and cancerous breast cells express MUC-1. However, breast cancer cells often express an aberrantly glycosylated form of MUC-1 [53]. The presence of circulating antibodies against MUC-1 at the time of breast cancer diagnosis has been correlated with a favorable outcome [54]. In addition to stimulating humoral immune responses, aberrantly glycosylated MUC-1 can also stimulate $CD8^+$ T cells [55]. While early MUC-1 vaccines failed to elicit effective antitumor immune responses in clinical trials, a glycosylated MUC-1-derived glycopeptide covalently linked to a Toll-like receptor (TLR) agonist has been recently shown to elicit potent humoral and cellular immune responses [56], highlighting the importance of maintaining conformational elements of MUC-1 to achieve successful vaccination.

23.10.2 HER2 Vaccines

Several clinical trials have investigated the use of immunogenic peptides derived from the HER2 protein in order to induce therapeutic vaccination against $HER2^+$ breast cancer. Different trials were conducted with increasing doses of peptide (AE37 or E75 or GP2 peptide) and varying amounts of the immune adjuvant granulocyte-macrophage colony-stimulating factor (GM-CSF) injected intradermally. All three peptide vaccines have been well tolerated [57]. Interestingly, the combination of trastuzumab and HER2 vaccine has also been recently investigated [58]. In a phase I/II study, 22 patients with advanced metastatic $HER2^+$ breast cancer already receiving trastuzumab were vaccinated with six inoculations of a HER2 peptide-based vaccine. The study revealed that preexisting immunity specific for HER2 (possibly as a result of previous trastuzumab therapy) could be significantly augmented with vaccination.

23.10.3 MAGE-A3 Vaccines

Vaccines targeting CT antigens, such as MAGE-A3, are also being tested in clinical studies. In a phase II trial of non-small cell lung cancer (NSCLC), it was reported that patients whose tumors had been removed by surgery experienced 25 % fewer recurrences following vaccination against MAGE-A3. A phase III trial involving 2,300 NSCLC patients positive for MAGE-A3 antigen is currently underway [59]. The outcome of this phase III clinical trial could be decisive in the development of tumor vaccines targeting MAGE-A3 or other CT antigens in breast cancer.

23.10.4 Targeting Immune Checkpoints

While correlative studies suggest that antitumor immunity can control breast cancer progression and patient outcome, tumors persist despite being infiltrated with tumor-specific $CD8^+$ T cells. This apparent paradox is at least partly due to the exhausted nature of tumor-infiltrating T cells and the presence of immunosuppressive factors in the tumor microenvironment. One of the most important means of immune regulation is a process known as "T cell exhaustion," which results from chronic exposure to antigens and is characterized by the upregulation of inhibitory receptors. Blocking of one or several of these inhibitory receptors, also known as "immune checkpoints," with mAbs has been the mainstay of recent developments in cancer immunotherapy.

23.10.5 Anti-CTLA-4

The first immune checkpoint inhibitor to be tested in clinical trial was ipilimumab (Yervoy, Bristol-Myers Squibb), an anti-CTLA-4 mAb. CTLA-4 belongs to the immunoglobulin superfamily of receptors, which also includes PD-1, B and T lymphocyte attenuator (BTLA), T cell immunoglobulin- and mucin domain-containing protein 3 (TIM-3), and V-domain immunoglobulin suppressor of T cell activation (VISTA). In 2011, the US Food and Drug Administration approved the use of ipilimumab for treatment of unresectable or metastatic melanoma, either as initial therapy or after relapse.

The mechanism of action of ipilimumab includes enhanced antitumor function of effector T cells, increased ratio of $CD8^+$ T cells to $Foxp3^+$ T regulatory cells (Tregs), and interference with the suppressive function of Tregs [60]. CTLA-4 blockade has been shown to expand a subpopulation of tumor-infiltrating $CD4^+$ T cells that express high levels of ICOS and secrete IFN-γ [61]. These $CD4^+$ $ICOS^+$ T cells might play a role in the therapeutic activity of anti-CTLA-4 mAb therapy, as there frequency correlates with survival in treated melanoma patients. Nevertheless, it is still not clear whether the activity of ipilimumab is dependent on the blockade of CTLA-4-mediated negative regulatory signal in effector T cells or to its interference with Treg function. Another potential mechanism of action includes Antibody-dependent cell-mediated cytotoxicity (ADCC) of CTLA-4-expressing cells. The major drawback to anti-CTLA-4 mAb therapy is the generation of immune-related toxicities due to on-target effects. It has been reported that up to 25 % of patients treated with ipilimumab developed serious grade 3–4 adverse events [62], reflecting the importance of CTLA-4 in maintaining immune homeostasis. Unfortunately, toxicity is not always associated with therapeutic benefit. Thus, a major challenge in the use of anti-CTLA-4 mAb is to define favorable clinical settings that strike an optimum balance between tumor immunity and autoimmunity.

23.10.6 Anti-PD-1

PD-1 is another inhibitory co-receptor expressed on activated and exhausted T cells. Its ligand, PD-L1, is often found overexpressed in various types of cancer. Administration of mAbs blocking anti-PD-1/anti-PD-L1 enhances adaptive antitumor immune responses by preventing T cell exhaustion [63]. Anti-PD-1 mAb blocks interactions between PD-1 and its ligands, PD-L1 and PD-L2, whereas anti-PD-L1 mAb blocks interactions between PD-L1 and both PD-1 and CD80. PD-1 is expressed by activated $CD4^+$ and $CD8^+$ T cells, B cells, monocytes, and NKT cells. It has two ligands, PD-L1 and PD-L2, with distinct expression profiles. Expression of PD-L1 has been shown to be associated with poor prognosis in melanoma and hepatocellular carcinoma [64, 65]. Notably, cytotoxic chemotherapeutics such as paclitaxel, etoposide, and 5-fluorouracil have been shown to upregulate PD-L1 expression on breast cancer cells [66].

There are currently six agents blocking the PD-1/PD-L1 pathway in clinical evaluation: MDX-1106/BMS-936558/ONO-4538 (fully human IgG4 from BMS), CT-011 (humanized IgG1 from CureTech/Teva), MK-3475 (human IgG4 from Merck), MPDL3280A/RG7446 (from Genentech), BMS-936559 (fully humanized IgG4), and AMP-224 (a B7-DC/IgG1 fusion protein licensed to GSK) [www.clinicaltrials.gov]. Two phase I trials recently reported clinical responses with anti-PD-1 or anti-PD-L1 mAb in pretreated patients with diverse tumor types [67, 68]. Anti-PD-1 mAb therapy was associated with objective responses in 18 % of patients with NSCLC (14 of 76 patients), 28 % of patients with melanoma (26 of 94 patients), and 27 % of patients with renal cell cancer (9 of 33 patients) [68]. Anti-PD-L1 mAb therapy was associated with objective responses in 17 % of patients with melanoma (9 of 52), 12 % of patients with renal cell cancer (2 of 17), 10 % of patients with NSCLC (5 of 49), and 6 % of ovarian cancer patients (1 of 17) [67]. Notably, anti-PD-1 and anti-PD-L1 mAb therapy caused drug-related grade 3 or 4 adverse events in 14 and 9 % of patients, respectively. Strikingly, in the context of

anti-PD-1 mAb therapy, objective responses occurred only in PD-L1$^+$ tumors (36 % response rate) compared to no clinical responses in PD-L1-negative tumors.

Recent safety and efficacy data from the phase I/II study using another anti-PD-1 antibody called lambrolizumab have been published [69]. A total of 135 patients with advanced melanoma were treated in a dose escalation followed by cohort expansion. Common adverse events attributed to treatment were fatigue, rash, pruritus, and diarrhea, all of which were low grade, with hypothyroidism, transaminitis, and pneumonitis also occurring (all grades, but <10 %). The confirmed response rate across all dose cohorts (RECIST 1.1) was 38 % (95 % confidence interval [CI] 25–44), with the highest response rate seen in the highest dose (10 mg/kg every 2 weeks). Responses were durable, with 81 % of patients who had a response (42 of 52) still receiving treatment (median progression-free survival was longer than 7 months). The majority of responses were seen at the time of first imaging at 12 weeks. Some patients with stable disease at first imaging also showed objective responses with further time, these being durable as well. Biopsies of regressing lesions revealed infiltration by CD8$^+$ T lymphocytes, consistent with the mode of action of the drug.

Recently, Muenst et al. [70] investigated the prognostic value of PD-1 expression in human breast cancer TILs. Using IHC analysis of a tissue microarray of 660 breast cancer cases, the study revealed that the presence of PD-1 expression on breast cancer TILs was associated with significantly worse overall survival in luminal B and basal-like breast cancer, but not in luminal A or HER2$^+$ breast cancer.

PD-L1 expression using IHC has also been examined in breast cancer. Expression was found in the tumor but also in the surrounding TILs. Tumoral expression was associated with high histologic grade and ER negativity, whereas PD-L1 expression in TILs was associated with larger tumor size, HER2 positivity, and high histologic grade [71]. Expression at the mRNA level is higher in breast cancer subtypes that are ER-negative and HER2-amplified compared with ER-positive tumors (unpublished data). PD-L1 in TILs in breast cancer may also present an exhausted T cell response resulting from chronic antigen exposure, suggesting that TILs represent previous activation of antitumor immunity with subsequent suppression. This suppression may also be enhanced by the tumor.

23.10.7 Combination of Checkpoint Inhibitors

While inhibition of a single immune checkpoint can prolong the survival of cancer patients, an important question that remains is whether combinatorial checkpoint blockade can be synergistic in promoting anticancer activity. The first combination of immune checkpoint inhibitors to be tested in mice was the combination of anti-CTLA-4 and anti-PD-1 mAbs. Curran et al. [70] demonstrated that blockade of CTLA-4 and PD-1 in mice allows CD8$^+$ and CD4$^+$ T cells to survive in the tumor microenvironment, proliferate, and carry out effector function. More recently, combination of anti-CTLA-4 and PD-1 in metastatic melanoma showed impressive tumor regressions and durable responses providing proof-of-concept of this hypothesis.

More recently, TIM-3 has been identified as another important inhibitory receptor expressed by exhausted CD8$^+$ T cells. In mouse models of cancer, it was shown that the most dysfunctional tumor-infiltrating CD8$^+$ T cells actually co-express PD-1 and TIM-3 [72]. Based on these findings, a direct comparison of the therapeutic activity of anti-CTLA-4, anti-PD-1, and anti-TIM-3 mAbs was made in various mouse models of cancer [73]. It was observed that the combination of anti-PD-1 and anti-TIM-3 mAbs had the most potent anticancer effect against well-established experimental and carcinogen-induced tumors. From a molecular point of view, a recent paper by Kuchroo and colleagues identified Bat3 as a key regulator of TIM-3 activity on T lymphocytes [74]. Bat3, through its binding to the intracellular tail of TIM-3, prevents TIM-3-mediated cell death or exhaustion in T lymphocytes. Interestingly, the authors demonstrated that Bat3

is highly downregulated in TIM-3$^+$/PD-1$^+$ TILs and that this downmodulation is associated with a decreased cytotoxic potential as revealed by a reduced secretion of IFN-γ and TNF-α.

In addition to its inhibitory role on CD8$^+$ T cells, TIM-3 has also been reported as a key regulator of nucleic acid-mediated antitumor immunity. In a very recent paper, TIM-3 was shown to be upregulated on tumor-associated dendritic cells (TADCs) extracted from both mouse and human tumors [75]. The authors identified IL-10, VEGF-A, and arginase I as the main tumor-released immunosuppressive factors responsible for TIM-3 upregulation on TADCs. TIM-3 expression in TADCs was linked to an impaired nucleic acid-mediated innate immune response as revealed by a reduced secretion of cytokines such as IFN-β or IL-12. Accordingly, it was proven that anti-TIM-3 mAb therapy greatly enhanced the antitumor efficacy of nucleic acid-based adjuvants in a B16F10 mouse melanoma model and that this synergistic activity depended on IFN-β and IL-12 secretion. More importantly, using a CD11c DTR mouse strain (in which CD11c can be depleted upon diphtheria toxin administration), it was demonstrated that TIM-3 expression on TADCs (and not on CD8 T cells) was the main limit to the triggering of a nucleic acid-mediated antitumor immune response. From a mechanistic point of view, TIM-3 limited DC innate immune response in a HMGB1-dependent fashion, restraining the HMGB1-mediated transport of nucleic acid into endosome and thus limiting the activation of cytosolic sensors responsible for nucleic acid-mediated immune response. Finally, the authors extend the relevance of their study showing that TIM-3 mAb therapy strongly synergizes with standard chemotherapy in a subcutaneous colon tumor model, which reinforces the rational for combining "immunogenic cell death" inducing chemotherapeutic agents with immune checkpoint inhibitors for cancer therapy.

