Advances in Experimental Medicine and Biology 779

Wafik S El-Deiry *Editor*

Impact of Genetic Targets on Cancer Therapy

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ISSN 0065-2598
ISBN 978-1-4614-6175-3 ISBN 978-1-4614-6176-0 (eBook)
DOI 10.1007/978-1-4614-6176-0
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2012952286

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Forward

In the last two decades, great strides have been made in unraveling molecular biology, immunology, genetics, and in some cases epigenetics as they apply to the cellular and organ pathology of cancer. This is hugely enabling in many ways as hematologists and oncologists throughout the world strive to develop the best and most personalized and often innovative recommendations to help their patients. A reasonable foundation for this and path forward is to understand what has been learned about various human tumors and to try to integrate this knowledge into medical practice. This is a daunting task by any standards in 2012 because there is simply a vast amount of genetic and genomic information upon which taking action remains unsupported by clinical evidence. This applies particularly well to individual patients whose tumors are not only unique but which can be heterogeneous and otherwise complex three-dimensional organ-like structures with multiple interacting cell types and local microenvironments. This is further amplified by the extremely rapid rate at which new information is accumulating. It should be stated that this book does not intend to set medical practice guidelines. However, progress will be less elusive and perhaps quicker if the scientific and medical communities take different approaches to accomplishing what both clinicians and patients want which is to benefit from the available knowledge. Importantly for those who treat cancer patients, our patients sadly and very often cannot wait and deserve every chance to benefit from the latest available information. Anecdotally this may be of benefit to individual patients and may lead to new directions for clinical or basic studies to move the field forward.

The authors of the various chapters were asked to comment on current practice in terms of standard of care approaches, to describe the molecular genetics and current understanding of tumor progression for their particular cancer type or hematological malignancy including the various key driver pathway alterations, to comment on cancer stem cells and the tumor microenvironment, and to include, to the best of their abilities, the available information on therapeutics targeting the molecular alterations in specific tumor types. I think this volume brings significant clinical insights for basic scientists and significant basic science and molecular

understanding for clinicians. The chapters are presented by both basic scientist and clinician authors who are using and studying the therapeutics targeting the genetic changes thereby making this volume very unique. I believe it is a very useful resource for seasoned investigators as well as students of all ages who care and who want to learn more and do more about the problem of cancer and its therapy. It is particularly rewarding that many of my colleagues at the Penn State Hershey Medical Center and Penn State Hershey Cancer Institute both in the Hematology/Oncology Division and other departments who are working on and treating the various malignancies graciously agreed to provide their valuable contributions to this effort. Hopefully you will appreciate what this volume brings, will enjoy reading it and learning from it, and perhaps might be inspired and/or motivated to get involved in the fight against cancer in your own way. There are many opportunities for doing so along many fronts despite the many challenges facing the field in terms of funding for research, access to health care and clinical trials, and costs of medical care.

Hershey, PA, USA Wafik S. El-Deiry, MD, Ph.D., FACP

Contents

Novel Antineoplastics Targeting Genetic Changes in Colorectal Cancer

Jamal Joudeh, Joshua E. Allen, Avisnata Das, Varun Prabhu, Michael Farbaniec, Jeffrey Adler, and Wafik S. El-Deiry

Abstract Cytotoxic chemotherapy remains the mainstay of the medical management of colorectal cancer (CRC). Research over the last two decades has led to a molecular understanding of the oncogenic mechanisms involved in CRC and has contributed to the rational development of antineoplastics that target these mechanisms. During carcinogenesis, genetic changes often occur in molecules that play key functional roles in cancer such as cell proliferation, angiogenesis, apoptosis, cell death and immune-mediated destruction of cancer cells. Here, we review novel antineoplastics that are approved or in development for CRC that target molecules associated with genetic aberrations in CRC. Some of these targeted antineoplastics have proven effective against other solid tumors and hold promise in treating CRC whereas others are now routinely used in combination with cytotoxic agents. This article reviews antineoplastics that target genetic changes in CRC, their antitumor mechanisms, and their stage of development.

Key words Colon cancer • Colorectal cancer • Clinical trial • Targeted agents • Cancer therapy • Cancer genetics

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W.S. El-Deiry (ed.), *Impact of Genetic Targets on Cancer Therapy*, Advances in Experimental Medicine and Biology 779, DOI 10.1007/978-1-4614-6176-0_1,

Introduction

Colon cancer has the third highest incidence and mortality among cancers in both men and women in the United States. There has been a recent decline in CRC mortality in developed countries because of increasingly better early detection methods and improved therapeutic options. Screening colonoscopy has decreased the mortality rate by 50% in CRC in United States [1]. Symptomatic patients usually present with hematochezia or melena, abdominal pain, unexplained iron deficiency anemia and/or a change in bowel habits. Left-sided cancer usually presents with changes in bowel movement while right-sided cancers often present with occult bleeding.

The majority of CRCs are adenocarcinomas with 70–90% being sporadic whereas less than 10% of patients have true inherited genetic factors linked to colorectal cancers. Most colorectal cancers progress from normal epithelium to invasive cancer via an intermediate precursor, the adenomatous polyp. This transformation was linked to a multistep process of specific genetic changes. Individuals with familial adenomatous polyposis (FAP) and individuals with hereditary nonpolyposis colon cancer syndrome (HNPCC, Lynch syndrome) were found to have an early single germline mutation while sporadic cancers result from the stepwise accumulation of multiple somatic mutations (Fig. 1). Studies showed that most colorectal cancers

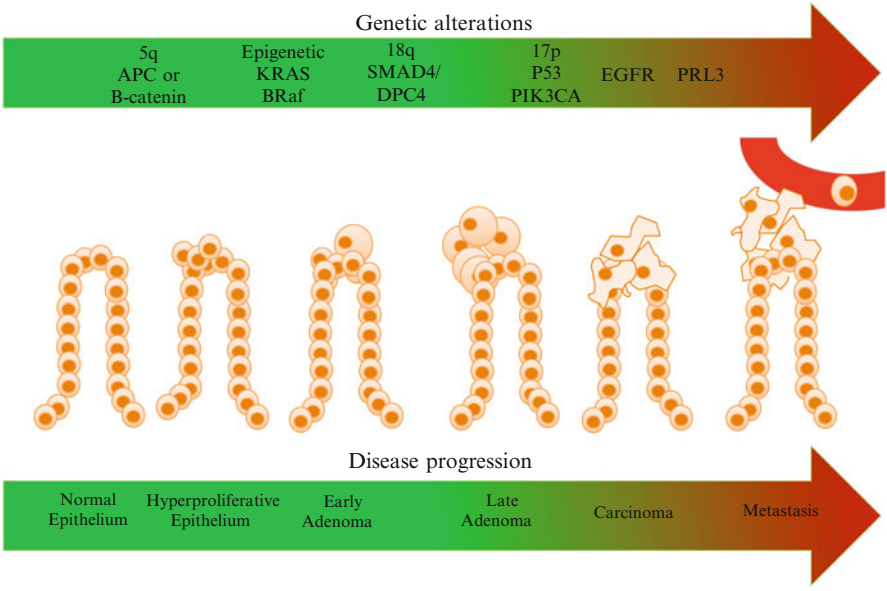

Fig. 1 Linear model of genetic changes that drive CRC. At the earliest stage of colon cancer genesis, normal colonic cells advance to a hyperproliferative state by mutations that inactivate either the APC gene on chromosome 5q or activate beta-catenin. Epigenetics and mutations in either KRAS or BRAF typically change the hyperproliferative cells into the early adenoma stage. SMAD4/DPC4 mutation on chromosome 18q then advances the mutant cells to a late adenoma stage. Finally, mutations in PIK3CA and p53 on chromosome 17p can transform late adenoma into carcinoma. Metastasis can occur during disease progression, which has been associated with PRL3 gene amplification

begin with inactivation (through a germline or sporadic mutation) of the APC gene. Chromosome 18 loss of heterozygosity (LOH), DCC deletion, KRAS oncogene mutation and p53 mutations were found to be a late event in colon carcinogenesis. The MSI-high (MSI-H) phenotype is associated with HNPCC syndrome but it is also found in 10–15% of sporadic colorectal cancers.

The TNM staging system is the international standard for staging colorectal cancer. The pathologic features at diagnosis (depth of bowel wall penetration (T), number of locoregional lymph nodes involved (N), and presence of extra-colonic metastases (M)) remain the best indicators of long-term prognosis for both colon and rectal cancer. Surgical resection is the only curative treatment for locoregional disease (stage I–III) and may be a curative option for patients with limited metastatic disease involving the liver and/or lungs (stage IV).

Adjuvant chemotherapy is usually reserved for patients with high-risk stage II and stage III (node-positive) disease. In the MOSAIC trial, 5-Fluorouracil, leucovorin, and oxaliplatin (FOLFOX versus 5-Fluorouracil, leucovorin (5-FU/LV), there was a trend toward improved disease-free survival with FOLFOX in the subgroup of stage II patients with high-risk tumors (clinical T4, poorly differentiated, perforation, obstruction, or <10 nodes in the surgical specimen). Overall survival was essentially the same in both groups [2]. On the other hand, adjuvant chemotherapy was evaluated in patients with stage II colon cancer with poor prognostic features; it did not substantially improve overall survival in stage II patients. Patients in this study were unlikely treated with oxaliplatin [3]. ECOG 5202 was designed to evaluate adjuvant chemotherapy in patients with stage II colon cancer by stratifying the patients as having low or high risk of recurrence depending on their molecular marker analysis. Loss of heterozygosity at chromosome 18q (LOH18q) and the lack of microsatellite instability (MSI) are potential markers for aggressive clinical disease that were used in the study. Patients who were in the high-risk category were prospectively stratified to treatment with FOLFOX with or without the addition of bevacizumab whereas low-risk patients were assigned to surveillance alone. The study was criticized for not having an observation arm in the high-risk category since adjuvant treatment is not standard of care in this group of patients. This study is currently closed to enrollment as one of the arms is no longer standard of care in the adjuvant setting [4].

For patients with stage III colon cancer, adjuvant chemotherapy was shown to reduce individual 5-year risk of cancer recurrence and mortality by about 30%. The addition of oxaliplatin to 5-FU showed a significant improvement in 3-year disease-free survival for patients with stage III colon cancer in two large randomized trials (MOSAIC and NASBP-C07) [2, 5]. There was an update for the MOSAIC study in 2009 that showed no benefit in overall survival with FOLFOX versus 5-FU/LV for patients with stage III who were more than 65 years old [6].

Bevacizumab is a humanized monoclonal antibody to vascular endothelial growth factor (VEGF), that was added to oxaliplatin-based chemotherapy in the NSABP C-08 and AVANT trials in patients with stage II or III colon cancer. It did not prolong disease-free survival or overall survival when compared to chemotherapy alone [7, 8].

Cetuximab is monoclonal antibody that targets the epidermal growth factor receptor (EGFR); its benefit in the adjuvant setting in combination with chemotherapy was tested in the N0147 trial. This trial was closed prematurely because of lack of benefit.

Patients with mutant KRAS had a worse disease-free survival and a trend toward worse overall survival [9]. Hence monoclonal antibodies that target EGFR are not currently indicated in any group of patients with resected colon cancer, though cetuximab is used in other settings.

The treatment of metastatic colorectal cancer (mCRC or stage IV) usually involves chemotherapy alone except in patients who have limited metastatic disease in the liver and/or lungs who are candidates for surgical resection. Triplet combination represents a standard option for first-line therapy to treat metastatic colorectal cancer. Many oncologists use FOLFOX in the first-line setting and FOLFIRI regimen (Irinotecan + 5-FU + leucovorin) in the second-line setting after failure of initial oxaliplatin-based therapy. However, the FOLFIRI regimen could be considered initially in a patient with a relative contraindication to oxaliplatin. Selection of oxaliplatin or irinotecan as part of cytotoxic backbone upfront in metastatic disease is mainly dependent on toxicity profile. In 2012, FOLFIRI plus cetuximab was approved as an option in the first line setting to treat metastatic CRC and can be considered especially when the KRAS mutation status is wild-type. FOLFOX or FOLFIRI plus bevacizumab remains as the most reasonable first line option for mCRC in 2012 especially in patients without known KRAS mutation status.

Patients who progress on FOLFIRI regimen initial therapy could benefit from FOLFOX regimen. In a study that evaluated the two sequences of FOLFIRI followed by FOLFOX, and FOLFOX followed by FOLFIRI, both sequences had similarly impressive survival benefits. In a pooled analysis of cohorts of older patients (aged 65 years or older) from two randomized clinical trials evaluated the benefit of bevacizumab plus 5-FU-based chemotherapy in first-line treatment of mCRC [10]. The study showed that adding bevacizumab to 5-FU-based chemotherapy improved overall survival and progression-free survival in older and younger patients. Bevacizumab is also approved for second-line therapy combined with other chemotherapy if it was not used with the first-line chemotherapy. There are some data that suggests a possible benefit for continued bevacizumab beyond first progression, though data from a randomized trial is lacking to corroborate this observation [11].

Two EGFR-targeted monoclonal antibodies are approved for metastatic colorectal cancer, though these therapies should be given only to patients with wild-type KRAS tumors. The addition of cetuximab to irinotecan-based chemotherapy improved median time to progression and median survival after failure of prior irinotecan-based chemotherapy [12, 13]. The addition of cetuximab to first-line oxaliplatin regimen showed mixed results in contrast to panitumumab, which significantly improved PFS in patients with wild-type KRAS tumors when combined with first-line oxaliplatin regimen. The combination of anti-EGFR antibody therapy and bevacizumab is not advised outside of clinical trials. The addition of panitumumab to bevacizumab resulted in increased toxicity and decreased PFS [14].

Dasatinib, a small molecule BCR-ABL and Src inihbitor, was found to sensitize mutant KRAS colorectal tumors to cetuximab in CRC lines [15]. The combination of dasatinib and cetuximab was shown to decrease prosurvival signaling through the MAPK, mTOR, and STAT pathways compared to untreated or monotherapies in preclinical studies. The combination also resulted in decreased cell proliferation and a higher amount of apoptosis [15]. A retrospective study evaluated the role of

PTEN loss, Akt phosphorylation, and KRAS mutations on the activity of cetuximab plus irinotecan in patients with mCRC. This study concluded that PTEN loss may be predictive of resistance to cetuximab plus irinotecan. Patients with PTEN-positive metastases and wild-type KRAS had longer PFS compared to other patients [16].

KRAS mutations and overexpression of EGFR were found to be important independent predictive markers in mCRC patients treated with cetuximab plus chemotherapy [17]. This study showed that tumors expressing high levels of EGFR or have wild-type KRAS are more likely to have a better PFS and OS when treated with cetuximab plus chemotherapy. In patients with wild-type KRAS tumor status, EGFR expression was a predictor of clinical response. Non-activating KRAS mutant tumor had better PFS and OS than patients with activating KRAS mutants [17]. BRAF mutations are mutually exclusive with KRAS mutations that are found in about 5–10% of mCRC. BRAF mutations are associated with poor prognosis overall but should not be used as predictive factor for patients with wild-type KRAS. In 2012, the presence of either a codon 12 or a codon 13 mutation in KRAS predicts resistance to anti-EGFR targeted therapy. Acquired resistance through KRAS mutation or EGFR extracellular domain mutation has been observed.

Inhibition of the $BRAF^{V600E}$ oncoprotein by the small-molecule vemurafenib in melanoma was shown to be highly effective, likely secondary to the low level of EGFR in melanoma [18]. On the other hand, inhibition of $BRAF^{V600E}$ in preclinical colon cancer models led to rapid feedback activation of EGFR [18]. This preclinical study proposed the benefit of adding BRAF and EGFR inhibitors for complete blockade of EFGR cascade. The role of genetics in the genesis, prognosis, and therapeutic sensitivity of colon cancer and other tumors is becoming increasingly important as we enhance our understanding of the disease. This has potentiated the field of personalized medicine, which is being vetted as a future direction in oncology and is becoming increasingly feasible with improvements in technology and associated costs.

Overview of Genetic Alterations in Colorectal Cancer

In Western countries, death rates associated with CRC have steadily declined over the past few decades [20]. This is likely a result of several factors that include improved screening techniques and participation, changes in lifestyle, and improved therapies. Despite improvements in treatment options, cytotoxic chemotherapy along with surgery or radiotherapy remains the most frequently deployed strategy in the management of colorectal cancer. Nevertheless, refractory disease and systemic side effects of chemotherapy that often limit its dose and tolerability among patients has left physicians searching for alternatives. The last two decades have yielded an increased understanding of the molecular basis of cancer that has driven the development of antitumor agents that target critical signaling pathways that drive the genesis, maintenance, and/or progression of the disease.

The incidence of CRC appears to be linked to environmental factors and genetics. While modernized countries have benefited from declining death rates in CRC, their incidence rate is higher and is attributed to increased sedentary lifestyles and

obesity. The risk of developing CRC increases substantially with incidence in first-degree relatives and several syndromes that confer a substantially increased predisposition to CRC have been identified. These include familial adenomatous polyposis (FAP), MUTYH-associated polyposis, Lynch syndrome and other more rare syndromes (reviewed in [21]) that have been rationalized at the genetic level. For instance, FAP is directly linked to germline mutations in the APC gene, a tumor suppressor that is frequently inactivated in CRC.

The evolution of CRC is thought to be a progression of concomitant molecular and macroscopic events that convert normal colorectal epithelial to adenoma, followed by an adenoma to carcinoma transformation [22]. At the genetic level, CRC is comprised of several cumulative oncogenic alterations that include inactivating tumor suppressors and activating oncogenes. One of the earliest canonical events in CRC genesis is the inactivation of APC, which cooperates with the kinase GSK3-beta to complex with and negatively regulate the activity of the pro-proliferation transcription factor beta-catenin. Mutation of the oncogene KRAS has been proposed as a major step in CRC that advances the disease to the adenoma stage and has been found in approximately half of colorectal adenomas and carcinomas [23–25]. KRAS is a GTPase that mediates the signal transduction of several prosurvival receptors such as EGFR. G12V is one of the most common oncogenic mutations in the KRAS gene, which results in constitutively active pro-survival signaling that is normally controlled by upstream receptor-ligand complexes. Inactivation of the "gate keeper" tumor suppressor p53 is thought to be a late-stage event in CRC and is associated with transition from adenoma to carcinoma.

The traditional linear model of CRC development is useful to describe common oncogenic alterations that fit observations across a large population and may fit many typical cases, though cancer is clearly not a homogeneous and linear process. The progression of these genetic events to induce CRC may occur out of order, cooperate with other alterations, and may be accomplished by various mechanisms such as genomic instability or mutagens. Other genetic alterations can substitute with these canonical alterations by themselves or act in concert such as PTEN, STK11, SMAD4, IGF1, and COX2. Interestingly, some genetic events that act on the same signaling pathway can substitute for others such as the inactivation of beta-catenin in lieu of APC inactivation.