LAG-3 is another recently identified inhibitory receptor that acts to limit effector T cell function and augment the suppressive activity of Tregs. Woo et al. [76] recently revealed that PD-1 and LAG-3 are extensively co-expressed by tumor-infiltrating T cells in mice and that combined blockade of PD-1 and LAG-3 provokes potent synergistic antitumor immune responses against mouse models of cancer. These studies suggest that combined blockade of immune checkpoint inhibitors may represent a promising strategy for cancer immunotherapy.

23.10.8 Agonistic of TNF Receptor Superfamily

Members of the TNF receptor superfamily also play an important role as regulators of T cell function [77]. Activation of these co-stimulatory receptors may further enhance the generation of tumor-reactive T cells in the context of cancer therapy. Co-stimulatory receptors of the TNF receptor family are composed of OX40 (CD134), 4-1BB (CD137), CD27, CD30, and HVEM. When activated, each of these receptors can enhance cytokine production and T cell proliferation in response to TCR signaling. OX40 and CD137 activation are particularly effective at allowing activated T cells to survive and proliferate in the late phase of immune responses. The administration of agonistic mAbs against OX40 or CD137 has been shown to enhance tumor immunity and induce regression of established mouse models of cancer [78–80]. Taken together, the use of agonists to co-stimulatory receptors or antagonists to inhibitory receptors may provide efficient means to rescue or enhance the activity of tumor-reactive T cells.

23.10.9 Blocking the Immunosuppressors

Targeting immunosuppression by soluble mediators is another attractive approach for cancer immunotherapy. A plethora of immunosuppressive factors has been associated with tumorigenesis, including TGF-β, indoleamine 2,3-dioxygenase (IDO), arginase, prostaglandin-E2 (PGE2), and extracellular adenosine [3]. Determining which immunosuppressive factors are minimally required for maintaining tumor tolerance in a given patients population remains a great challenge. Recent studies in mouse models of cancer and clinical

correlative studies suggest that IL-23 may be a key cytokine governing the balance between pro- and anti-tumorigenic immune responses [81–83]. In support of this model, mice genetically deficient in IL-23 are significantly protected against a wide range of malignancies, and mice treated with a blocking antibody against IL-23 have a decreased risk of tumor formation and a faster elimination of transplanted tumor cells [82].

Enzymes that metabolize L-arginine (such as arginase I), the tryptophan-catabolizing enzyme IDO, and enzymes that regulate extracellular adenosine levels (such as the ecto-nucleotidases CD39 and CD73) also significantly contribute to the inhibition of anticancer immune responses [43, 84]. CD73 is at a critical checkpoint in the conversion of immune-activating ATP into immunosuppressive adenosine, making it a potential therapeutic target. Tumors often overexpress CD73 as a consequence of tissue hypoxia or, in the case of breast cancer, consequent to the loss of ER expression. CD73 expression on tumor cells and host cells (including FOXP3$^+$ Tregs) is a significant contributor to immune escape. Given the promising results of anti-CD73 targeted therapy in mice [43–45] and the prognostic importance of CD73 in TNBC [48], future studies aimed at translating this approach into the clinic are warranted. Inhibitors of IDO1 are also currently in phase I/II testing in metastatic breast cancer in combination with docetaxel (clinicaltrials.gov identifier NCT 01792050)

23.10.10 Adoptive T Cell Therapy

Another promising immunotherapy consists of the adoptive transfer of tumor-specific T cells. Work pioneered by the Rosenberg lab established that autologous tumor-infiltrating lymphocytes (TIL) can be isolated from primary tumors, expanded *ex vivo* and adoptively transferred back to patients following lymphodepletion. For metastatic melanoma patients capable of withstanding treatment, TIL therapy combined to IL-2 is the best available option, with response rates ranging from 49 to 72 % [85]. TIL therapy, however, has not proven to be readily applicable in most clinical settings, and, to date, only melanoma patients have consistently demonstrated favorable outcomes.

Building up from the successes of TIL therapy in melanoma patients, the past decade has seen an emerge of a novel form of adoptive cell therapy based on the transfer of genetically engineered T cells expressing a single-chain antibody structure fused to an intracellular T cell receptor signaling domain called chimeric antigen receptor (CAR) [86]. The inclusion of CD28 and CD137 co-stimulatory signaling in the intracellular domain of CAR has substantially improved their therapeutic efficacy. The therapeutic potential of CAR-expressing T cells for treatment of Chronic lymphocytic leukemia (CLL) was recently revealed in a clinical study where infusion of autologous T cell genes modified to express a CD19-specific CAR induced complete regression of a patient with refractory disease [87]. In a breast cancer clinical trial, T cells genetically engineered to target HER2$^+$ tumors resulted in the death of patients presumably due to the cross-reactivity of CAR T cells with normal cells expressing low levels of HER2 [88]. CAR T cell therapy is also being investigated for treatment of TNBC. A search for tumor antigens associated with TNBC identified mesothelin as a potential target antigen [89]. While mesothelin is rarely found expressed in ER$^+$ or HER2$^+$ breast cancers, 67 % (29 out of 43) TNBC samples have been found to express mesothelin in at least 5 % of the tumor, with 19 % of TNBC samples expressing mesothelin in over 50 % of the tumor. In proof-of-concept experiments, it was shown that cytotoxicity of mesothelin-positive breast cancer cells can be successfully achieved by CAR T cells specific for mesothelin. While further validation is needed, this study suggests that mesothelin might represent a valid target for adoptive T cell therapy of TNBC.

23.11 Concluding Remarks

In conclusion, data have been presented here to demonstrate that immunomodulatory approaches are worth investigating in certain

types of breast cancer. Particularly, the ER-negative and HER2-amplified breast cancers seem to employ tumor-mediated immunosuppression to facilitate their growth. This is evident by the large amount of preclinical and correlative data that associates immune infiltrates, cells, and signatures with prognosis and therapeutic efficacy in ER^- and $HER2^+$ breast cancer, as well as demonstrating the efficacy of T cell enhancement in combination with trastuzumab in $HER2^+$ disease.

The presence of TILs in primary breast tumors is intriguing and may identify a population most amendable to some immunomodulatory approaches, such as T cell checkpoint inhibition. Current data suggests that its evaluation is warranted in clinical samples due to its association with improved prognosis. Further work will need to be done between pathologists to ensure reproducibility and robustness of TILs measurement prior to widespread clinical implementation. Understanding why some patients have TILs at diagnosis and others do not could be critical to improved outcome in TNBC, which currently has a dearth of treatment options, as well as $HER2^+$ disease. While TILs may not be able to mediate primary tumor regressions, this could be due to other reasons such as the effector response was too late, not durable, or too weak, particularly if the primary tumor is of a large size. Furthermore, it could be that the breast is a site that is not well served by the immune system (unlike sites such as lung or skin). However, the generation of immune memory in these patients may suppress the development of microscopic metastatic cells at other sites. Further research is warranted to functionally delineate the key players in breast cancer in order to develop suitable immune therapies, as well as clinical trials evaluating immunocombination approaches in breast cancer.

References

1. Penn I. Tumors of the immunocompromised patient. Annu Rev Med. 1988;39:63–73.
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74.
3. Stagg J, Johnstone RW, Smyth MJ. From cancer immunosurveillance to cancer immunotherapy. Immunol Rev. 2007;220:82–101.
4. Mlecnik B, Bindea G, Pages F, Galon J. Tumor immunosurveillance in human cancers. Cancer Metastasis Rev. 2011;30(1):5–12.
5. Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer. 2012;12(4):298–306.
6. Denkert C, Loibl S, Noske A, Roller M, Muller BM, Komor M, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol. 2010;28(1):105–13.
7. Mahmoud SM, Paish EC, Powe DG, Macmillan RD, Grainge MJ, Lee AH, et al. Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. J Clin Oncol. 2011;29(15):1949–55.
8. Loi S, Sirtaine N, Piette F, Salgado R, Viale G, Van Eenoo F, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. J Clin Oncol. 2013;31(7):860–7.
9. Finak G, Bertos N, Pepin F, Sadekova S, Souleimanova M, Zhao H, et al. Stromal gene expression predicts clinical outcome in breast cancer. Nat Med. 2008;14(5):518–27.
10. Baker K, Lachapelle J, Zlobec I, Bismar TA, Terracciano L, Foulkes WD. Prognostic significance of CD8+ T lymphocytes in breast cancer depends upon both oestrogen receptor status and histological grade. Histopathology. 2011;58(7):1107–16.
11. Teschendorff AE, Miremadi A, Pinder SE, Ellis IO, Caldas C. An immune response gene expression module identifies a good prognosis subtype in estrogen receptor negative breast cancer. Genome Biol. 2007;8(8):R157.
12. DeNardo DG, Brennan DJ, Rexhepaj E, Ruffell B, Shiao SL, Madden SF, et al. Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. Cancer Discov. 2011;1(1):54–67.
13. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol. 1995;155(3):1151–64.
14. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet. 2001;27(1):20–1.
15. Brunkow ME, Jeffery EW, Hjerrild KA, Paeper B, Clark LB, Yasayko SA, et al. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet. 2001;27(1):68–73.

16. Wildin RS, Ramsdell F, Peake J, Faravelli F, Casanova JL, Buist N, et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nat Genet. 2001;27(1):18–20.
17. Mahmoud SM, Paish EC, Powe DG, Macmillan RD, Lee AH, Ellis IO, et al. An evaluation of the clinical significance of FOXP3+ infiltrating cells in human breast cancer. Breast Cancer Res Treat. 2011;127(1): 99–108.
18. Liu F, Lang R, Zhao J, Zhang X, Pringle GA, Fan Y, et al. CD8(+) cytotoxic T cell and FOXP3(+) regulatory T cell infiltration in relation to breast cancer survival and molecular subtypes. Breast Cancer Res Treat. 2011;130(2):645–55.
19. Liu F, Li Y, Ren M, Zhang X, Guo X, Lang R, et al. Peritumoral FOXP3(+) regulatory T cell is sensitive to chemotherapy while intratumoral FOXP3(+) regulatory T cell is prognostic predictor of breast cancer patients. Breast Cancer Res Treat. 2012;135(2):459–67.
20. Ladoire S, Mignot G, Dabakuyo S, Arnould L, Apetoh L, Rebe C, et al. In situ immune response after neoadjuvant chemotherapy for breast cancer predicts survival. J Pathol. 2011;224(3):389–400.
21. Oda N, Shimazu K, Naoi Y, Morimoto K, Shimomura A, Shimoda M, et al. Intratumoral regulatory T cells as an independent predictive factor for pathological complete response to neoadjuvant paclitaxel followed by 5-FU/epirubicin/cyclophosphamide in breast cancer patients. Breast Cancer Res Treat. 2012;136(1):107–16.
22. Lee S, Cho EY, Park YH, Ahn JS, Im YH. Prognostic impact of FOXP3 expression in triple-negative breast cancer. Acta Oncol. 2013;52(1):73–81.
23. West NR, Kost SE, Martin SD, Milne K, Deleeuw RJ, Nelson BH, et al. Tumour-infiltrating FOXP3(+) lymphocytes are associated with cytotoxic immune responses and good clinical outcome in oestrogen receptor-negative breast cancer. Br J Cancer. 2013;108(1):155–62.
24. Gu-Trantien C, Loi S, Garaud S, Equeter C, Libin M, de Wind A, et al. CD4+ follicular helper T cell infiltration predicts breast cancer survival. J Clin Invest. 2013;123(7):2873–92.
25. Schmidt M, Bohm D, von Torne C, Steiner E, Puhl A, Pilch H, et al. The humoral immune system has a key prognostic impact in node-negative breast cancer. Cancer Res. 2008;68(13):5405–13.
26. Schmidt M, Hellwig B, Hammad S, Othman A, Lohr M, Chen Z, et al. A comprehensive analysis of human gene expression profiles identifies stromal immunoglobulin kappa C as a compatible prognostic marker in human solid tumors. Clin Cancer Res. 2012;18(9):2695–703.
27. Mahmoud SM, Lee AH, Paish EC, Macmillan RD, Ellis IO, Green AR. The prognostic significance of B lymphocytes in invasive carcinoma of the breast. Breast Cancer Res Treat. 2012;132(2):545–53.
28. DeNardo DG, Barreto JB, Andreu P, Vasquez L, Tawfik D, Kolhatkar N, et al. CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. Cancer Cell. 2009;16(2):91–102.
29. Bunt SK, Yang L, Sinha P, Clements VK, Leips J, Ostrand-Rosenberg S. Reduced inflammation in the tumor microenvironment delays the accumulation of myeloid-derived suppressor cells and limits tumor progression. Cancer Res. 2007;67(20):10019–26.
30. Serafini P, Borrello I, Bronte V. Myeloid suppressor cells in cancer: recruitment, phenotype, properties, and mechanisms of immune suppression. Semin Cancer Biol. 2006;16(1):53–65.
31. Sceneay J, Chow MT, Chen A, Halse HM, Wong CS, Andrews DM, et al. Primary tumor hypoxia recruits CD11b+/Ly6Cmed/Ly6G+ immune suppressor cells and compromises NK cell cytotoxicity in the premetastatic niche. Cancer Res. 2012;72(16):3906–11.
32. Filipazzi P, Valenti R, Huber V, Pilla L, Canese P, Iero M, et al. Identification of a new subset of myeloid suppressor cells in peripheral blood of melanoma patients with modulation by a granulocyte-macrophage colony-stimulation factor-based antitumor vaccine. J Clin Oncol. 2007;25(18):2546–53.
33. Desmedt C, Haibe-Kains B, Wirapati P, Buyse M, Larsimont D, Bontempi G, et al. Biological processes associated with breast cancer clinical outcome depend on the molecular subtypes. Clin Cancer Res. 2008;14(16):5158–65.
34. Ignatiadis M, Singhal SK, Desmedt C, Haibe-Kains B, Criscitiello C, Andre F, et al. Gene modules and response to neoadjuvant chemotherapy in breast cancer subtypes: a pooled analysis. J Clin Oncol. 2012;30(16):1996–2004.
35. Liu S, Lachapelle J, Leung S, Gao D, Foulkes WD, Nielsen TO. CD8+ lymphocyte infiltration is an independent favorable prognostic indicator in basal-like breast cancer. Breast Cancer Res. 2012;14(2):R48.
36. Rody A, Karn T, Liedtke C, Pusztai L, Ruckhaeberle E, Hanker L, et al. A clinically relevant gene signature in triple negative and basal-like breast cancer. Breast Cancer Res. 2011;13(5):R97.
37. Ha NH, Nair VS, Reddy DN, Mudvari P, Ohshiro K, Ghanta KS, et al. Lactoferrin-endothelin-1 axis contributes to the development and invasiveness of triple-negative breast cancer phenotypes. Cancer Res. 2011;71(23):7259–69.
38. Grigoriadis A, Caballero OL, Hoek KS, da Silva L, Chen YT, Shin SJ, et al. CT-X antigen expression in human breast cancer. Proc Natl Acad Sci U S A. 2009;106(32):13493–8.
39. Curigliano G, Viale G, Ghioni M, Jungbluth AA, Bagnardi V, Spagnoli GC, et al. Cancer-testis antigen expression in triple-negative breast cancer. Ann Oncol Off J Eur Soc Med Oncol ESMO. 2011;22(1):98–103.
40. Chen YT, Ross DS, Chiu R, Zhou XK, Chen YY, Lee P, et al. Multiple cancer/testis antigens are preferentially expressed in hormone-receptor negative and high-grade breast cancers. PLoS One. 2011;6(3): e17876.
41. Karn T, Pusztai L, Ruckhaberle E, Liedtke C, Muller V, Schmidt M, et al. Melanoma antigen family A