Numerous therapeutic targets have arisen by coupling the knowledge of the molecular events that drive CRC with other molecules that play an essential role in cancer. Significant insight has been gained regarding molecules that regulate key cellular processes conserved in cancer such as evading apoptosis, escaping immune surveillance, increasing cell proliferation through growth factor signaling, and angiogenesis (Fig. 2). These include molecules that are typically altered in CRC and other molecules that act on the same signaling pathway to drive the same phenotype. Novel targeted agents that inhibit the function or production of these key molecules are being pursued and have been approved in some cases such as bevacizumab, which inhibits angiogenesis by sequestering VEGF. Clinical trials are being pursued with these targeted agents as a monotherapy and in combination with standard of care therapies. Here, we review novel targeted agents that are currently being explored in CRC that exploit genetic alterations in cancer.

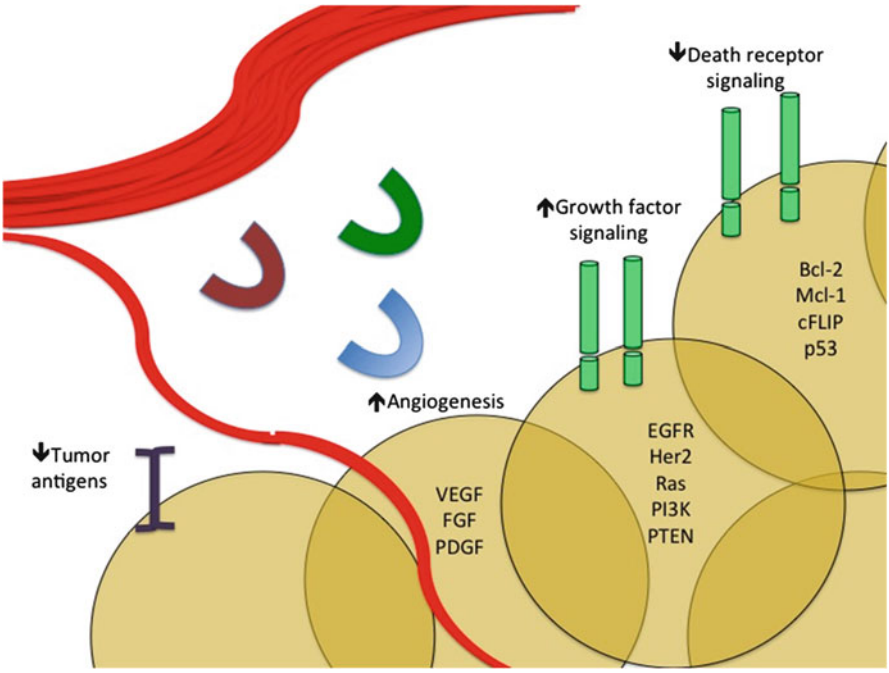

Fig. 2 Molecular targets that drive CRC tumor initiation and maintenance. Tumor cells downregulate death receptor signaling to avoid induction of apoptosis and upregulate growth factor signaling in order to divide more rapidly and in an unregulated manner. The increased proliferation rate of tumor cells requires an increased supply of oxygen and nutrients. This increased supply is provided by new blood vessels formed by upregulating cytokines involved in angiogenesis such as VEGF, PDGF, and FGF. Tumor cells also downregulate surface antigens that are recognized and attacked by the immune system so that the tumor can evade the immune surveillance of cancer

Targeting Cell Death Pathways

Agonistic TRAIL Death Receptor Antibodies

Apoptosis is a naturally occurring process that is necessary for homeostasis of multicellular organisms. Apoptosis occurs by the activation of effector caspases through either the intrinsic, mitochondria-dependent pathway or the extrinsic death pathway. Cancer cells can escape the cytotoxic effects of various conventional chemotherapies by bypassing the intrinsic apoptotic response to the DNA damage. Depending on the cell type, either the intrinsic or extrinsic death pathways can be initiated by binding of ligands or agonistic antibodies to specific death receptors on the cell surface. These death receptor-mediated pathways that induce apoptosis provide an alternative route to target cancer cell that become resistant to traditional chemotherapy (Fig. 3) [26].

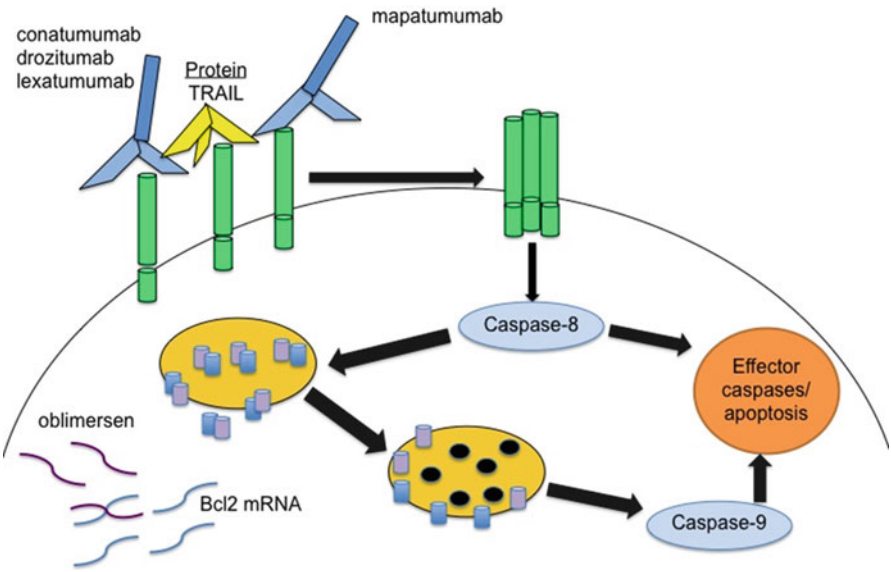

Fig. 3 Antineoplastics that target death receptor signaling in CRC. The pro-apoptotic TRAIL death receptors are engaged by several antibodies that are in clinical trials as antitumor agents. Death receptor 5 (DR5) is engaged by conatumumab, drozitumab, and lexatumumab. Death receptor 4 (DR4) is engaged by mapatumumab. Binding to these death receptors induces death receptor homotrimerization, which activates caspase-8 to trigger apoptosis through pathways that may or may not involve the permeabilization of mitochondria. The mitochondria permeabilization process is regulated by Bcl-2 family members, including Bcl-2 itself. Oblimersen is an antisense drug that targets the Bcl-2 transcript (mRNA) to prevent its translation and therefore downregulates its expression

While there are several death receptor ligands, tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a member of the TNF receptor superfamily, is an attractive antitumor protein as it exerts differential cytotoxicity to cancer and normal cells. In most contexts, TRAIL binds two decoy receptors (DcR1 and DcR2) and two death receptors (DR4 or DR5), which results in the formation of the death-inducing signaling complex (DISC). DISC formation results in activation of the initiator caspase-8, which ultimately leads to activation of effector caspases-3, -6, and -7 (Fig. 3). Normal cells are thought to express higher levels of decoy receptors, which lack the intracellular death domains that, form the DISC and therefore do not initiate apoptosis [27]. Cancer cells evade cell death through a variety of resistance mechanisms such as loss of p53 function. The majority of GI cancers show alterations in the CD95 pathway molecules that impact on TRAIL sensitivity by alter the inhibitory effect of FLICE/caspase-8 inhibitory protein (c-FLIP or CFLAR) or the Bcl-2 family of proteins [28]. In addition to recombinant TRAIL, the death receptor pathway may also be accomplished by the agonistic activity of antibodies against DR4 or DR5.

Conatumumab is a fully humanized agonist antibody against DR5 that induces apoptosis via caspase activation in human tumor cell lines in vitro and demonstrated

anti-tumor efficacy in xenograft models of colon, lung, and pancreatic tumors. A link between the increase in serum caspase-3/7 activity and M30 level in the activation of the extrinsic apoptotic pathway by an anti-DR5 agonist antibody in a preclinical cancer model, which could be used as cell death biomarkers [29]. A phase Ib study of another DR5 agonist antibody, drozitumab, was conducted with first-line FOLFOX plus bevacizumab (BV) in patients with mCRC. The combination was well tolerated and no adverse interactions were found between drozitumab and the chemotherapy. This abstract was presented at 2011 Gastrointestinal Cancers Symposium [30]. In another phase Ib study, drozitumab was combined with cetuximab plus irinotecan or with FOLFIRI with or without bevacizumab in previously treated mCRC patients. This trial also reported no adverse interactions between drozitumab and the chemotherapy.

Lexatumumab (HGS-ETR2 developed by Human Genome Sciences) is another anti-DR5 agonist antibody that has been studied in a phase Ib trial. Lexatumumab was well tolerated and tumor regression was observed in two patients with CRC receiving lexatumumab in combination with folate, 5-FU and irinotecan. This study suggested that further evaluation of lexatumumab in combination with chemotherapeutic agents in phase II studies to evaluate efficacy is warranted [31]. Mapatumumab (HGS-ETR1) is the only DR4 antibody in clinical trials. Preclinically it showed cytotoxic activity against cancer cells but no objective response was found in a phase I study [32].

Most clinical studies showed that these antibodies are not effective when used as monotherapy in patients with gastrointestinal (GI) cancer. Combining TRAIL with other agents may overcome resistance mechanisms, such as combination of TRAIL-based therapies with c-FLIP inhibitors or the multi-kinase inhibitor sorafenib, which down regulates Bcl-2 and Mcl-1. Preclinical studies have shown that the TRAIL-DR5 pathway can cause hepatotoxicity and bile duct toxicity at high doses in mice treated with an anti-mouse DR5 monoclonal antibody.

Bcl-2

Oblimersen is an antisense agent that inhibits the translation of the anti-apoptotic Bcl-2. Oblimersen inhibits Bcl-2 protein production via providing a complementary genetic strand to the messenger RNA encoding for Bcl-2, which renders the cancer cell more sensitive to chemotherapy. In a phase I study the pharmacokinetic and biological effects of oblimersen were evaluated in combination with irinotecan in mCRC patients [33]. This combination was found to be safe and moderately active in patients with previously treated CRC. The recommended dose of oblimersen was determined to be 7 mg/kg/day for days 1–8 with irinotecan 280 mg/m^2/day on day 6 once every 3 weeks. Phase I/II studies with oblimersen are in progress in melanoma [34, 35], small cell lung cancer (SCLC) [36], prostate cancer, refractory acute leukemia and chronic lymphocytic leukemia (CLL). A phase I/II study is evaluating the effectiveness of combining oxaliplatin, fluorouracil, and leucovorin with oblimersen in patients with advanced CRC [37].

Targeting Growth Factor Signaling

The process of cell division is tightly controlled and normally requires stimuli. Growth factor signaling typically involves the binding of an extracellular ligand, such as EGF, to a receptor tyrosine kinase. Binding results in the homo- or hetero-oligomerization of the receptor and autophosphorylation events that activate downstream signaling molecules that lead to prosurvival effects (Fig. 4). Therefore it is unsurprising that cancers, including CRC, typically harbor genetic aberrations that

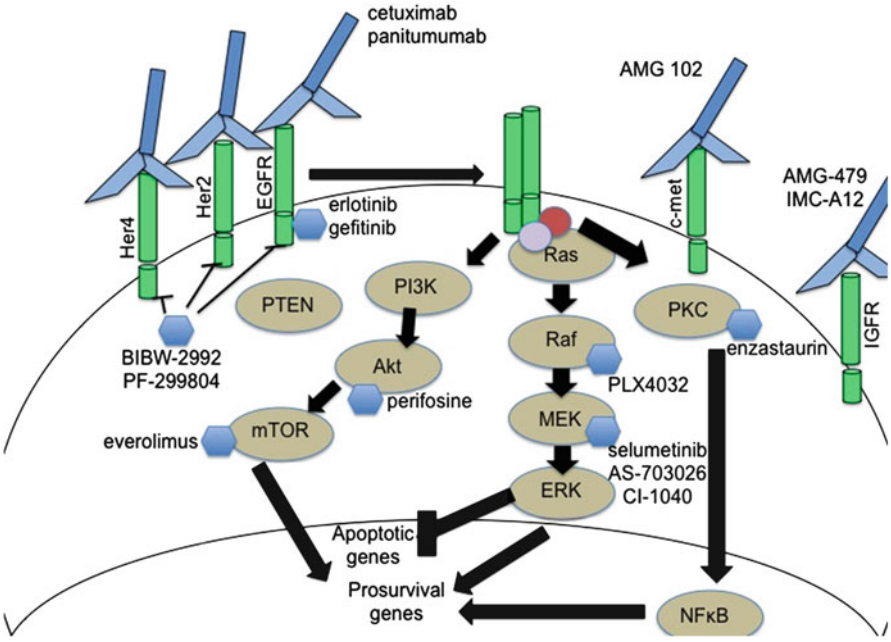

Fig. 4 Antineoplastics targeting growth factor signaling in CRC. Tumor cells require growth factor-independent signaling to increase their proliferation rate. Growth factor signaling typically involves a receptor kinase localize to the cell surface such as the EGFR family members, IGFR, or c-met. These receptors normally bind to secreted growth factors followed by events that turn on intracellular signaling. Several EGFR inhibitors have been developed including intracellular small molecule inhibitors such as erlotinib and gefitinib as well as antibodies such as cetuximab and panitumumab, which bind to EGFR to prevent ligand binding without turning on EGFR signaling. BIBW-2992 and PF-299804 are small molecules that inhibit multiple members of the EGFR family. AMG 102 binds and inhibits the c-met surface receptor. AMG479 and IMC-A12 are antibodies that bind to IGFR. Ligand-receptor complexes involved in growth factor signaling often activate the GTPase Ras, which activates PKC, PI3K/Akt/mTOR signaling, and the MAPK signaling pathway that involves the sequential phosphorylation of Raf, MEK, and ERK. The signaling pathways ultimately turn on genes that have oncogenic consequences such as upregulating prosurvival gene transcription and downregulating apoptotic genes. Enzastaurin is a small molecule inhibitor of PKC. Perifosine is a small molecule indirect inhibitor of Akt and everolimus is a small molecule mTOR inhibitor. Among the MAPK members, PLX4032 is a small molecule specific inhibitor of the V600E mutant form of BRAF where as MEK is inhibited by several small molecules such as selumetinib, AS-703026, and CI-1040

allow cancer cells to grow in the absence of such stimuli. This relationship has led to the development of several agents targeting growth factor signaling, which has generated the most successful targeted agents in terms of FDA approval to date.

The EGFR Family

The EGFR family has been a successful target for targeted cancer therapies. Increased EGFR signaling is particularly common in lung, breast, and CRCs through one or more of the family members, which includes HER1 (EGFR, ErbB-1), HER2 (ErbB-2), HER3 (ErbB-3), and HER4 (ErbB-4) [38]. Agents that inhibit EGFR signaling have been approved by the FDA such as cetuximab in colon cancer and erlotinib and gefitinib in non-small cell lung cancer. However, responders to these inhibitors almost universally develop resistance through acquired mutations in these receptors after long-term use of EGFR inhibitors [39]. This has led to development of inhibitors to multiple EGFR family members such as BIBW-2992, which is an inhibitor of EGFR and HER2 as well as PF-299804, an inhibitor of EGFR, HER2, and HER4.

BIBW-2992 is an irreversible small molecule inhibitor of EGFR and HER2 that has efficacy against first generation EGFR inhibitor-resistant cancers in cell-based assays [40]. Increased efficacy was also noted in xenografts resistant to first generation EGFR inhibitors with significant regression as compared to erlotinib [41]. Other preclinical studies found significant tumor regressions in epidermoid carcinoma xenografts in mice. A Phase I trial of PF-299804, an EGFR, HER2, and HER4 inhibitor, studied 121 patients with advanced solid malignancies, 22 of which being mCRC. In this study, four patients with non-small cell lung cancer had a partial remission but no CRC patients experienced remission with the oral therapy. However, 44 patients of the 121 had stable disease that did not occur with previous treatment [42].

IGF Receptors

The insulin-like growth factor receptor (IGFR) is a family of receptor tyrosine kinases that bind insulin-like growth factors. Ligand binding activates two kinase cascades, the mitogen-activated protein kinase (MAPK) pathway and the phosphatidylinositol 3-kinase (PI3K)/Akt pathway [43]. The MAPK pathway regulates cellular metabolism and is known to promote cell growth and survival whereas the PI3K/Akt pathway is involved in regulation proliferation and apoptosis. This receptor quickly became a cancer therapy target as many early studies found elevated receptor expression in colon carcinoma cell lines. One of the earliest studies in 1986 showed that 20% of colon cancer lines have a mild to moderate increase in IGF1 mRNA and 40% showed an increase in IGF2 mRNA relative to the surrounding normal colonic mucosa. There was a significant increase in IGF1 receptor (IGF1-R) staining in higher stage and metastatic colon carcinomas as compared to normal colonic cell

lines [44]. Based on some of this preclinical data, IGF1-R inhibitors are in development including monoclonal antibody antagonistic ligands that irreversibly bind the receptor to prevent downstream signaling.

One such monoclonal antibody is AMG-479, a fully human antibody produced by Amgen with an IC50 of 0.53 nanomolar against IGF1-R. A 2009 phase I trial with this antibody showed one complete response and one partial response in Ewing's sarcoma out of 15 patients with soft tissue sarcomas. Patients received dose escalations every 2 weeks with intravenous infusions of 1–20 mg/kg. After day 80 of treatment, one patient with Ewing's sarcoma had complete response of all pulmonary metastases and has maintained this remission. One of the five patients with neuroendocrine tumors had a partial response. While the four CRC patients enrolled in the trial did not respond, evidence in other cancers shows promise for IGF1-R monoclonal antibodies [45].

In 2010, a Phase II trial of the IGF1-R monoclonal antibody IMC-A12 compared cetuximab to the combination of cetuximab and this antibody in CRC patients refractory to cetuximab alone. In this study, none of the patients who received IMC-A12 monotherapy had a response. One out of 21 patients had a partial response to the combinatorial therapy that lasted approximately 6 months after treatment initiation. This patient was also noted to have KRAS wild type CRC [46]. One of the reasons why IGF1 receptor monoclonal antibodies seemed so promising in preclinical trials but in clinical trials with CRC have not been as successful could be the large amount of KRAS mutations found in late stage CRCs. KRAS is one of the downstream activators in the EGFR tyrosine kinase pathway and are found in 40–50% of CRCs [47], which confers resistance to IGF1R mAbs.