identified by the bimodality index defines a subset of triple negative breast cancers as candidates for immune response augmentation. Eur J Cancer. 2012;48(1):12–23.
42. Spychala J, Lazarowski E, Ostapkowicz A, Ayscue LH, Jin A, Mitchell BS. Role of estrogen receptor in the regulation of ecto-5′-nucleotidase and adenosine in breast cancer. Clin Cancer Res. 2004;10(2): 708–17.
43. Stagg J, Divisekera U, McLaughlin N, Sharkey J, Pommey S, Denoyer D, et al. Anti-CD73 antibody therapy inhibits breast tumor growth and metastasis. Proc Natl Acad Sci U S A. 2010;107(4):1547–52.
44. Stagg J, Divisekera U, Duret H, Sparwasser T, Teng MW, Darcy PK, et al. CD73-deficient mice have increased antitumor immunity and are resistant to experimental metastasis. Cancer Res. 2011;71(8):2892–900.
45. Stagg J, Beavis PA, Divisekera U, Liu MC, Moller A, Darcy PK, et al. CD73-deficient mice are resistant to carcinogenesis. Cancer Res. 2012;72(9):2190–6.
46. Wang L, Fan J, Thompson LF, Zhang Y, Shin T, Curiel TJ, et al. CD73 has distinct roles in nonhematopoietic and hematopoietic cells to promote tumor growth in mice. J Clin Invest. 2011;121(6):2371–82.
47. Yegutkin GG, Marttila-Ichihara F, Karikoski M, Niemela J, Laurila JP, Elima K, et al. Altered purinergic signaling in CD73-deficient mice inhibits tumor progression. Eur J Immunol. 2011;41(5):1231–41.
48. Ferris RL, Jaffee EM, Ferrone S. Tumor antigen-targeted, monoclonal antibody-based immunotherapy: clinical response, cellular immunity, and immunoescape. J Clin Oncol. 2010;28(28):4390–9.
49. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. Nat Med. 2000;6(4): 443–6.
50. Park S, Jiang Z, Mortenson ED, Deng L, Radkevich-Brown O, Yang X, et al. The therapeutic effect of anti-HER2/neu antibody depends on both innate and adaptive immunity. Cancer Cell. 2010;18(2):160–70.
51. Stagg J, Loi S, Divisekera U, Ngiow SF, Duret H, Yagita H, et al. Anti-ErbB-2 mAb therapy requires type I and II interferons and synergizes with anti-PD-1 or anti-CD137 mAb therapy. Proc Natl Acad Sci U S A. 2011;108(17):7142–7.
52. Pencina MJ, d'Agostino RB. Overall C as a measure of discrimination in survival analysis: model specific population value and confidence interval estimation. Stat Med. 2004;23:2109–23.
53. Ju T, Cummings RD. A unique molecular chaperone Cosmc required for activity of the mammalian core 1 beta 3-galactosyltransferase. Proc Natl Acad Sci U S A. 2002;99(26):16613–8.
54. von Mensdorff-Pouilly S, Verstraeten AA, Kenemans P, Snijdewint FG, Kok A, Van Kamp GJ, et al. Survival in early breast cancer patients is favorably influenced by a natural humoral immune response to polymorphic epithelial mucin. J Clin Oncol. 2000;18(3):574–83.
55. Brossart P, Heinrich KS, Stuhler G, Behnke L, Reichardt VL, Stevanovic S, et al. Identification of HLA-A2-restricted T-cell epitopes derived from the MUC1 tumor antigen for broadly applicable vaccine therapies. Blood. 1999;93(12):4309–17.
56. Lakshminarayanan V, Thompson P, Wolfert MA, Buskas T, Bradley JM, Pathangey LB, et al. Immune recognition of tumor-associated mucin MUC1 is achieved by a fully synthetic aberrantly glycosylated MUC1 tripartite vaccine. Proc Natl Acad Sci U S A. 2012;109(1):261–6.
57. Arteaga CL, Sliwkowski MX, Osborne CK, Perez EA, Puglisi F, Gianni L. Treatment of HER2-positive breast cancer: current status and future perspectives. Nat Rev Clin Oncol. 2012;9(1):16–32.
58. Disis ML, Wallace DR, Gooley TA, Dang Y, Slota M, Lu H, et al. Concurrent trastuzumab and HER2/neu-specific vaccination in patients with metastatic breast cancer. J Clin Oncol. 2009;27(28):4685–92.
59. Tyagi P, Mirakhur B. MAGRIT: the largest-ever phase III lung cancer trial aims to establish a novel tumor-specific approach to therapy. Clin Lung Cancer. 2009;10(5):371–4.
60. Quezada SA, Peggs KS, Curran MA, Allison JP. CTLA4 blockade and GM-CSF combination immunotherapy alters the intratumor balance of effector and regulatory T cells. J Clin Invest. 2006;116(7): 1935–45.
61. Chen H, Liakou CI, Kamat A, Pettaway C, Ward JF, Tang DN, et al. Anti-CTLA-4 therapy results in higher CD4+ICOShi T cell frequency and IFN-gamma levels in both nonmalignant and malignant prostate tissues. Proc Natl Acad Sci U S A. 2009;106(8):2729–34.
62. Kahler KC, Hauschild A. Treatment and side effect management of CTLA-4 antibody therapy in metastatic melanoma. J Dtsch Dermatol Ges. 2011;9(4):277–86.
63. Hirano F, Kaneko K, Tamura H, Dong H, Wang S, Ichikawa M, et al. Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. Cancer Res. 2005;65(3):1089–96.
64. Gao Q, Wang XY, Qiu SJ, Yamato I, Sho M, Nakajima Y, et al. Overexpression of PD-L1 significantly associates with tumor aggressiveness and postoperative recurrence in human hepatocellular carcinoma. Clin Cancer Res. 2009;15(3):971–9.
65. Gadiot J, Hooijkaas AI, Kaiser AD, van Tinteren H, van Boven H, Blank C. Overall survival and PD-L1 expression in metastasized malignant melanoma. Cancer. 2011;117(10):2192–201.
66. Zhang P, Su DM, Liang M, Fu J. Chemopreventive agents induce programmed death-1-ligand 1 (PD-L1) surface expression in breast cancer cells and promote PD-L1-mediated T cell apoptosis. Mol Immunol. 2008;45(5):1470–6.
67. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366(26):2455–65.
68. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366(26):2443–54.

69. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, et al. Safety and tumor responses with lambrolizumab (Anti-PD-1) in melanoma. N Engl J Med. 2013;369(2):134–44.
70. Curran MA, Montalvo W, Yagita H, Allison JP. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. Proc Natl Acad Sci U S A. 2010;107(9):4275–80.
71. Ghebeh H, Lehe C, Barhoush E, Al-Romaih K, Tulbah A, Al-Alwan M, et al. Doxorubicin downregulates cell surface B7-H1 expression and upregulates its nuclear expression in breast cancer cells: role of B7-H1 as an anti-apoptotic molecule. Breast Cancer Res. 2010;12(4):R48.
72. Sakuishi K, Apetoh L, Sullivan JM, Blazar BR, Kuchroo VK, Anderson AC. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. J Exp Med. 2010; 207(10):2187–94.
73. Ngiow SF, von Scheidt B, Akiba H, Yagita H, Teng MW, Smyth MJ. Anti-TIM3 antibody promotes T cell IFN-gamma-mediated antitumor immunity and suppresses established tumors. Cancer Res. 2011;71(10): 3540–51.
74. Rangachari M, Zhu C, Sakuishi K, Xiao S, Karman J, Chen A, et al. Bat3 promotes T cell responses and autoimmunity by repressing Tim-3-mediated cell death and exhaustion. Nat Med. 2012;18(9):1394–400.
75. Chiba S, Baghdadi M, Akiba H, Yoshiyama H, Kinoshita I, Dosaka-Akita H, et al. Tumor-infiltrating DCs suppress nucleic acid-mediated innate immune responses through interactions between the receptor TIM-3 and the alarmin HMGB1. Nat Immunol. 2012;13(9):832–42.
76. Woo SR, Turnis ME, Goldberg MV, Bankoti J, Selby M, Nirschl CJ, et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. Cancer Res. 2012;72(4):917–27.
77. Croft M, Duan W, Choi H, Eun SY, Madireddi S, Mehta A. TNF superfamily in inflammatory disease: translating basic insights. Trends Immunol. 2012;33(3):144–52.
78. Piconese S, Valzasina B, Colombo MP. OX40 triggering blocks suppression by regulatory T cells and facilitates tumor rejection. J Exp Med. 2008;205(4):825–39.
79. Narazaki H, Zhu Y, Luo L, Zhu G, Chen L. CD137 agonist antibody prevents cancer recurrence: contribution of CD137 on both hematopoietic and nonhematopoietic cells. Blood. 2010;115(10):1941–8.
80. Palazon A, Teijeira A, Martinez-Forero I, Hervas-Stubbs S, Roncal C, Penuelas I, et al. Agonist anti-CD137 mAb act on tumor endothelial cells to enhance recruitment of activated T lymphocytes. Cancer Res. 2011;71(3):801–11.
81. Langowski JL, Zhang X, Wu L, Mattson JD, Chen T, Smith K, et al. IL-23 promotes tumour incidence and growth. Nature. 2006;442(7101):461–5.
82. Teng MW, von Scheidt B, Duret H, Towne JE, Smyth MJ. Anti-IL-23 monoclonal antibody synergizes in combination with targeted therapies or IL-2 to suppress tumor growth and metastases. Cancer Res. 2011;71(6):2077–86.
83. Gangemi S, Minciullo P, Adamo B, Franchina T, Ricciardi GR, Ferraro M, et al. Clinical significance of circulating interleukin-23 as a prognostic factor in breast cancer patients. J Cell Biochem. 2012;113(6): 2122–5.
84. Singer K, Gottfried E, Kreutz M, Mackensen A. Suppression of T-cell responses by tumor metabolites. Cancer Immunol Immunother. 2011;60(3): 425–31.
85. Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res. 2011;17(13):4550–7.
86. Jena B, Dotti G, Cooper LJ. Redirecting T-cell specificity by introducing a tumor-specific chimeric antigen receptor. Blood. 2010;116(7):1035–44.
87. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med. 2011;365(8):725–33.
88. Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. Mol Ther. 2010;18(4):843–51.
89. Tchou J, Wang LC, Selven B, Zhang H, Conejo-Garcia J, Borghaei H, et al. Mesothelin, a novel immunotherapy target for triple negative breast cancer. Breast Cancer Res Treat. 2012;133(2):799–804.