Hepatocyte Growth Factor (HGF)

Hepatocyte growth factor (HGF) has been shown to increase the motility of human colon cancer cells in vitro, which can be blocked by an anti-HGF antibody [48]. The HGF receptor is encoded by the c-Met proto-oncogene, which cross-talks with beta-catenin signaling to sustain and enhance CRC cell invasiveness [49]. A Phase Ib study of AMG 102, a fully human monoclonal antibody against HGF, in combination with bevacizumab found the combination to have an acceptable toxicity profile. Two of the 14 CRC patients in the study had a mean progression-free survival of approximately 36 weeks on the combination. Treatment-induced side effects were mild and included fatigue, nausea, constipation and peripheral edema and no anti-AMG antibodies were detected [50].

Mutant BRAF

KRAS mutations are present in 40–50% of the patients with mCRC, while the mutually exclusive BRAF activating mutation is present in up to 10% of mCRC and

confer a poor prognosis. BRAF mutations are associated with some response to treatment with monoclonal antibodies against EGFR. PLX4032/RG7204 is an oral small molecule inhibitor of mutant BRAF that has demonstrated efficacy in melanoma, thyroid cancer, and CRC among others. The activity of PLX4032 as monotherapy or in combination with capecitabine with or without bevacizumab was evaluated in a CRC xenograft model. Monotherapy was found to have superior activity to capecitabine or bevacizumab alone that was enhanced in combination with capecitabine ± bevacizumab [51].

In a phase I study, patients with mCRC with mutant BRAF were treated with PLX4032 at the previously determined maximum tolerated dose of 960 mg BID. As compared to the 81% response rate in metastatic melanoma, responses in this study were heterogeneous. The clinical activity was found to be more modest than previously seen in melanoma patients with mutant BRAF. This was rationalized by the increased heterogeneity of the biological consequences of BRAF activation in CRC patients compared to melanoma patients [52]. In 2012 it has become clear from preclinical studies that targeting EGFR may help with response of BRAF mutant colon cancer cells to BRAFtargeted agents.

MEK

Aberrant expression of EGFR is common in human cancers, particularly in CRCs. EGFR family members signal by a pathway that is similar to IGFR signaling by acting through the Ras-Raf-MEK-ERK and PI3K signaling pathways, leading to cell proliferation and evasion of apoptosis. Due to this fact, the EGFR receptor has been a hotly pursued drug target for antineoplastics. Such drugs include cetuximab and panitumumab, which are monoclonal antibodies against the receptor [53, 54]. Unfortunately only about 8–23% of cancer patients respond to EGFR-targeting treatments due to activating mutations KRAS that cause resistance to EGFR monoclonal antibody therapy since Ras activation occurs downstream of the EGFR receptor as well IGF1R. These resistance mechanisms make therapies targeting activators downstream of Ras a priority. MAP kinase kinase (MEK) is an integral part of the Ras signaling pathway as a downstream signal transducer that has been pursued as cancer drug target.

A recent study described two highly potent small molecule inhibitors of MEK, selumetinib (AZD-6244) and AS-703026. In vitro studies demonstrated that both molecules reduced the proliferation of mutant KRAS cancer cells by 63–67%. As expected, there was no reduction in proliferation of mutant KRAS cells treated with cetuximab. In vivo studies using mouse models found that selumetinib decreased tumor size by 60–70% in mutant KRAS tumors [55]. Selumetinib may also increase radiation responsiveness of lung cancers and CRCs to two highly potent small molecule inhibitors by decreasing cellular response to hypoxia that induces therapeutic resistance. Tumor growth was delayed approximately 25 days more than controls in xenografts treated with both selumetinib and ionizing radiation, which is 15 more

days than radiation alone. There was also a significant decrease in the tumor density of blood vessels after 5 days of treatment with both selumetinib and radiation [55].

In 2009, a phase II trial was performed with selumetinib in CRC patients refractory to one or two previous therapies. In this study, the effects of oral selumetinib on disease progression were compared with that of capecitabine. There was no significant difference in disease-free survival between the two randomized groups receiving either therapy. There was one partial response out of the 35 patients in the capecitabine group and no responses in the selumetinib group. Unfortunately, approximately 80% of the patients experienced disease progression within the 2 year study while the others had stable disease.

Despite very promising preclinical data, several phase I and phase II trials of MEK inhibitors have been less than encouraging. A phase II trial of an oral MEK inhibitor, CI-1040, in non-small cell lung cancer, breast, colon, and pancreatic cancers was conducted in 2004. The oral therapy was well tolerated with minimal side effects; however this MEK inhibitor yielded no complete or partial responses [56]. A phase I trial of a MEK inhibitor was later attempted in the 2009 AS-703026 trial in advanced solid tumors. 78% of these patients had CRC and the other 22% had melanoma. There were two partial responses out of 15 previously treated advanced melanomas, however there was no documented response in CRC [57].

Akt

The PI3K/Akt pathway is a prosurvival signaling pathway downstream of many receptors that bind growth factors such as EGFR. Perifosine is a small molecule that inhibits the activation of Akt by a poorly understood mechanism and has been studied in melanoma, multiple myeloma, and sarcoma. In vitro effects on colon cancer cell lines have been reported [58]. Perifosine continues to be tested in clinical trials.

Mammalian Target of Rapamycin (mTOR)

mTOR is a substrate of Akt and the mTOR pathway is involved in several aspects of cancer cell survival and proliferation. Everolimus is a rapamycin analog that binds with a high affinity to FK-507 binding protein-12, which forms a complex that interacts with mTOR to block signaling by inhibiting the phosphorylation of S6K1 and 4E-BP1 by mTOR. Inhibiting the mTOR pathway impacts the expression of proteins involved in angiogenesis, cell growth and proliferation, and metabolism [59]. Everolimus is FDA approved for subependymal giant cell astrocytoma associated with tuberous sclerosis that cannot be surgically removed and advanced renal cell carcinoma after failure of sunitinib or sorafenib. It has been investigated in other solid tumors including CRC. A phase II trial of everolimus in combination with bevacizumab in refractory mCRC demonstrated a promising disease control rate [60].

Results showing safety and efficacy have been reported in a phase I trial of everolimus with irinotecan and cetuximab as second-line treatment in mCRC. A phase II study is planned [61].

Protein Kinase C (PKC)

PKC plays a role in the signaling of growth factor receptors that has cross talk with both Akt and mTOR. Enzastaurin was developed as an oral ATP-competitive selective inhibitor of the serine/threonine kinase protein kinase C-beta (PKC-beta) that was subsequently shown to inhibit multiple PKC isoforms, suppress the phosphorylation of Akt, GSK3β, and ribosomal protein S6. Enzastaruin has demonstrated pro-apoptotic and anti-proliferative effects on an array of cultured human tumor cells including CRC [62]. Several phase II trials failed to produce any promising signs of efficacy in solid tumors. The addition of enzastaurin to pemetrexed as second-line therapy in advanced non-small cell lung cancer failed to improve progression-free survival or overall survival [63]. The addition of enzastaurin to pemetrexed, carboplatin, and bevacizumab in stage IIIB/IV non-small cell lung cancer failed to improve progression-free survival [64]. Enzastaurin also failed to show sufficient single agent activity in recurrent high-grade gliomas. A trial investigating the addition of enzastaurin to capecitabine in metastatic or recurrent breast cancer after prior cytotoxic therapy was stopped early after finding no median overall survival benefit and shorter progressional-free survival in the enzastaurin arm [65, 66]. A phase III trial failed to show superior efficacy of enzastaurin compared to lomustin in recurrent intracranial glioblastoma [67]. On the other hand, enzastaurin has shown activity in prolonging freedom from progression in relapsed or refractory diffuse large B-cell lymphoma in a small subset of patients, and in relapsed or refractory mantle cell lymphoma [68, 69]. A 'window of opportunity' trial in chemonaïve asymptomatic mCRC patients showed that enzastaurin may have single agent activity [70]. However, a recent placebo controlled phase II trial of maintenance enzastaurin in combination with 5-FU, leucovorin, and bevacizumab following first-line chemotherapy in mCRC, failed to demonstrate a PFS advantage [71].

Targeting Angiogenesis

VEGF Receptors

In order for neoplasms to continue to propagate they require an adequate blood supply, which is accomplished by inducing angiogenesis (Fig. 5). One of the most important factors involved in angiogenesis is vascular endothelial growth factor (VEGF), which is sufficient in vitro to cause angiogenesis [72]. Due to the importance of angiogenesis in cancer, a number of therapies have been developed