Immunology and Immunotherapy of Graft-Versus-Host Disease

24

Doreen Haase

Contents

D. Haase, PhD
SIgN (Singapore Immunology Network),
(A*STAR) Agency for Science, Technology and Research, 8A Biomedical Grove, #04 Immunos, Biopolis, Singapore 138648, Singapore
e-mail: doreen_haase@immunol.a-star.edu.sg

24.1 Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is often the last resort for treatment of patients with different malignant diseases as well as inherited immune disorders and autoimmune diseases, for example, leukemia, lymphoma, sickle cell anemia, and multiple sclerosis. A common complication is graft-versus-host disease (GVHD) which accounts for a major cause of morbidity and mortality after HSCT [1]. GVHD can occur whenever immunologically competent cells are transferred into an immune-incompetent recipient where they attack specific tissues of the host, mainly the gut, skin, and liver. This multistep inflammatory response is directed against HLA disparities between donor and recipient and involves a network of cellular players which, after activation, release a surge of cytokines with detrimental effects for the patient.

Although the current management of GVHD starts already at the time of HSCT with preemptive immunosuppression, this treatment cannot prevent the onset of GVHD in all patients. After diagnosis of GVHD, the immunosuppressive therapy is further intensified. Different regimens contain steroids and calcineurin inhibitors which lead to the broad suppression necessary to stop the ongoing inflammation. However, none of the drugs or combinations currently in use is completely efficient in abrogating GVHD, but all increase the risk of life-threatening infections, sepsis, and recurrence of the underlying disease.

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6_24, © Springer-Verlag Berlin Heidelberg 2015

Novel strategies to prevent and treat GVHD therefore target more specific mechanisms in the pathogenesis of GVHD without affecting general immunity and if possible antitumor responses.

24.2 GVHD

Not all patients have the same risk of developing GVHD. Apart from genetic differences in pathways involved in the pathogenesis, the main risk factors include the source and composition of the stem cells transferred, the degree of discrepancy for both major and minor histocompatibility antigens (MHC/MiHA) between donor and recipient, the conditioning treatment, as well as the age of the recipient [2].

Two separate forms of GVHD have been described with different implications for patients. Traditionally, acute GVHD is defined by the early occurrence within the first 100 days after HCST, while the chronic form develops later. However, the preventive treatment with potent immunosuppressive drugs can complicate the diagnosis by delaying the onset of acute GVHD beyond this threshold. Still there are distinct histopathological characteristics of acute and chronic GVHD. While acute GVHD is mainly considered a TH1- and TH17-type inflammatory condition [3] which is described in detail in the following paragraphs, chronic GVHD resembles more a TH2-inflicted autoimmune disease which often succeeds the acute form of GVHD. In addition to the organs involved in classical acute GVHD – skin, liver, and gut – also the lung, eyes, and mucous membranes are typically affected by chronic GVHD [4]. Several symptoms have been linked to autoantibodies [5, 6]. In general, chronic GVHD leads to an imbalance of the immune system with an increase in serious infections [7]. This has fundamental impact on the long-term management of patients.

Acute GVHD is usually diagnosed first by skin lesions in the form of rashes that can develop into generalized erythroderma and bullous formation in severe cases. The second organ affected by acute GVHD is the liver with patients presenting with jaundice and increased bilirubin levels. Differential diagnosis is important in these cases as these symptoms can also be caused by chemotherapy, immunosuppression, or infections. GVHD of the gut occurs with symptoms like cramps and diarrhea. Especially the latter can be severe and life-threatening due to immense loss of fluids. All these manifestations require symptomatic treatment in addition to suppressing the causative GVHD [2].

According to the severity of symptoms in the organs affected, acute GVHD is graded into stages I–IV, with grade I only affecting the skin and grade IV showing severe manifestations in two or more organs [8].

24.3 Pathogenesis of Acute GVHD

Acute GVHD is a multistep process that involves different cell types as well as soluble factors. Already in 1966, Billingham formulated three factors that define the development of GVHD:

1. The graft contains immunocompetent cells which are able to elicit an allogeneic response.
2. There are genetic difference between donor and recipient.
3. The host is unable to reject the donor cells [9].

Three decades later, Ferrara described the pathogenesis of GVHD in three phases [10] (Fig. 24.1): The first phase is induced by the conditioning treatment that prepares the patient for HSCT and lays the ground for a general inflammation. In phase II, allogeneic cells of the transplant are activated in the host and migrate to the target tissues of GVHD. Here effector cells, mainly T cells and natural killer (NK) cells, execute their cytotoxic function in the third phase.

24.3.1 Phase I: Conditioning

The first phase of GVHD started already before the actual HSCT with the conditioning regime. This treatment depletes the patient's own bone marrow cells to allow engraftment of the donor's stem cells and prevent their rejection. It also aims at clearing any remaining pathological cells in the patient. The myeloablation is either

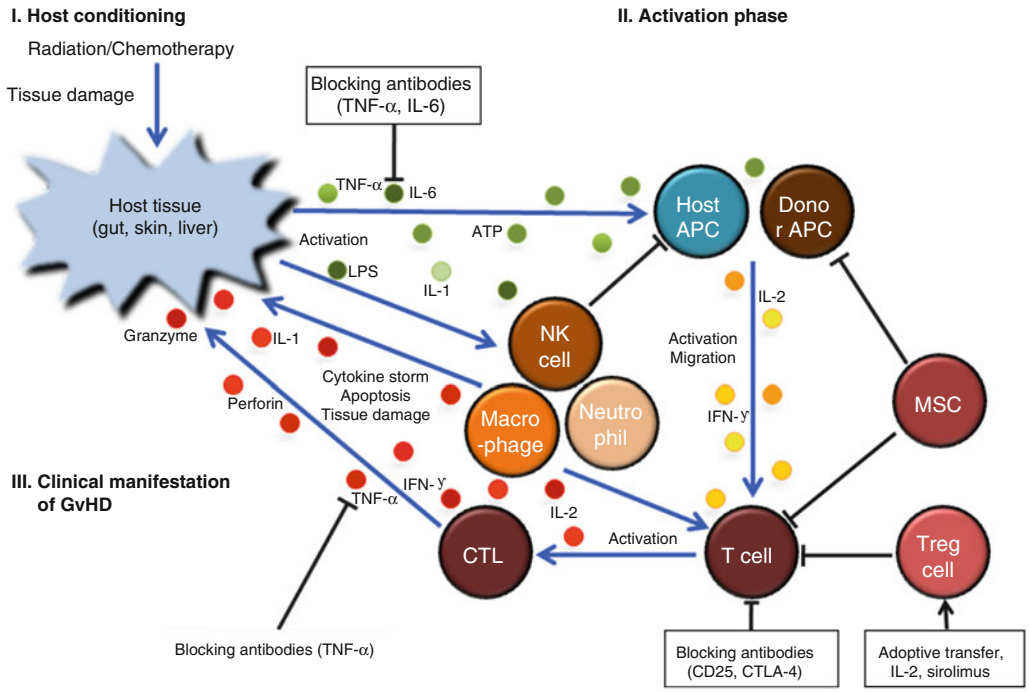


Fig. 24.1 Pathogenesis of graft-versus-host disease (*GVHD*) can be divided into three phases. In the first phase, the host is conditioned by myeloablative treatment. This leads to tissue damage with the release of proinflammatory cytokines and the danger signals LPS and ATP. The stem cell transplant induces phase II, when these molecules activate the transferred allogeneic donor cells. In phase III, donor NK cells, macrophages, and neutrophils directly attack host tissues by triggering apoptosis and releasing additional cytokines which leads to a cytokine storm. Both host and donor APC in their turn activate T cells which then exert their cytotoxic activity causing the typical symptoms of GVHD. All three phases are targeted by approaches to treat GVHD. Blocking antibodies against cytokines, co-stimulation molecules, and activation markers interfere with specific activation pathways of GVHD. Cell therapy with Treg cells, mesenchymal stem cells (*MSC*), and NK cells suppresses additional mediators

done by total-body irradiation or high-dose chemotherapy. However, neither strategy is targeted and causes also injury to other tissues, particularly the gut mucosa, skin, and liver. The damaged cells in response secrete a massive amount of cytokines, especially interleukin (IL)-1, IL-6, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α [11].

Several polymorphisms for genes of these cytokines have been associated with an increased risk for GVHD. While polymorphisms for TNF-α, IFN-γ, IL-6, IL-10, and a natural occurring IL-1 receptor antagonist were associated with severity of acute GVHD [12–15], IL-6 also correlates with the incidence of chronic GVHD [16–18].

Not only cytokines but also other danger signals contribute to the proinflammatory environment. One molecule that is released from dying cells and has been implicated in the pathogenesis of GVHD is ATP [19]. It can itself induce the secretion of the proinflammatory cytokines IFN-γ, TNF-α, and IL-6 and activate antigen-presenting cells (APC). Also the ATP receptor P2X7 is upregulated during GVHD, leaving cells more susceptible to the effects of ATP and creating a positive feedback loop. Thus, blocking of P2X7 as well as reduction of ATP concentration can improve GVHD symptoms. Even increased numbers of regulatory T (Treg) cells, which are known to control GVHD, are detected. Notably,

Treg cells are also one of the main populations expressing the ATP-hydrolyzing enzyme CD39 on their surface [20] as they are particularly sensitive to ATP [21].

In the intestine, the tissue damage leads to the release of microbial lipopolysaccharides (LPS) which is recognized by Toll-like receptors (TLR) on APC. Polymorphisms in genes for several of these TLR, namely, TLR4, TLR7, and TLR9, were described to influence the incidence and severity of GVHD [22–24]. Furthermore, flagellin, an agonist for TLR5, was able to reduce GVHD in a mouse model [25]. Although the TLR/APC axis undoubtedly plays an important role in the onset of GVHD, it also develops without intact TLR signaling [26].

The presence this plethora of cytokines and danger signals present at these sites of acute inflammation leads to the activation of APC. These comprise dendritic cells (DC), monocytes, macrophages, and B cells which present antigens to T lymphocytes and activate these. APC and other local cells upregulate the MHC (major histocompatibility complex) and co-stimulatory and adhesion molecules [27] and thereby set the stage for the next phase of GVHD.

24.3.2 Phase II: Activation

The second phase is initiated when donor stem cells are transferred into this proinflammatory setting. They are attracted by the presence of cytokines, chemokines, and adhesion molecules at the sites of tissue injury. Here they come into contact with the activated host APC that present both MHC molecules and self-antigens. Any self-antigen derived from a protein that is different between donor and recipient can give rise to minor histocompatibility antigens (MiHA). Allogeneic donor cells recognize disparities in MHC for HLA mismatched donor/patient pairs or in MiHA with their T-cell receptor (TCR) [28]. In addition, also non-hematopoietic host tissue cells [29, 30] and donor APC [31, 32] can be involved in the presentation of host antigens. As all of these cell types are involved in the presentation of alloantigens, targeting only one cell type is not sufficient in abrogating GVHD [33, 34].

In addition to TCR engagement, alloreactive T cells require co-stimulatory signals for their activation; these are also provided by activated APC. The main pathway for this co-stimulation involves the engagement of CD28 on T lymphocytes by its B7 ligands CD80 and CD86 on APC [35]. Also CD40 and its ligand CD40L play a role in providing positive co-stimulation for T cells [36].

After activation by antigen recognition plus co-stimulation, allogeneic T cells differentiate into effector cells [37] and secrete TH1 cytokines, mainly IFN-γ, IL-2, and TNF-α, as well as the TH17 cytokine IL-17 [38], and thereby execute phase III of GVHD.

24.3.3 Phase III: Effector Phase

The clinical symptoms of acute GVHD are mainly caused by the action of T cells. It was shown that only the naïve pool of donor T cells is responsible for the development of GVHD but not transplanted effector and central memory T cells [39]. Other important cell subsets responsible for the tissue damage in the effector phase of GVHD are natural killer (NK) cells, macrophages, and neutrophils [40]. Also B cells were reported to play a role [41].

Alloreactive $CD8^+$ T cells use two main mechanisms for their cytotoxic action, both of which induce the activation of the caspase cascade in target cells by direct cell contact. One pathway induces the release of perforin and granzymes which trigger cell lysis [42]. The other mechanism requires engagement of the death receptor, Fas, with the Fas ligand (FasL) leading to apoptosis [43].

Tissue damage is also induced indirectly by the cytokines present in abundance from the preconditioning (TNF-α, IFN-γ and IL-1).

These cytokines themselves trigger the release of a surge of additional cytokines from allogeneic donor cells in a self-amplifying cycle that can lead to a cytokine storm [44] with deleterious effects on the patient.

TNF-α is the key cytokine involved in all three phases of the pathogenesis of GVHD [45]. It was reported as an independent predictor of GVHD as

it correlates with its severity, time of onset, and mortality when measured with the help of the surrogate marker TNF receptor 1 (TNFR1). TNFR1 binds TNF-α and is shed into the plasma where it can easily be measured [46–49]. Another member of the tumor necrosis factor receptor family, CD30, has been described as a marker for GVHD. Higher levels of CD30 were found both in plasma and on $CD8^+$ T cells of patients with acute GVHD [50]. A more comprehensive combination of the four biomarkers IL-8, hepatocyte growth factor, TNFR1, and IL-2 receptor-α was suggested to predict the occurrence of GVHD [51].

24.4 Natural Control of GVHD

Natural suppression of GVHD is conferred by Treg cells. These cells regulate both innate and adaptive immune responses and confer peripheral tolerance by suppressing activated T cells [52, 53]. Their modes of action include a variety of mechanisms involving both soluble factors and direct cell-cell contact [54, 55]. In mouse models, depletion of Treg cells exacerbated GVHD, while infusion of these cells improved its outcome [56, 57]. However, in the overwhelming inflammation during GVHD, the balance of the immune system is grossly shifted toward the proinflammatory response and apparently leaves Treg cells insufficient. Nevertheless, Treg cells have been associated with the outcome of GVHD [57, 58]. Even specific markers in the transcriptome of Treg cells were able to predict both acute and chronic GVHD, among these were activation markers (PIP5Kγ, FAS, CD44, CD69), cyclins, and the transcription factor Eos which is important for the suppressive capacity of Treg cells [59].

24.5 Graft-Versus-Tumor Effect

The undesirable graft-versus-host syndrome is closely intertwined with the graft-versus-tumor (GVT) response, which is part of the curative effect of stem cell transplantation especially in patients with malignancies. T cells from the graft not only recognize alloantigens but also tumor antigens and are thus able to act against leukemia or tumor cells that escaped the conditioning regime. Also NK cells [60–62] and NKT cells were implicated in the GVT activity [63]. The greater the MHC mismatch between donor and patient, the stronger the GVT response is. This explains the advantage of allogeneic over autologous HSCT and the higher relapse rate of patients receiving HSCT from a related donor [1]. Especially after non-myeloablative pretreatment of the patient, GVT is indispensable to clear any remaining malignant cell. The strong general immunosuppression as well as T-cell depletion to prevent GVHD also inhibits GVT [64]. Thus, the challenge is to find a balance between the negative impact of GVHD and the positive effects of GVT. The incidence of GVHD is considered as an indication of an ongoing GVT activity and was shown to correlate with a reduced risk of relapse [65, 66]. An option to treat cancer relapse after HSCT is donor lymphocyte infusion which has a high risk of GVHD but also a highly effective GVT response [67].

Approaches to separate GVHD from GVT are difficult as both share similar mechanisms. However, attempts are being made to interfere with differences in the organ-specific proinflammatory environment, specific cells involved, and selective migration of effector cells to the target tissues [68].

24.6 Prevention of GVHD

Without any preventive treatment, acute GVHD would occur in practically every patient receiving allogeneic HSCT. Even with preemptive general immunosuppression, acute GVHD is still a major complication after HSCT. The standard treatment includes corticosteroids, cyclosporine A, and methotrexate (MTX). The combination of these has proven to be most effective [2]. Most novel approaches for the treatment of GVHD discussed below are also tested for its prevention.

A non-pharmacological approach to prevent the occurrence of GVHD is depletion of T cells, either *ex vivo* from the graft or *in vivo*. This depletion can be achieved by different methods, like

anti-thymocyte globulin (ATG) or monoclonal antibodies (mAbs) directed against CD4, CD5, CD8, and/or CD52 (Campath/alemtuzumab) [69, 70]. Although depletion of lymphocytes was shown to reduce GVHD, it also increases the risk of graft rejection by the recipient's T cells and of recurrence of the underlying disease as also GVT responses are diminished. It needs to be shown whether the attempts to selectively deplete alloreactive T cells can provide a tool to reduce the incidence and severity of GVHD.

24.7 Treatment of Acute GVHD

Even with these comprehensive measures of prevention, between 10 and 50 % of patients still develop forms of GVHD severe enough to require additional treatment. The first-line therapy remains administration of steroids, in particular methylprednisolone. However, the high doses necessary to abrogate the ongoing immune reaction lead to a generalized immunosuppression with the risk of opportunistic infections [2].

Other routine medications like the calcineurin inhibitor cyclosporine A inhibit T-cell activation and block IL-2 transcription [71]. The macrolide antibiotic tacrolimus or FK-506 also interferes with IL-2 production [72, 73] but appears to have less toxic side effects [74]. A different pathway is targeted by mycophenolate mofetil, which inhibits activation and proliferation of lymphocytes by blocking their purine synthesis [75]. These standard drugs, even in combination, are not sufficient for all patients and have serious side effects in addition to the immunosuppression [76–78]. Often renal and other toxicities limit their use.

Another approach is the depletion of T lymphocytes by the polyclonal anti-thymocyte globulin (ATG). A meta-analysis of six prospective randomized controlled trials found that ATG was able to lower incidence of grade II–IV GVHD with a stronger effect seen for rabbit compared to equine ATG but no impact on overall GVHD, survival, or relapse [79]. Higher doses of ATG can further decrease incidence of GVHD, but with a higher risk of serious infections [80]. A more recent study with a different ATG preparation could confirm the reduction in GVHD and reported a reduction in tumor relapse and infections [81]. Also a reduction of chronic GVHD was reported [82].

An interesting therapeutic modality is the extracorporeal photochemotherapy (ECP), a combination of a photosensitizing drug with exposure to UVA light. This has been demonstrated to inactivate lymphocytes although the mechanism still needs to be elucidated. However, in several clinical trials, symptoms of both acute and chronic GVHD were improved without negatively affecting GVT or relapse incidence [83, 84].

Also statins were shown to have an immunomodulatory effect by reducing cytokine secretion and T-cell activation [85]. In a mouse model, they reduced GVHD incidence [86]. A correlation study in patients could attribute a decreased risk for acute GVHD to the use of statins by the donor [87]. The risk for chronic GVHD was diminished if the recipient took statins [88].

A novel mechanism is targeted by the proteasome inhibitor bortezomib which is approved for treatment of myeloma. In a mouse model, early administration promoted the apoptosis of alloreactive T cells and thus prevented acute GVHD [89]. These results could be corroborated in a small clinical trial which also suggested an improvement of immune reconstitution [90]. However, timing is crucial as delayed and continued administration enhanced GVT but also aggravated GVHD symptoms [89].

24.8 Targeted Approaches

GVHD is well known as a multifactorial process with a variety of players involved, and most of these have been targeted in mouse models. However, not all could be transferred into clinical applications. Therefore, this chapter will focus on approaches for which clinical trials have been reported.

24.8.1 Targeting Cytokines

One approach is targeting the cytokines involved in the pathogenesis of GVHD. Best studied is

TNF-α, a cytokine implicated in all three stages of GVHD. Antibodies against TNF-α or other neutralizing reagents are used routinely to treat inflammatory autoimmune disease. One of them, etanercept, is a TNF-α receptor 2 fusion protein that competes for binding with TNF-α. In combination with steroids, it elicited good response rates in two studies without increased incidence of infections or relapse [91, 92]. The neutralizing TNF-α antibody, infliximab, showed efficacy in several studies, although this was accompanied by a high incidence of infections [93–97]. This can be attributed to the ability of infliximab to additionally deplete monocytes and macrophages that present membrane bound TNF-α [98]. However, it needs to be noted that none of the antibodies was superior to steroids alone [99].

Another critical cytokine in GVHD is IL-2. Its receptor α-chain, CD25, is upregulated on activated $CD4^+$ T cells. IL-2 is indispensable for the survival and proliferation of these cells. Therefore, CD25 is an interesting molecule to specifically target effector T cells. A monoclonal CD25 antibody, daclizumab, proved to be efficient in first clinical trials [100, 101]. However, a larger multicenter trial was halted due to lower survival in the daclizumab arm [102]. This could be due to the fact that Treg cells constitutively express high CD25 levels and could be preferentially depleted by the antibody. Treg cells are able to control GVHD by conferring tolerance after HSCT [103], and the depletion of beneficial Treg cells might outweigh the positive effects of depletion of effector T cells.

The receptor for IL-6 has been targeted by the mAb tocilizumab and could improve GVHD by shifting the balance of the immune response from proinflammatory effector cells toward the suppressive Treg cells [104]. The first clinical studies in patients with steroid refractory GVHD have promising results [105].

24.8.2 Targeting Co-stimulation

Specificity for the allogeneic responses in GVHD can be achieved by interfering with the activation of effector cells. Apart from engagement of the T-cell receptor by alloantigens, co-stimulation is essential for T cells to become activated and to prevent anergy and apoptosis.

The main co-stimulatory molecules expressed on T cells are the activating CD28 and its inhibitory counterpart CTLA4 (cytotoxic T-lymphocyte antigen 4). Their B7 ligands, CD80 and CD86, are provided by APC. But blocking the CD28-B7 axis affects not only the co-stimulation but also the inhibition pathway affecting Treg cells and thus exacerbating GVHD. Instead, one trial targeted CTLA4-mediated inhibition in a small group of patients and showed low incidence of GVHD. However, the procedure requires coculturing of donor and patient cells in the presence of a CTLA4-immunoglobulin fusion protein to induce tolerance of alloreactive donor cells and thus is not feasible for a larger cohort of patients [106]. Abatacept, a CTLA4-immunoglobulin fusion protein that acts *in vivo*, has shown efficacy in autoimmune disease [107] but has yet to be tested in GVHD.

The second important co-stimulatory pathway CD40/CD40L can be blocked by an antibody directed against CD40L. This was able to reduce GVHD and has a beneficial effect on Treg cells [108]. Unfortunately, clinical studies with a CD40L antibody had to be halted due to serious thrombotic side effects [109].

24.8.3 Targeting Cell Subsets

24.8.3.1 B Cells

The role of B cells in the pathogenesis of GVHD is not yet fully understood. They play an important role not only in the humoral immune response but also act as antigen-presenting cells and produce cytokines, thereby modulating GVHD [41]. Treatment of B-cell malignancies, one indication for HSCT, includes depletion of B cells with the mAb rituximab. In one study, high doses of rituximab given before HSCT were correlated with a reduced incidence of acute GVHD [110]. However, these findings could not be confirmed by all studies [111–114]. Also the effect of rituximab on chronic GVHD is controversial as two studies showed a decrease in the incidence

of chronic GVHD [111], while one group even reported an increase. However, this was blamed on additional donor lymphocytes given to a subset of these patients [112, 115]. In summary, it seems that B-cell depletion with high doses of rituximab prior to HSCT is effective and does not affect engraftment, although it delays B-cell recovery [41].

24.8.3.2 NK Cells

A novel strategy to treat GVHD is the adoptive transfer of immune cells. Natural killer (NK) cells are one of the main effectors of GVHD and GVT. Therefore, they were successfully used in clinical trials to treat patients suffering from cancer relapse [116–118]. Surprisingly, in addition to promoting GVT, they were also able to abrogate GVHD [119, 120] and improved engraftment [62] by killing recipient APC which are implicated in the induction of GVHD [121]. The beneficial effect of NK cells is most profound in an unrelated donor/recipient setting [122].

24.8.3.3 Mesenchymal Stem Cells

The most straightforward approach of cell therapy is the adoptive transfer of inhibitory cells that are able to suppress the ongoing inflammatory process. One subset suggested for this strategy is mesenchymal stem cells (MSC). This immunomodulatory cell type can induce a tolerogenic phenotype in other immune cells [123, 124] and increase Treg numbers [125]. As they act in an MHC-independent manner and do not induce alloreactive responses, they were considered an ideal target for cell therapy [126]. MSC could indeed inhibit GVHD in one mouse model [127]. But although another model could confirm the suppressive function of MSC, no effect on GVHD was observed [128]. A canine GVHD model was also unable to detect any benefit of treatment with MSC [129]. Several small clinical trials confirmed that MSC can improve GVHD [130–132] with pediatric patients in general responding better to the treatment. However, a large phase III trial failed to show any benefit of MSC transfer in GVHD patients. Further studies need to demonstrate whether optimizing the dosage and timing of treatment with MSC can provide more reliable data [133].

24.8.3.4 Treg Cells

Treg cells are the main suppressors of the immune system and have been implicated in the control of GVHD. Transfer of Treg cells can rescue GVHD while maintaining the GVT effect [56, 134, 135]. The main problem for employing Treg cells for clinical application is that their only lineage-specific marker, the intracellular transcription factor FoxP3 [136], cannot be used for isolation of live cells. Thus, Treg cells have to be selected by surrogate surface markers. First studies used expression of CD25 for enrichment [137]. Although Treg cells express high levels of CD25, also activated T-effector cells upregulate CD25 and are thus indistinguishable from their regulatory counterparts. Nevertheless, the first phase I clinical trial was performed with cells from apheresis blood that were positively selected for CD25 after depletion of CD19+ B cells. This study with nine patients confirmed that adoptive transfer of these Treg cells is safe and feasible [138]. Another group used a similar approach to isolated Treg cells. These cells were administered as sole prevention measure against GVHD in 28 patients with high-risk malignancies who had received HLA-haploidentical HSCT. In these patients, a reduction in GVHD and CMV reactivation without loss of GVT was reported [139].