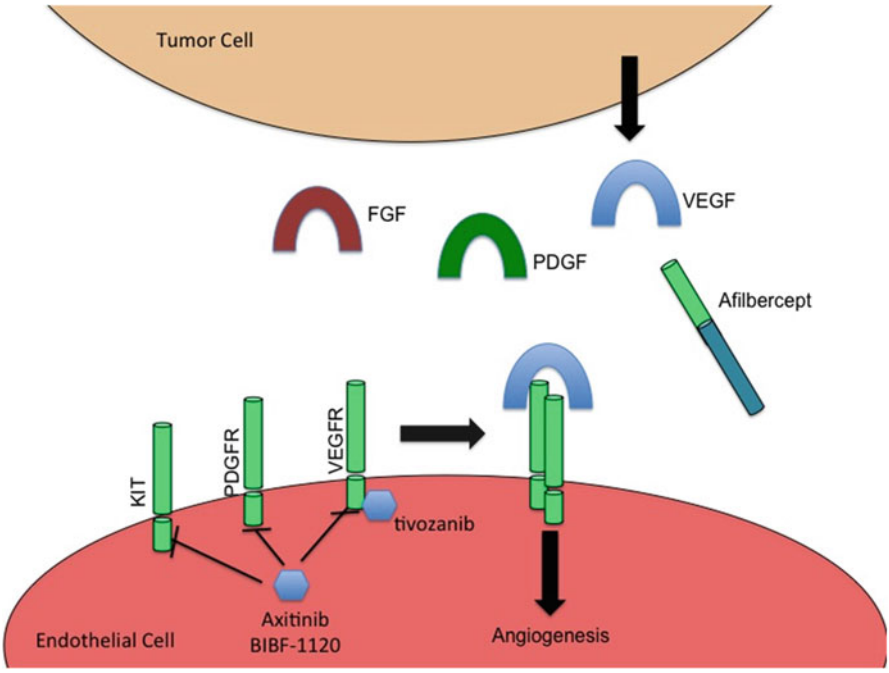

Fig. 5 Antineoplastics targeting angiogenesis in CRC. Several cytokines are secreted by tumor cells to induce angiogenesis. These cytokines such as VEGF are bound by surface receptor on endothelial cells that include KIT, PDGFR, and VEGFR. Aflibercept is a fusion protein that mimic two VEGF receptors. Tivozanib is a small molecule inhibitor of VEGFR and the small molecules axitinib and BIBF-1120 inhibit VEGFR, PDGFR, and KIT

to target VEGF and its cognate receptor VEGFR. Aflibercept is a recombinant fusion protein consisting of the Fc portion of IgG1 combined with the third domain of VEGFR2 and the second domain of VEGFR1. This allows aflibercept to mimic VEGFR2 and VEGFR1 to prevent VEGF from binding to those receptors, thereby inhibiting angiogenesis. Preclinical studies have shown that aflibercept is an effective inhibitor of angiogenesis and tumor growth in animal models [73]. Aflibercept has shown tolerability in phase I trials in patients with solid tumors [74–77]. Aflibercept has shown clinical efficacy in recurrent platinum-resistant epithelial ovarian cancer and prolonged time to repeat paracentesis in advanced epithelial ovarian cancer with symptomatic malignant ascites [78, 79]. Clinical efficacy has also been shown in several other phase II trials including patients with platinum- or erlotinib-resistant locally advanced or metastatic non-small cell lung cancer, uterine leiomyosarcoma, inoperable stage II or IV melanoma, temozolomide-resistant recurrent glioblastoma, and anaplastic glioma at first relapse [80–83]. Limited clinical efficacy has been reported with aflibercept as a single agent in patients with recurrent metastatic urothelial cancer previously treated with a platinum-containing regimen [84]. A phase II trial showed efficacy in patients with

mCRC previously treated with bevacizumab and a recent phase I trial investigating aflibercept in combination with FOLFIRI in mCRC showed tolerability [85, 86]. The phase III VELOUR trial investigating aflibercept in combination with FOLFIRI as a second-line regimen in mCRC is anticipated to report its results during the second half of 2011. Similarly, the phase II AFFIRM investigating aflibercept in combination with FOLFOX as a first-line treatment for mCRC is also expected to have results by late 2011. In 2012, aflibercept was approved by the FDA in combination with FOLFIRI as a therapeutic option for patients with metastatic CRC, including about a 10% response rate in patients who were previously treated with Avastin in combination chemotherapy.

Tivozanib is an oral, ATP-competitive, small molecule inhibitor of VEGFR [87]. A phase II trial and subgroup analysis found that tivozanib as monotherapy achieved disease control for patients with different histological types of renal cell carcinoma (RCC), with longer PFS seen in patients with clear cell RCC compared to non-clear cell variants [88, 89]. Tivozanib has also been studied in phase Ib trials in combination with temsirolimus in metastatic RCC, in combination with paclitaxel in metastatic breast cancer, and as a monotherapy in non-small cell lung cancer [90–92]. A phase III randomized, controlled trial comparing tivozanib with sorafenib in patients with advanced RCC is pending results [93]. Recently presented in abstract form, an open-label phase Ib trial of tivozanib in combination with FOLFOX in patients with advanced gastrointestinal tumors showed safety and tolerability [94]. A phase Ib trial investigating tivozanib in combination with capecitabine for patients with advanced solid tumors including CRC is currently recruiting patients [94].

Axitinib (AG-013736) is an oral selective inhibitor of VEGF receptors [95, 96]. Axitinib inhibits the autophosphorylation of VEGF receptors (VEGFR) that normally occurs upon ligand binding, interferes with eNOS/AKT mediated signal transduction, decreases vascular permeability, and prevents VEGF-mediated endothelial cell survival. Axitinib demonstrates dose-dependent anti-tumor activity that is associated with a reduction in angiogenesis, tumor cell proliferation, and increased apoptosis. At higher concentrations, axitinib also has activity against PDGF receptors and KIT, which are also receptors involved in angiogenesis, may enhance its anti-tumor efficacy. However, it is likely that the principal effects of axitinib are mediated through the VEGF receptors when considering the pharmacokinetic/pharmacodynamic data where it has shown efficacy [97]. Several phase II studies have shown clinical efficacy in a variety of solid tumors including advanced non-small cell lung cancer, cytokine-refractory metastatic RCC, advanced thyroid cancer, advanced pancreatic cancer, and metastatic melanoma [98–101]. Axitinib has also shown activity in human breast cancer models in mice [102]. Recently, axitinib has been investigated as a second-line agent in mCRC. This open-label, randomized phase II trial compared axitinib to bevacizumab in combination with either FOLFOX or FOLFIRI. The study failed to show a difference between axitinib and bevacizumab with respect to either progression-free survival or median overall survival. However, a trend towards improved median overall survival was seen with axitinib in combination with FOLFOX in comparison to bevacizumab in combination with FOLFOX. Conversely, a trend towards reduced

median overall survival was seen with axitinib in combination with FOLFIRI in comparison to bevacizumab in combination with FOLFIRI [103].

Other Receptors That Mediate Angiogenesis

VEGF receptor inhibitors have proven to be effective targeted therapies. However, some tumors are still able to sustain angiogenesis by upregulating other vascular growth factors such as platelet derived growth factor (PDGF) and fibroblast growth factor (FGF) [104]. This resistance mechanism led to the development of a triple angiokinase receptor inhibitor, BIBF-1120, which irreversibly inhibits VEGF, PDGF, and FGF receptors.

A phase I trial in 2009 investigated oral BIBF-1120 in 61 patients with advanced solid tumors, 30 of which were CRC. Of these patients, 56 had prior therapy with surgery or chemotherapy and continued to have disease progression. There were two partial responses, one in a patient with CRC and one in a patient with RCC. There was a complete response in a RCC patient, whose lung metastases disappeared 2 months post-treatment. BIBF-1120 was well tolerated when its MTD of 250 mg was split into twice daily dosing [105], though other phase I trials were not as successful. In a group of 21 patients with advanced solid tumors, there were no complete or partial responses but 16 patients had stabilization of disease for at least 56 days or a total of two cycles [106]. BIBF-1120 can also be combined with other common chemotherapeutic regimens for CRC. One study showed no additional adverse effects when BIBF-1120 was added to FOLFOX [107]. BIBF-1120 was also successfully added to the EGFR/HER2 inhibitor BIBW-2992 in alternating regimens with diarrhea and vomiting being the most common side effects. In this 2008 study, patients with advanced CRC that continued to progress on two to three therapies, including bevacizumab and cetuximab for 89% of patients, had alternating oral regimens of BIBF-1120 and BIBW-2992 and managed to have disease stabilization for at least 2 months. Unfortunately, there were no partial or complete responses in this patient population [108].

Multi-Targeted Agents

Sorafenib

Sorafenib is an oral multi-kinase inhibitor of VEGFR2, VEGFR3, Flt-3, PDGFR-β and c-KIT BRAF, RAF-1, and RET with demonstrable anti-angiogenic and anti-tumor activity. Sorafenib is FDA approved for use in advanced RCC and surgically unresectable hepatocellular carcinoma [109–111]. The utility of sorafenib in CRC is an active area of research. Recently published, the addition of sorafenib to

cetuximab in patients with mCRC improved overall survival by 2 months [112]. The combination of sorafenib and radiation has shown efficacy in human CRC xenografts and a phase I/II trial currently recruiting participants is investigating sorafenib, capecitabine, and external beam radiation in patients with locally advanced rectal cancer [113, 114]. The combination of sorafenib and bevacizumab as a salvage therapy in heavily pretreated mCRC patients showed promise of clinical activity that is still being evaluated in trials [115, 116]. A phase I/II trial of sorafenib in combination with cetuximab and irinotecan in patients with advanced mCRC has recently reported that the regimen was well-tolerated following amendment of the irinotecan dose/schedule; however, the phase II portion is unlikely to be opened due to limited responses [117]. A phase II trial of sorafenib in combination with FOLFIRI for patients with advanced CRC after failing treatment with oxaliplatin is currently recruiting participants [118]. Also currently ongoing, a trial investigating sorafenib in combination with irinotecan as second-line therapy in mCRC with mutant KRAS has reported favorable phase I results, and interim phase II reports showing evidence of disease control [119, 120]. A phase II efficacy assessment trial of sorafenib in combination with capecitabine in advanced pretreated CRC is currently recruiting patients [121]. Sorafenib is also being investigated in two phase II trials in combination with FOLFOX6 or FOLFIRI as second-line treatment in mCRC [122, 123]. A related kinase inhibitor called regorafenib was approved by the FDA in 2012 as single agent salvage therapy in metastatic CRC and was shown to benefit overall survival including in patients who had previously progressed on bevacizumab.