Another approach is the isolation of Treg cells not from adults but from umbilical cord blood. As those T cells are mainly in an antigen inexperienced naïve state, the majority remains CD25 negative [140]. Thus, selection of CD25-positive cells yields a relatively pure population of Treg cells. The drawback of the usage of cord blood is the low number of cells available in one unit. Therefore, the isolated cells were expanded to obtain sufficient numbers. Transfer of these cord blood-derived Treg cells into patients who had previously received a transplant of cord blood stem cells resulted in a reduction in GVHD incidence [141]. However, the low yield of Treg cells from umbilical cord blood and the need for cell expansion make the application for a larger patient cohort challenging.

The report that the IL-7 receptor, CD127, is expressed on all effector T cells but Treg cells

lack its expression [142] suggested a different strategy for isolation. Depletion of CD127-expressing cells followed by positive selection of CD25 cells yields functionally suppressive Treg cells. In a case study, these cells were expanded and transferred into one patient with acute and one with chronic GVHD. In both, symptoms of GVHD improved at least transiently [143].

Combination with a second marker that is selectively absent on Treg cells, namely, CD49d, the α-chain of the integrin VLA-4, allows the isolation of untouched Treg cells. Single-step depletion of all CD127- and CD49d-expressing cells yields highly pure functional Treg cells [134]. A trial using these cells is in preparation.

Apart from adoptive transfer of Treg cells, several attempts have been made to target these cells *in vivo*. The cytokine IL-2 is indispensible for the function and survival of these cells, and they express high levels of its receptor CD25. This distinguishes them from CD4 effector cells which only after activation upregulate CD25. However, they require much higher concentrations of IL-2 than Treg cells. To exploit this difference, in two human trials, patients were treated with low-dose IL-2. Both studies detected increased numbers of Treg cells [144], and in one of them, symptoms of chronic GVHD were alleviated [145].

Treg and T-effector cells also use different signaling pathways. Sirolimus or rapamycin is an immunosuppressant that blocks the mammalian target of rapamycin (mTOR) and thereby cytokine secretion of T cells. Treg cells instead signal via STAT3 and STAT5 after activation and are spared from the suppressive effect of sirolimus. Some reports even find a preferential expansion of Treg cells in its presence [146, 147]. Clinical trials have confirmed the efficacy of sirolimus to prevent GVHD although toxicities limit its usage [148, 149].

A combination of sirolimus and IL-2 was reported as particularly efficient in promoting Treg cells by enhancing proliferation of natural Treg cells and conversion of induced Treg cells in a mouse model [150], but clinical data are still pending.

24.9 Concluding Remarks

Hematopoietic stem cell transplantation is often the last resort for patients with malignancies although the long-term outcome is still disappointing. One major complication is GVHD, a fulminant inflammatory response that is difficult to treat with conventional immunosuppression. New approaches target the different pathways involved in GVHD. Results of first clinical trials are promising, but no therapy is efficient for all patients. Heterogeneous patient cohorts and concomitant treatments make it difficult to compare results from different studies. The networks involved in the pathogenesis of GVHD are complimentary, and it is unlikely that targeting only one pathway will be sufficient. It is also possible that certain pathways are more important in one patient group than in others. In addition, genetic differences contribute to this heterogeneity and might be responsible for discrepancies observed in many trials. A more thorough selection of patients for specific therapies might give clearer results in the future. It might also prove helpful to identify relevant pathways to provide a more tailored therapy for individual patients which would not only improve efficacy of the therapy but also help to reduce side effects which are a huge problem with current medications. An additional challenge is to specifically prevent GVHD without affecting general immunity and the GVT effect. Eventually, combination of different therapeutic strategies and more individualized treatment of patients could improve the outcome and therefore boost the success story of HSCT.

References

1. Pasquini MC, Wang Z. Current use and outcome of hematopoietic stem cell transplantation: CIBMTR Summary Slides. 2012. http://www.cibmtr.org.
2. Goker H, Haznedaroglu IC, Chao NJ. Acute graft-vs-host disease: pathobiology and management. Exp Hematol. 2001;29(3):259–77.

3. Teshima T. Th1 and Th17 join forces for acute GVHD. Blood. 2011;118(18):4765–7.
4. Inamoto Y, Flowers ME. Treatment of chronic graft-versus-host disease in 2011. Curr Opin Hematol. 2011;18(6):414–20.
5. Soderberg C, Larsson S, Rozell BL, Sumitran-Karuppan S, Ljungman P, Moller E. Cytomegalovirus-induced CD13-specific autoimmunity–a possible cause of chronic graft-vs-host disease. Transplantation. 1996;61(4):600–9.
6. Patriarca F, Skert C, Sperotto A, Zaja F, Falleti E, Mestroni R, et al. The development of autoantibodies after allogeneic stem cell transplantation is related with chronic graft-vs-host disease and immune recovery. Exp Hematol. 2006;34(3):389–96.
7. Lee SJ, Vogelsang G, Flowers ME. Chronic graft-versus-host disease. Biol Blood Marrow Transplant. 2003;9(4):215–33.
8. Jacobsohn DA, Vogelsang GB. Acute graft versus host disease. Orphanet J Rare Dis. 2007;2:35.
9. Billingham RE. The biology of graft-versus-host reactions. Harvey Lect. 1966;62:21–78.
10. Ferrara JL, Cooke KR, Pan L, Krenger W. The immunopathophysiology of acute graft-versus-host-disease. Stem Cells. 1996;14(5):473–89.
11. Ferrara JL, Levy R, Chao NJ. Pathophysiologic mechanisms of acute graft-vs.-host disease. Biol Blood Marrow Transplant. 1999;5(6):347–56.
12. Cavet J, Middleton PG, Segall M, Noreen H, Davies SM, Dickinson AM. Recipient tumor necrosis factor-alpha and interleukin-10 gene polymorphisms associate with early mortality and acute graft-versus-host disease severity in HLA-matched sibling bone marrow transplants. Blood. 1999;94(11):3941–6.
13. Middleton PG, Taylor PR, Jackson G, Proctor SJ, Dickinson AM. Cytokine gene polymorphisms associating with severe acute graft-versus-host disease in HLA-identical sibling transplants. Blood. 1998;92(10):3943–8.
14. Symington FW, Symington BE, Liu PY, Viguet H, Santhanam U, Sehgal PB. The relationship of serum IL-6 levels to acute graft-versus-host disease and hepatorenal disease after human bone marrow transplantation. Transplantation. 1992;54(3):457–62.
15. Cullup H, Dickinson AM, Jackson GH, Taylor PR, Cavet J, Middleton PG. Donor interleukin 1 receptor antagonist genotype associated with acute graft-versus-host disease in human leucocyte antigen-matched sibling allogeneic transplants. Br J Haematol. 2001;113(3):807–13.
16. Cavet J, Dickinson AM, Norden J, Taylor PR, Jackson GH, Middleton PG. Interferon-gamma and interleukin-6 gene polymorphisms associate with graft-versus-host disease in HLA-matched sibling bone marrow transplantation. Blood. 2001;98(5):1594–600.
17. Ambruzova Z, Mrazek F, Raida L, Jindra P, Vidan-Jeras B, Faber E, et al. Association of IL6 and CCL2 gene polymorphisms with the outcome of allogeneic haematopoietic stem cell transplantation. Bone Marrow Transplant. 2009;44(4):227–35.
18. Socie G, Loiseau P, Tamouza R, Janin A, Busson M, Gluckman E, et al. Both genetic and clinical factors predict the development of graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. Transplantation. 2001;72(4):699–706.
19. Wilhelm K, Ganesan J, Muller T, Durr C, Grimm M, Beilhack A, et al. Graft-versus-host disease is enhanced by extracellular ATP activating P2X7R. Nat Med. 2010;16(12):1434–8.
20. Borsellino G, Kleinewietfeld M, Di Mitri D, Sternjak A, Diamantini A, Giometto R, et al. Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression. Blood. 2007;110(4):1225–32.
21. Aswad F, Kawamura H, Dennert G. High sensitivity of CD4+CD25+ regulatory T cells to extracellular metabolites nicotinamide adenine dinucleotide and ATP: a role for P2X7 receptors. J Immunol. 2005;175(5):3075–83.
22. Heimesaat MM, Nogai A, Bereswill S, Plickert R, Fischer A, Loddenkemper C, et al. MyD88/TLR9 mediated immunopathology and gut microbiota dynamics in a novel murine model of intestinal graft-versus-host disease. Gut. 2010;59(8):1079–87.
23. Calcaterra C, Sfondrini L, Rossini A, Sommariva M, Rumio C, Menard S, et al. Critical role of TLR9 in acute graft-versus-host disease. J Immunol. 2008;181(9):6132–9.
24. Imado T, Iwasaki T, Kitano S, Satake A, Kuroiwa T, Tsunemi S, et al. The protective role of host Toll-like receptor-4 in acute graft-versus-host disease. Transplantation. 2010;90(10):1063–70.
25. Hossain MS, Jaye DL, Pollack BP, Farris AB, Tselanyane ML, David E, et al. Flagellin, a TLR5 agonist, reduces graft-versus-host disease in allogeneic hematopoietic stem cell transplantation recipients while enhancing antiviral immunity. J Immunol. 2011;187(10):5130–40.
26. Li H, Matte-Martone C, Tan HS, Venkatesan S, McNiff J, Demetris AJ, et al. Graft-versus-host disease is independent of innate signaling pathways triggered by pathogens in host hematopoietic cells. J Immunol. 2011;186(1):230–41.
27. Chakraverty R, Sykes M. The role of antigen-presenting cells in triggering graft-versus-host disease and graft-versus-leukemia. Blood. 2007;110(1):9–17.
28. den Haan JM, Sherman NE, Blokland E, Huczko E, Koning F, Drijfhout JW, et al. Identification of a graft versus host disease-associated human minor histocompatibility antigen. Science. 1995;268(5216):1476–80.
29. Koyama M, Kuns RD, Olver SD, Raffelt NC, Wilson YA, Don AL, et al. Recipient nonhematopoietic antigen-presenting cells are sufficient to induce lethal acute graft-versus-host disease. Nat Med. 2012;18(1):135–42.
30. Toubai T, Tawara I, Sun Y, Liu C, Nieves E, Evers R, et al. Induction of acute GVHD by sex-mismatched H-Y antigens in the absence of functional radiosensitive host hematopoietic-derived antigen-presenting cells. Blood. 2012;119(16):3844–53.

31. Matte CC, Liu J, Cormier J, Anderson BE, Athanasiadis I, Jain D, et al. Donor APCs are required for maximal GVHD but not for GVL. Nat Med. 2004;10(9):987–92.
32. Shlomchik WD, Couzens MS, Tang CB, McNiff J, Robert ME, Liu J, et al. Prevention of graft versus host disease by inactivation of host antigen-presenting cells. Science. 1999;285(5426):412–5.
33. Teshima T, Ordemann R, Reddy P, Gagin S, Liu C, Cooke KR, et al. Acute graft-versus-host disease does not require alloantigen expression on host epithelium. Nat Med. 2002;8(6):575–81.
34. Wang X, Li H, Matte-Martone C, Cui W, Li N, Tan HS, et al. Mechanisms of antigen presentation to T cells in murine graft-versus-host disease: cross-presentation and the appearance of cross-presentation. Blood. 2011;118(24):6426–37.
35. Blazar BR, Taylor PA, Panoskaltsis-Mortari A, Gray GS, Vallera DA. Coblockade of the LFA1:ICAM and CD28/CTLA4:B7 pathways is a highly effective means of preventing acute lethal graft-versus-host disease induced by fully major histocompatibility complex-disparate donor grafts. Blood. 1995;85(9):2607–18.
36. Yang Y, Wilson JM. CD40 ligand-dependent T cell activation: requirement of B7-CD28 signaling through CD40. Science. 1996;273(5283):1862–4.
37. Baxter AG, Hodgkin PD. Activation rules: the two-signal theories of immune activation. Nat Rev Immunol. 2002;2(6):439–46.
38. Lai HY, Chou TY, Tzeng CH, Lee OK. Cytokine profiles in various graft-versus-host disease target organs following hematopoietic stem cell transplantation. Cell Transplant. 2012;21(9):2033–45.
39. Zheng H, Matte-Martone C, Li H, Anderson BE, Venketesan S, Sheng Tan H, et al. Effector memory CD4+ T cells mediate graft-versus-leukemia without inducing graft-versus-host disease. Blood. 2008; 111(4):2476–84.
40. Blazar BR, Murphy WJ, Abedi M. Advances in graft-versus-host disease biology and therapy. Nat Rev Immunol. 2012;12(6):443–58.
41. Shimabukuro-Vornhagen A, Hallek MJ, Storb RF, von Bergwelt-Baildon MS. The role of B cells in the pathogenesis of graft-versus-host disease. Blood. 2009;114(24):4919–27.
42. Lowin B, Hahne M, Mattmann C, Tschopp J. Cytolytic T-cell cytotoxicity is mediated through perforin and Fas lytic pathways. Nature. 1994;370(6491):650–2.
43. Via CS, Nguyen P, Shustov A, Drappa J, Elkon KB. A major role for the Fas pathway in acute graft-versus-host disease. J Immunol. 1996;157(12): 5387–93.
44. Reddy P. Pathophysiology of acute graft-versus-host disease. Hematol Oncol. 2003;21(4):149–61.
45. Nestel FP, Price KS, Seemayer TA, Lapp WS. Macrophage priming and lipopolysaccharide-triggered release of tumor necrosis factor alpha during graft-versus-host disease. J Exp Med. 1992;175(2):405–13.
46. Kitko CL, Paczesny S, Yanik G, Braun T, Jones D, Whitfield J, et al. Plasma elevations of tumor necrosis factor-receptor-1 at day 7 postallogeneic transplant correlate with graft-versus-host disease severity and overall survival in pediatric patients. Biol Blood Marrow Transplant. 2008;14(7):759–65.
47. Choi SW, Kitko CL, Braun T, Paczesny S, Yanik G, Mineishi S, et al. Change in plasma tumor necrosis factor receptor 1 levels in the first week after myeloablative allogeneic transplantation correlates with severity and incidence of GVHD and survival. Blood. 2008;112(4):1539–42.
48. Holler E, Kolb HJ, Moller A, Kempeni J, Liesenfeld S, Pechumer H, et al. Increased serum levels of tumor necrosis factor alpha precede major complications of bone marrow transplantation. Blood. 1990;75(4):1011–6.
49. Cooke KR, Hill GR, Crawford JM, Bungard D, Brinson YS, Delmonte Jr J, et al. Tumor necrosis factor- alpha production to lipopolysaccharide stimulation by donor cells predicts the severity of experimental acute graft-versus-host disease. J Clin Invest. 1998;102(10):1882–91.
50. Chen YB, McDonough S, Hasserjian R, Chen H, Coughlin E, Illiano C, et al. Expression of CD30 in patients with acute graft-versus-host disease. Blood. 2012;120(3):691–6.
51. Paczesny S, Krijanovski OI, Braun TM, Choi SW, Clouthier SG, Kuick R, et al. A biomarker panel for acute graft-versus-host disease. Blood. 2009;113(2):273–8.
52. Wing K, Sakaguchi S. Regulatory T cells exert checks and balances on self tolerance and autoimmunity. Nat Immunol. 2010;11(1):7–13.
53. Modigliani Y, Coutinho A, Pereira P, Le Douarin N, Thomas-Vaslin V, Burlen-Defranoux O, et al. Establishment of tissue-specific tolerance is driven by regulatory T cells selected by thymic epithelium. Eur J Immunol. 1996;26(8):1807–15.
54. O'Garra A, Vieira P. Regulatory T cells and mechanisms of immune system control. Nat Med. 2004;10(8):801–5.
55. Tang Q, Bluestone JA. The Foxp3+ regulatory T cell: a jack of all trades, master of regulation. Nat Immunol. 2008;9(3):239–44.
56. Taylor PA, Lees CJ, Blazar BR. The infusion of ex vivo activated and expanded CD4(+)CD25(+) immune regulatory cells inhibits graft-versus-host disease lethality. Blood. 2002;99(10):3493–9.
57. Hoffmann P, Ermann J, Edinger M, Fathman CG, Strober S. Donor-type CD4(+)CD25(+) regulatory T cells suppress lethal acute graft-versus-host disease after allogeneic bone marrow transplantation. J Exp Med. 2002;196(3):389–99.
58. Miura Y, Thoburn CJ, Bright EC, Phelps ML, Shin T, Matsui EC, et al. Association of Foxp3 regulatory gene expression with graft-versus-host disease. Blood. 2004;104(7):2187–93.
59. Ukena SN, Geffers R, Buchholz S, Stadler M, Franzke A. Biomarkers for acute and chronic

graft-versus-host disease in regulatory T cells. Transpl Immunol. 2012;27(4):179–83.
60. Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey J, Kolb HJ, et al. Graft-versus-leukemia reactions after bone marrow transplantation. Blood. 1990;75(3):555–62.
61. Valcarcel D, Martino R, Caballero D, Martin J, Ferra C, Nieto JB, et al. Sustained remissions of high-risk acute myeloid leukemia and myelodysplastic syndrome after reduced-intensity conditioning allogeneic hematopoietic transplantation: chronic graft-versus-host disease is the strongest factor improving survival. J Clin Oncol. 2008;26(4):577–84.
62. Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science. 2002;295(5562):2097–100.
63. Saito TI, Li HW, Sykes M. Invariant NKT cells are required for antitumor responses induced by host-versus-graft responses. J Immunol. 2010;185(4):2099–105.
64. Bacigalupo A, Van Lint MT, Occhini D, Gualandi F, Lamparelli T, Sogno G, et al. Increased risk of leukemia relapse with high-dose cyclosporine A after allogeneic marrow transplantation for acute leukemia. Blood. 1991;77(7):1423–8.
65. Baron F, Maris MB, Sandmaier BM, Storer BE, Sorror M, Diaconescu R, et al. Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. J Clin Oncol. 2005;23(9):1993–2003.
66. Toze CL, Galal A, Barnett MJ, Shepherd JD, Conneally EA, Hogge DE, et al. Myeloablative allografting for chronic lymphocytic leukemia: evidence for a potent graft-versus-leukemia effect associated with graft-versus-host disease. Bone Marrow Transplant. 2005;36(9):825–30.
67. Kolb HJ, Mittermuller J, Clemm C, Holler E, Ledderose G, Brehm G, et al. Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. Blood. 1990;76(12):2462–5.
68. Kotsiou E, Davies JK. New ways to separate graft-versus-host disease and graft-versus-tumour effects after allogeneic haematopoietic stem cell transplantation. Br J Haematol. 2013;160(2):133–45.
69. Mielke S, Solomon SR, Barrett AJ. Selective depletion strategies in allogeneic stem cell transplantation. Cytotherapy. 2005;7(2):109–15.
70. Bastien JP, Roy J, Roy DC. Selective T-cell depletion for haplotype-mismatched allogeneic stem cell transplantation. Semin Oncol. 2012;39(6):674–82.
71. Bram RJ, Hung DT, Martin PK, Schreiber SL, Crabtree GR. Identification of the immunophilins capable of mediating inhibition of signal transduction by cyclosporin A and FK506: roles of calcineurin binding and cellular location. Mol Cell Biol. 1993;13(8):4760–9.
72. Ratanatharathorn V, Nash RA, Przepiorka D, Devine SM, Klein JL, Weisdorf D, et al. Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. Blood. 1998;92(7):2303–14.
73. Nash RA, Antin JH, Karanes C, Fay JW, Avalos BR, Yeager AM, et al. Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. Blood. 2000;96(6):2062–8.
74. Thomson AW. FK-506 enters the clinic. Immunol Today. 1990;11(2):35–6.
75. Morris RE, Hoyt EG, Murphy MP, Eugui EM, Allison AC. Mycophenolic acid morpholinoethylester (RS-61443) is a new immunosuppressant that prevents and halts heart allograft rejection by selective inhibition of T- and B-cell purine synthesis. Transplant Proc. 1990;22(4):1659–62.
76. Deeg HJ, Lin D, Leisenring W, Boeckh M, Anasetti C, Appelbaum FR, et al. Cyclosporine or cyclosporine plus methylprednisolone for prophylaxis of graft-versus-host disease: a prospective, randomized trial. Blood. 1997;89(10):3880–7.
77. Alyea EP, Li S, Kim HT, Cutler C, Ho V, Soiffer RJ, et al. Sirolimus, tacrolimus, and low-dose methotrexate as graft-versus-host disease prophylaxis in related and unrelated donor reduced-intensity conditioning allogeneic peripheral blood stem cell transplantation. Biol Blood Marrow Transplant. 2008;14(8):920–6.
78. Schleuning M, Judith D, Jedlickova Z, Stubig T, Heshmat M, Baurmann H, et al. Calcineurin inhibitor-free GVHD prophylaxis with sirolimus, mycophenolate mofetil and ATG in Allo-SCT for leukemia patients with high relapse risk: an observational cohort study. Bone Marrow Transplant. 2009;43(9):717–23.
79. Theurich S, Fischmann H, Chakupurakal G, Shimabukuro-Vornhagen A, Chemnitz JM, Holtick U, et al. Anti-thymocyte globulins for post-transplant graft-versus-host disease prophylaxis-A systematic review and meta-analysis. Crit Rev Oncol Hematol. 2013;88(1):178–86.
80. Bacigalupo A, Lamparelli T, Gualandi F, Bregante S, Raiola AM, Di Grazia C, et al. Prophylactic antithymocyte globulin reduces the risk of chronic graft-versus-host disease in alternative-donor bone marrow transplants. Biol Blood Marrow Transplant. 2002;8(12):656–61.
81. Finke J, Bethge WA, Schmoor C, Ottinger HD, Stelljes M, Zander AR, et al. Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. Lancet Oncol. 2009;10(9):855–64.
82. Socie G, Schmoor C, Bethge WA, Ottinger HD, Stelljes M, Zander AR, et al. Chronic graft-versus-host disease: long-term results from a randomized trial on graft-versus-host disease prophylaxis with or

without anti-T-cell globulin ATG-Fresenius. Blood. 2011;117(23):6375–82.
83. Dall'Amico R, Messina C. Extracorporeal photochemotherapy for the treatment of graft-versus-host disease. Ther Apher. 2002;6(4):296–304.
84. Marshall SR. Technology insight: ECP for the treatment of GvHD–can we offer selective immune control without generalized immunosuppression? Nat Clin Pract Oncol. 2006;3(6):302–14.
85. Schonbeck U, Libby P. Inflammation, immunity, and HMG-CoA reductase inhibitors: statins as antiinflammatory agents? Circulation. 2004;109(21 Suppl 1):II18–26.
86. Zeiser R, Youssef S, Baker J, Kambham N, Steinman L, Negrin RS. Preemptive HMG-CoA reductase inhibition provides graft-versus-host disease protection by Th-2 polarization while sparing graft-versus-leukemia activity. Blood. 2007;110(13):4588–98.
87. Rotta M, Storer BE, Storb RF, Martin PJ, Heimfeld S, Peffer A, et al. Donor statin treatment protects against severe acute graft-versus-host disease after related allogeneic hematopoietic cell transplantation. Blood. 2010;115(6):1288–95.
88. Rotta M, Storer BE, Storb R, Martin PJ, Flowers ME, Vernon MS, et al. Impact of recipient statin treatment on graft-versus-host disease after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2010;16(10):1463–6.
89. Sun K, Li M, Sayers TJ, Welniak LA, Murphy WJ. Differential effects of donor T-cell cytokines on outcome with continuous bortezomib administration after allogeneic bone marrow transplantation. Blood. 2008;112(4):1522–9.
90. Koreth J, Stevenson KE, Kim HT, McDonough SM, Bindra B, Armand P, et al. Bortezomib-based graft-versus-host disease prophylaxis in HLA-mismatched unrelated donor transplantation. J Clin Oncol. 2012;30(26):3202–8.
91. Levine JE, Paczesny S, Mineishi S, Braun T, Choi SW, Hutchinson RJ, et al. Etanercept plus methylprednisolone as initial therapy for acute graft-versus-host disease. Blood. 2008;111(4):2470–5.
92. Alousi AM, Weisdorf DJ, Logan BR, Bolanos-Meade J, Carter S, Difronzo N, et al. Etanercept, mycophenolate, denileukin, or pentostatin plus corticosteroids for acute graft-versus-host disease: a randomized phase 2 trial from the blood and marrow transplant clinical trials network. Blood. 2009;114(3):511–7.
93. Couriel DR, Saliba R, de Lima M, Giralt S, Andersson B, Khouri I, et al. A phase III study of infliximab and corticosteroids for the initial treatment of acute graft-versus-host disease. Biol Blood Marrow Transplant. 2009;15(12):1555–62.
94. Busca A, Locatelli F, Marmont F, Ceretto C, Falda M. Recombinant human soluble tumor necrosis factor receptor fusion protein as treatment for steroid refractory graft-versus-host disease following allogeneic hematopoietic stem cell transplantation. Am J Hematol. 2007;82(1):45–52.
95. Sleight BS, Chan KW, Braun TM, Serrano A, Gilman AL. Infliximab for GVHD therapy in children. Bone Marrow Transplant. 2007;40(5):473–80.
96. Patriarca F, Sperotto A, Damiani D, Morreale G, Bonifazi F, Olivieri A, et al. Infliximab treatment for steroid-refractory acute graft-versus-host disease. Haematologica. 2004;89(11):1352–59.
97. Jacobsohn DA, Hallick J, Anders V, McMillan S, Morris L, Vogelsang GB. Infliximab for steroid-refractory acute GVHD: a case series. Am J Hematol. 2003;74(2):119–24.
98. Ehlers S. Tumor necrosis factor and its blockade in granulomatous infections: differential modes of action of infliximab and etanercept? Clin Infect Dis. 2005;41 Suppl 3:S199–203.
99. Levine JE. Implications of TNF-alpha in the pathogenesis and management of GVHD. Int J Hematol. 2011;93(5):571–7.
100. Bordigoni P, Dimicoli S, Clement L, Baumann C, Salmon A, Witz F, et al. Daclizumab, an efficient treatment for steroid-refractory acute graft-versus-host disease. Br J Haematol. 2006;135(3):382–5.
101. Przepiorka D, Kernan NA, Ippoliti C, Papadopoulos EB, Giralt S, Khouri I, et al. Daclizumab, a humanized anti-interleukin-2 receptor alpha chain antibody, for treatment of acute graft-versus-host disease. Blood. 2000;95:83–9.
102. Lee SJ, Zahrieh D, Agura E, MacMillan ML, Maziarz RT, McCarthy Jr PL, et al. Effect of up-front daclizumab when combined with steroids for the treatment of acute graft-versus-host disease: results of a randomized trial. Blood. 2004;104:1559–64.
103. Zeiser R, Nguyen VH, Beilhack A, Buess M, Schulz S, Baker J, et al. Inhibition of CD4+CD25+ regulatory T-cell function by calcineurin-dependent interleukin-2 production. Blood. 2006;108(1):390–9.
104. Chen X, Das R, Komorowski R, Beres A, Hessner MJ, Mihara M, et al. Blockade of interleukin-6 signaling augments regulatory T-cell reconstitution and attenuates the severity of graft-versus-host disease. Blood. 2009;114:891–900.
105. Drobyski WR, Pasquini M, Kovatovic K, Palmer J, Douglas Rizzo J, Saad A, et al. Tocilizumab for the treatment of steroid refractory graft-versus-host disease. Biol Blood Marrow Transplant. 2011;17(12):1862–8.
106. Guinan EC, Boussiotis VA, Neuberg D, Brennan LL, Hirano N, Nadler LM, et al. Transplantation of anergic histoincompatible bone marrow allografts. N Engl J Med. 1999;340(22):1704–14.
107. Weinblatt ME, Moreland LW, Westhovens R, Cohen RB, Kelly SM, Khan N, et al. Safety of abatacept administered intravenously in treatment of rheumatoid arthritis: integrated analyses of up to 8 years of treatment from the abatacept clinical trial program. J Rheumatol. 2013;40(6):787–97.
108. Taylor PA, Friedman TM, Korngold R, Noelle RJ, Blazar BR. Tolerance induction of alloreactive T cells via ex vivo blockade of the CD40:CD40L costimulatory pathway results in the generation of a