Sunitinib

Sunitinib is an oral tyrosine kinase inhibitor of VEGFR 1,2,3, PDGFRα, PDGFRβ, KIT, FLT3, RET, and the CSF1 receptor (CSF1R), that is approved for the treatment of advanced clear cell RCC and advanced GI stromal tumors after failure or intolerance to imatinib [124]. The role of sunitinib in the treatment and management of CRC is an active area of research. In human CRC xenograft models, sunitinib demonstrated single agent antitumor activity that synergized with TRAIL [125]. An early phase II trial of sunitinib in heavily pretreated mCRC patients failed to demonstrate a single-agent objective response rate. Subsequently, phase I results of sunitinib in combination with FOLFIRI in treatment-naïve mCRC showed tolerability and promising anti-tumor activity [126]. Sunitinib has also been investigated in combination with FOLFOX6 in mCRC as first-line treatment and is currently undergoing investigation in combination with FOLFOX in comparison to bevacizumab plus FOLFOX as first-line treatment in mCRC [127, 128]. Recently, a phase II study of sunitinib in combination with capecitabine in patients with mCRC refractory to prior treatment with 5-FU/irinotecan/oxaliplatin demonstrated feasibility and a high level of disease stability [129].

Dasatinib

Dasatinib is an oral ATP-competitive tyrosine kinase inhibitor of all members of the Src family of kinases as well as Abl, c-KIT, PDGFR, and EphA2 [130]. A phase I dose-escalation study of dasatinib in combination with capecitabine, oxaliplatin, and bevacizumab as first-line therapy in CRC identified a well-tolerated dose recommended for a phase II dose/schedule [131]. Recently reported was the premature termination of a phase II study of dasatinib in previously treated mCRC due to lack of efficacy [132]. A preclinical study showed that dasatinib sensitizes KRAS mutant CRC cells to cetuximab in vitro and in vivo [133]. Currently, a phase I study is recruiting patients for dasatinib and cetuximab as single agents or in combination for patients with CRC and resectable liver metastasis [134].

Harnessing the Immune System

Epithelial Cell Adhesion Molecule (EpCAM)

Catumaxomab is a trifunctional monoclonal antibody that recruits and activates different immune effector cells at the surface of tumor cells. Tripartite binding is accomplished by paratopes against CD3 to allow for binding to T-cells, an anti-EpCAM paratope to target tumor cells, and the Fc domain that is bound by Fc-receptor I-, IIa-, and III-positive antigen-presenting cells [135–137]. Catumaxomab has been studied in patients with malignant ascites due to peritoneal carcinomatosis. In one study of eight patients with peritoneal carcinomatosis of solid tumors including breast, ovarian, gastric, and one adenocarcinoma of unknown primary, patients were treated with intraperitoneal injections of either catumaxomab alone (4/8), another trifunctional antibody rexomun that targets Her2 instead of EpCAM alone (1/8), or a combination of the two antibodies (3/8). The therapy was found to be both well tolerated and clinically effective [138]. A phase I/II study of 23 women with malignant ascites due to ovarian cancer, showed that intraperitoneal administration of catumaxomab effectively induced tumor cell destruction, decreased ascites production, and reduced the necessity for repeat paracentesis [139]. In a recent study of patients with peritoneal carcinomatosis secondary to colon cancer, catumaxomab alone or in combination with chemotherapy was evaluated in comparison to cytoreductive surgery and hyperthermic chemoperfusion (HIPEC) with or without systemic chemotherapy and systemic chemotherapy alone. Their findings, which have been presented in abstract form, showed that catumaxomab had a preventative effect on the accumulation of malignant ascites, the development of intestinal obstruction, and conferred a survival benefit when compared to systemic chemotherapy alone [140]. A survival advantage when compared to paracentesis alone was shown in a recent study of catumaxomab in patients with malignant ascites due to GI cancers including colon, esophageal, pancreatic, gastric, and rectal cancers [141].

Toll-Like Receptor 9 (TLR9)

Toll-like receptors (TLR) are a family of specialized immune receptors that recognize pathogen-expressed molecules and elicit an immune response upon such pattern recognition. Each member of this family can detect one or more distinct pathogen-expressed molecules [142]. TLR 9 is exclusively expressed in human immune cells, B cells, and plasmacytoid dendritic cells. TLR9 detects unmethylated CpG dinucleotides, which are prevalent in bacterial and viral genomic DNAs but are uncommon in vertebrate genomes. TRL9 is stimulated by introducing synthetic oligodeoxynucleotides that contain unmethylated CpG dinucleotides [143]. Hence the novel idea of activating immune cells that express TLR9 was proposed in cancer therapy to enhance antigen-specific $CD4^+$ and $CD8^+$ T cells. Increased numbers of T cells with higher avidity are required in vivo as ineffective T cell triggering leads to much lower numbers of T cells that are less active killers and might tolerate the tumor [144].

Two types of TLR9 agonists were assessed in an in vitro study of CRC using a traditional CpG oligonucleotide and an immunomodulatory oligonucleotide [145]. This study showed that TLR agonists have antitumor activity regardless of p53, are cytotoxic in CRC cell lines, and synergize with radiation and chemotherapy. When TLR9 agonists were added to cetuximab or gefitinib, a small molecule EGFR inhibitor, the combination showed synergistic inhibition of tumor growth, downstream signaling proteins, and angiogenesis in colon cancer xenografts. The combination decreased resistance to cetuximab as well as to other EGFR inhibitors by decreasing the aberrant expression of downstream signaling proteins [146].

A33

The A33 antigen is a glycoprotein that was sequenced and cloned because of its significantly elevated expression in the epithelia of the lower GI tract in mCRC. A study conducted in 1996 found that 95% of mCRC had increased expression of the A33 antigen [147]. Although much has been learned about the antigen itself, its exact function remains unclear. A33 is a cell surface protein that appears to be internalized into cytoplasmic vesicles as determined by fluorescence microscopy [148]. A monoclonal humanized antibody against A33 was developed and was promising in preclinical studies targeting CRC cells and subsequent lysis with high expression of surface A33 [149]. A phase I trial of the A33 antibody was performed with eleven patients with advanced, chemotherapy-resistant CRC patients. Unfortunately, eight of the patients developed toxicity secondary to human anti-human antibody (HAHA) response. Of the three patients who tolerated the therapy, one achieved a partial response seen radiographically along with a significant reduction of carcinoembryonic antigen (CEA). Four of eleven patients had disease stabilization from 2 to 12 months with two cases having significant reduction in CEA [150]. Although the results from this phase I trial are promising, the significant toxicity of the antibody has limited its clinical use.

Other Approaches

Wnt and Hedgehog Signaling Pathways

Wnt and Hedgehog (Hh) signaling pathways play key roles in self-renewal and embryonic growth and patterning. Due to their role in development, there is a high frequency of human cancers harboring mutations that activate transcriptional responses of these pathways [151]. In fact, this signaling pathway is commonly activated in the early stages of CRC through inactivation of APC. Apoptosis has been noted in both adenoma- and carcinoma-derived CRC cell lines treated with the Hh inhibitor cyclopamine [152]. GDC-0449, another known inhibitor of the Hh pathway, is being studied as an agent for treating mCRC. Genentech has been conducting a randomized, placebo-controlled double blind study of GDC-0449 added to standard-of-care regimens for mCRC with treatment until disease progression [153]. Patients on this trial are receiving FOLFOX/FOLFIRI with bevacizumab and are stratified based on the chemotherapy regimen chosen and presence of RECIST measurable disease at baseline [154]. Data from this study will be valuable in understanding potential of this class of targeted agents against mCRC, which remains largely unconquered.

COX-2

Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit cyclooxygenases and have been recently linked to decreased risk of colon cancer and as a result such drugs are being explored as a treatment for CRC, particularly inhibitors of cyclooxygenase-2 (COX-2). Anti-tumor effects of such COX-2 inhibitors have been observed in colon cancer cell lines. Celecoxib, a selective COX-2 inhibitor, decreased lipid fluidity in the cancer cell lines irrespective of COX-2 expression status and decreased the proliferation, migration, and invasiveness of the tested CRC cell lines in vitro. The authors proposed that the cytotoxic effect of celecoxib is mediated by alterations in cellular membrane fluidity [155].

One study of selective COX-2 inhibitor, NS398, analyzed adenoma- and CRC-associated gene expression profiles in colon adenocarcinoma cell lines. A dose-dependent inhibition of COX-2 protein expression resulted in a reverse effect on the expression of CRC-associated genes, suggests its use in the chemoprevention. The authors proposed that the anti-tumor activity of NS398 is mediated through accumulation of arachidonic acid in cancer cells that causes apoptosis [156]. Another study evaluated long-term treatment with celecoxib in humans and mouse models of colon cancer. This study showed that long-term administration can oppose effects observed with short-term use, which includes anti-inflammatory and tumor suppressor activity. Long-term use was associated with resistance to the antitumor effects of celecoxib through inhibition of NF-κB signaling in enterocytes and decreased COX-2 expression, which resulted in chronic inflammation and tumor promotion [157].