potent immune regulatory cell. Blood. 2002; 99(12):4601–9.
109. Sidiropoulos PI, Boumpas DT. Lessons learned from anti-CD40L treatment in systemic lupus erythematosus patients. Lupus. 2004;13(5):391–7.
110. Ratanatharathorn V, Logan B, Wang D, Horowitz M, Uberti JP, Ringden O, et al. Prior rituximab correlates with less acute graft-versus-host disease and better survival in B-cell lymphoma patients who received allogeneic peripheral blood stem cell transplantation. Br J Haematol. 2009;145:816–24.
111. van Dorp S, Pietersma F, Wolfl M, Verdonck LF, Petersen EJ, Lokhorst HM, et al. Rituximab treatment before reduced-intensity conditioning transplantation associates with a decreased incidence of extensive chronic GVHD. Biol Blood Marrow Transplant. 2009;15(6):671–8.
112. Kharfan-Dabaja MA, Cutler CS. Rituximab for prevention and treatment of graft-versus-host disease. Int J Hematol. 2011;93(5):578–85.
113. Khouri IF, Saliba RM, Giralt SA, Lee MS, Okoroji GJ, Hagemeister FB, et al. Nonablative allogeneic hematopoietic transplantation as adoptive immunotherapy for indolent lymphoma: low incidence of toxicity, acute graft-versus-host disease, and treatment-related mortality. Blood. 2001;98(13):3595–9.
114. Khouri IF, McLaughlin P, Saliba RM, Hosing C, Korbling M, Lee MS, et al. Eight-year experience with allogeneic stem cell transplantation for relapsed follicular lymphoma after nonmyeloablative conditioning with fludarabine, cyclophosphamide, and rituximab. Blood. 2008;111:5530–6.
115. Khouri IF, Saliba RM, Admirand J, O'Brien S, Lee MS, Korbling M, et al. Graft-versus-leukaemia effect after non-myeloablative haematopoietic transplantation can overcome the unfavourable expression of ZAP-70 in refractory chronic lymphocytic leukaemia. Br J Haematol. 2007;137:355–63.
116. Shook DR, Campana D. Natural killer cell engineering for cellular therapy of cancer. Tissue Antigens. 2011;78(6):409–15.
117. Geller MA, Cooley S, Judson PL, Ghebre R, Carson LF, Argenta PA, et al. A phase II study of allogeneic natural killer cell therapy to treat patients with recurrent ovarian and breast cancer. Cytotherapy. 2011;13(1):98–107.
118. Bachanova V, Burns LJ, McKenna DH, Curtsinger J, Panoskaltsis-Mortari A, Lindgren BR, et al. Allogeneic natural killer cells for refractory lymphoma. Cancer Immunol Immunother. 2010;59(11): 1739–44.
119. Asai O, Longo DL, Tian ZG, Hornung RL, Taub DD, Ruscetti FW, et al. Suppression of graft-versus-host disease and amplification of graft-versus-tumor effects by activated natural killer cells after allogeneic bone marrow transplantation. J Clin Invest. 1998;101(9):1835–42.
120. Olson JA, Leveson-Gower DB, Gill S, Baker J, Beilhack A, Negrin RS. NK cells mediate reduction of GVHD by inhibiting activated, alloreactive T cells while retaining GVT effects. Blood. 2010; 115(21):4293–301.
121. Ruggeri L, Mancusi A, Burchielli E, Capanni M, Carotti A, Aloisi T, et al. NK cell alloreactivity and allogeneic hematopoietic stem cell transplantation. Blood Cells Mol Dis. 2008;40(1):84–90.
122. Tanaka M, Kobayashi S, Numata A, Tachibana T, Takasaki H, Maruta A, et al. The impact of the dose of natural killer cells in the graft on severe acute graft-versus-host disease after unrelated bone marrow transplantation. Leuk Res. 2012;36(6): 699–703.
123. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood. 2005;105(4):1815–22.
124. Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood. 2002;99(10):3838–43.
125. Prevosto C, Zancolli M, Canevali P, Zocchi MR, Poggi A. Generation of CD4+ or CD8+ regulatory T cells upon mesenchymal stem cell-lymphocyte interaction. Haematologica. 2007;92(7):881–8.
126. Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringden O. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. Exp Hematol. 2003;31(10): 890–6.
127. Polchert D, Sobinsky J, Douglas G, Kidd M, Moadsiri A, Reina E, et al. IFN-gamma activation of mesenchymal stem cells for treatment and prevention of graft versus host disease. Eur J Immunol. 2008;38(6):1745–55.
128. Sudres M, Norol F, Trenado A, Gregoire S, Charlotte F, Levacher B, et al. Bone marrow mesenchymal stem cells suppress lymphocyte proliferation in vitro but fail to prevent graft-versus-host disease in mice. J Immunol. 2006;176:7761–7.
129. Mielcarek M, Storb R, Georges GE, Golubev L, Nikitine A, Hwang B, et al. Mesenchymal stromal cells fail to prevent acute graft-versus-host disease and graft rejection after dog leukocyte antigen-haploidentical bone marrow transplantation. Biol Blood Marrow Transplant. 2011;17(2):214–25.
130. Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet. 2008; 371(9624):1579–86.
131. Le Blanc K, Rasmusson I, Sundberg B, Gotherstrom C, Hassan M, Uzunel M, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet. 2004;363(9419):1439–41.
132. von Bonin M, Stolzel F, Goedecke A, Richter K, Wuschek N, Holig K, et al. Treatment of refractory acute GVHD with third-party MSC expanded in platelet lysate-containing medium. Bone Marrow Transplant. 2009;43:245–51.

133. Kebriaei P, Robinson S. Mesenchymal stem cell therapy in the treatment of acute and chronic graft versus host disease. Front Oncol. 2011;1:16.
134. Kleinewietfeld M, Starke M, Di Mitri D, Borsellino G, Battistini L, Rotzschke O, et al. CD49d provides access to "untouched" human Foxp3+ Treg free of contaminating effector cells. Blood. 2009;113:827–36.
135. Joffre O, Santolaria T, Calise D, Al Saati T, Hudrisier D, Romagnoli P, et al. Prevention of acute and chronic allograft rejection with CD4+CD25+Foxp3+ regulatory T lymphocytes. Nat Med. 2008;14(1): 88–92.
136. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol. 2003;4(4):330–6. ni904 [pii].
137. Hoffmann P, Boeld TJ, Eder R, Albrecht J, Doser K, Piseshka B, et al. Isolation of CD4+CD25+ regulatory T cells for clinical trials. Biol Blood Marrow Transplant. 2006;12:267–74.
138. Edinger M, Hoffmann P. Regulatory T cells in stem cell transplantation: strategies and first clinical experiences. Curr Opin Immunol. 2011;23:679–84.
139. Di Ianni M, Falzetti F, Carotti A, Terenzi A, Castellino F, Bonifacio E, et al. Tregs prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation. Blood. 2011;117: 3921–8.
140. Takahata Y, Nomura A, Takada H, Ohga S, Furuno K, Hikino S, et al. CD25+CD4+ T cells in human cord blood: an immunoregulatory subset with naive phenotype and specific expression of forkhead box p3 (Foxp3) gene. Exp Hematol. 2004;32:622–9.
141. Brunstein CG, Miller JS, Cao Q, McKenna DH, Hippen KL, Curtsinger J, et al. Infusion of ex vivo expanded T regulatory cells in adults transplanted with umbilical cord blood: safety profile and detection kinetics. Blood. 2011;117:1061–70.
142. Seddiki N, Santner-Nanan B, Martinson J, Zaunders J, Sasson S, Landay A, et al. Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. J Exp Med. 2006;203:1693–700.
143. Trzonkowski P, Bieniaszewska M, Juscinska J, Dobyszuk A, Krzystyniak A, Marek N, et al. First-in-man clinical results of the treatment of patients with graft versus host disease with human ex vivo expanded CD4+CD25+CD127- T regulatory cells. Clin Immunol. 2009;133:22–6.
144. Zorn E, Mohseni M, Kim H, Porcheray F, Lynch A, Bellucci R, et al. Combined CD4+ donor lymphocyte infusion and low-dose recombinant IL-2 expand FOXP3+ regulatory T cells following allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2009;15:382–8.
145. Koreth J, Matsuoka K, Kim HT, McDonough SM, Bindra B, Alyea 3rd EP, et al. Interleukin-2 and regulatory T cells in graft-versus-host disease. N Engl J Med. 2011;365:2055–66.
146. Coenen JJ, Koenen HJ, van Rijssen E, Kasran A, Boon L, Hilbrands LB, et al. Rapamycin, not cyclosporine, permits thymic generation and peripheral preservation of CD4+ CD25+ FoxP3+ T cells. Bone Marrow Transplant. 2007;39:537–45.
147. Battaglia M, Stabilini A, Migliavacca B, Horejs-Hoeck J, Kaupper T, Roncarolo MG. Rapamycin promotes expansion of functional CD4+CD25+FOXP3+ regulatory T cells of both healthy subjects and type 1 diabetic patients. J Immunol. 2006;177:8338–47.
148. Johnston L, Florek M, Armstrong R, McCune JS, Arai S, Brown J, et al. Sirolimus and mycophenolate mofetil as GVHD prophylaxis in myeloablative, matched-related donor hematopoietic cell transplantation. Bone Marrow Transplant. 2012;47:581–8.
149. Rodriguez R, Nakamura R, Palmer JM, Parker P, Shayani S, Nademanee A, et al. A phase II pilot study of tacrolimus/sirolimus GVHD prophylaxis for sibling donor hematopoietic stem cell transplantation using 3 conditioning regimens. Blood. 2010;115:1098–105.
150. Shin HJ, Baker J, Leveson-Gower DB, Smith AT, Sega EI, Negrin RS. Rapamycin and IL-2 reduce lethal acute graft-versus-host disease associated with increased expansion of donor type CD4+CD25+Foxp3+ regulatory T cells. Blood. 2011;118:2342–50.

Index

N. Rezaei (ed.), *Cancer Immunology: Cancer Immunotherapy for Organ-Specific Tumors*,
DOI 10.1007/978-3-662-46410-6, © Springer-Verlag Berlin Heidelberg 2015