Heat Shock Protein 90 (Hsp90)

Cancer typically exhibits an increased proliferation rate compared to normal cells, which in turn requires the cell to produce proteins at a more rapid rate as it progresses through the cell cycle at a higher rate. This increased protein production along with acquired mutations that often subtly or grossly alter protein secondary and/or tertiary structure increase the demand for molecular chaperones, which are a class of proteins that assist in and allow for proper folding of other proteins. Hsp90 is a molecular chaperone that has recently emerged as an important molecule in some malignancies. 17-allylamino 17-demethoxygeldnamycin is a Hsp90 inhibitor shown to inhibit signal transduction in four colon cancer cell lines by depleting c-Raf-1 and Akt [158]. The drug also enhances the cytotoxicity of oxaliplatin in colon cancer cell lines through NF-kB inhibition, particularly in TRAIL-resistant cell lines [159]. Evidence from preclinical studies combining cisplatin and Hsp90, inhibitors suggest synergistic activity in colon cancer [160]. STS-9090, another small molecule inhibitor of Hsp90, is a promising agent being investigated in a variety of solid tumors.

Virotherapy

Virotherapy is a novel approach of treating cancers that is based on the selective infection of cancer cells with natural or engineered lytic viruses that induce oncolysis, promote apoptosis in tumor cells, or cause synctitium formation [161]. Vaccinia virus has desirable characteristics of an oncolytic virus including a short, well-characterized life cycle, high cytolytic activity, genetic stability, lack of a natural host, non-pathogenicity in humans, and a well-documented side effect profile from its previous extensive use as pox vaccine [161]. JX-594, an oncolytic poxvirus engineered to be selectively replication-competent in tumor cells, has been used by intravenous administration in phase I trials for some cancers [162]. Studies in patients with metastatic liver tumors including those with colonic primary have shown good tolerance and acceptable safety with intravenous administration. However, direct hyperbilirubinemia was the dose-limiting toxicity. Progress has been made to phase II trials [163]. Recent results of a JX-594 clinical trial demonstrated safety, dose-dependent infectivity, and preliminary responses with JX-594 in patients with solid tumors [163].

Colorectal Cancer Stem Cells (CRCSCs)

Tumors are composed of a heterogeneous population of cells with varying capacity for pluri-potency or multi-potency and self-renewal. Tumors mainly consist of rapidly proliferating differentiated cells with limited capacity for self-renewal. The cancer stem cell (CSC) hypothesis indicates that tumors originate from a small

population of stem/progenitor cells capable of self-renewal. The CSCs are multipotent or pluripotent cells that are essential for tumor initiation and long term tumor maintenance. CSCs could also arise from dedifferentiation of existing cancer cells. Thus, a CSC may not necessarily be the cell of origin for a tumor. CSCs are resistant to chemotherapy and radiation, and are thought to be responsible for local tumor recurrence and metastatic relapse. Selective targeting of CSCs in combination with conventional therapy could improve treatment outcomes and prolong patient survival [164, 165].

Multipotent stem cells in the colonic crypt give rise to all the epithelial cell lineages in the crypt. The multipotent stem cells undergo asymmetric division and give rise to transit amplifying cells that proliferate and differentiate into various epithelial cell types. According to the CSC hypothesis, the origin of colorectal cancer (CRC) lies in oncogenic mutations that accumulate in the colorectal stem cells. These mutated CRC stem cells (CRCSCs) can then undergo symmetric and asymmetric division to initiate tumor formation. These multipotent cells give rise to progenitors, transit amplifying cells as well as more differentiated cells within the tumor resulting in tumor progression [166].

Several markers such as CD133, CD44, CD166, Epithelial cell adhesion molecule (EpCAM) and Aldehyde dehydrogenase-1 (ALDH1) have been used in various combinations to identify the CRCSCs phenotype. Such markers are also involved in regulation of the CRCSC phenotype and could be potential targets for cancer therapy. However, the tumor specificity of such markers along with adverse effects on normal colon stem cells needs considerable evaluation [166, 167].

CRCSCs are resistant to chemotherapeutic drugs used in the clinic for colorectal cancer such as 5-FU and oxaliplatin. The chemotherapeutic drugs cause an enrichment of CRCSCs, perhaps contributing to more aggressive disease. Several approaches are currently being explored to specifically target CRCSCs [168–173]. Further studies are essential to identify CSC-specific targets, novel therapies and evaluate the promise of targeting CRCSC's in the clinic.

Stem Cell Signaling in CRC

TGF-β and its mediators known as Smad proteins are important for stem cell maintenance and function in the colonic crypt. TGF-β serves as a tumor suppressor in the intestinal epithelium. Mutations in the genes encoding TGF-β and Smad4 are frequently observed in CRC. Wnt/β-catenin pathway is known to regulate several target genes involved in the regulation of stem cell self-renewal and differentiation. Wnt signaling is essential for maintenance of the colonic crypt. It is frequently turned on in crypt stem cells and is switched off in more differentiated cells of the intestinal epithelium. Constitutively activated Wnt signaling and mutations in beta-catenin commonly occur during colorectal carcinogenesis involving Adenomatous polyposis coli (APC) tumor suppressor loss. TGF-β and Wnt signaling are known to co-operate in CRC tumorigenesis [176, 177]. Notch and Hedgehog signaling

pathways are also known to regulate self-renewal and differentiation of intestinal stem cells. Dysregulation of Notch and Hedgehog pathways has been shown to be associated with CRC [174, 175]. p53 mutations and pathway inactivations frequently occur in colorectal cancer [176]. p53 is known to regulate several aspects of stem cell biology including self-renewal, differentiation and reprogramming [177]. Loss of p53 is known to enrich for CRCSCs [178]. Targeting of stem cell signaling pathways such as TGF-β, Wnt, Notch, Hedgehog and p53 is being evaluated in various stages of clinical development. Thus, the stem cell signaling network represents the major network of therapeutic targets for CRCSCs [176, 179–184].

Stem Cell Microenvironment in CRC

Intestinal stem cells rely on extrinsic signaling from the surrounding microenvironment to maintain stemness or undergo differentiation and proliferation. The stem cell niche is the surrounding stromal microenvironment composed of extracellular matrix, immune cells, endothelial cells, smooth muscle cells and fibroblasts. The niche provides stem cells with essential morphogenetic signals such as Wnt, Notch, bone morphogenetic proteins (BMPs) and Hedgehog signals. Elevated Wnt signaling along with Notch activity helps in maintenance and proliferation of intestinal stem cells while BMPs counteract Wnt signaling to promote differentiation. All these signals from the microenvironment via growth factors and cytokines are crucial for crypt maintenance and cell lineage determination. CRCSCs also rely on the surrounding microenvironment [185–187]. A recent study demonstrated the importance of myofibroblast-mediated Wnt signaling in the maintenance of CRCSCs. Myofibroblasts secrete HGF to maintain Wnt signaling in CRCSCs. Myfibroblast-secreted HGF also stimulates dedifferentiation of differentiated tumor cells to induce a CSC phenotype [188]. The microenvironment signals that regulate CRCSCs serve as potential therapeutic targets for therapy. Wnt and Notch inhibitors and BMP receptor agonists could be used to target the CRCSC niche [185].

Acknowledgements W.S.E-D. is an American Cancer Society Research Professor

References

1. Desch CE, Benson AB, Somerfield MR, et al. Colorectal cancer surveillance: 2005 update of an American society of clinical oncology practice guideline. J Clin Oncol. 2005; 23(33):8512–9.
2. André T, Boni C, Mounedji-Boudiaf L, et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. N Engl J Med. 2004;350(23):2343–51.
3. O'Connor ES, Greenblatt DY, LoConte NK, Gangnon RE, Liou JI, Heise CP, et al. Adjuvant chemotherapy for stage II colon cancer with poor prognostic features. J Clin Oncol. 2011;29(25):3381.

4. Benson A, O'Dwyer P, Hamilton S. Oxaliplatin, Leucovorin, and Fluorouracil With or Without Bevacizumab in Treating Patients Who Have Undergone Surgery for Stage II Colon Cancer. 2012; NCT00217737.
5. Kuebler JP, Wieand HS, O'Connell MJ, et al. Oxaliplatin combined with weekly bolus fluorouracil and leucovorin as surgical adjuvant chemotherapy for stage II and III colon cancer: results from NSABP C-07. J Clin Oncol. 2007;25(16):2198.
6. André T, Boni C, Navarro M, Tabernero J, Hickish T, Topham C, et al. Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial. J Clin Oncol. 2009;27:3109–16.
7. Allegra CJ, Yothers G, O'Connell MJ, et al. Initial safety report of NSABP C-08: a randomized phase III study of modified FOLFOX6 with or without bevacizumab for the adjuvant treatment of patients with stage II or III colon cancer. J Clin Oncol. 2009;27(20):3385.
8. De Gramont A, van Cutsem E, Tabernero J, et al. AVANT: results from a randomized, three-arm multinational phase III study to investigate bevacizumab with either XELOX or FOLFOX4 versus FOLFOX4 alone as adjuvant treatment for colon cancer (abstract). Data presented at the 2011 ASCO GI Cancers Symposium; 2011 Jan 20–22; San Francisco, CA; 2011. Abstract available online at http://www.asco.org/ASCOv2/Meetings/Abstracts?&vmview=abst_detail_view&confID=103&abstractID=71344.
9. Alberts SR, Sargent DJ, Smyrk TC, et al. Adjuvant mFOLFOX6 with and without cetuximab (Cmab) in KRAS wild-type (WT) patients with resected stage III colon cancer: results from NCCTG Intergroup Phase III Trial N0147 9abstract CRA3507). J Clin Oncol. 2010;28:959s. (Abstract available online at http://abstract.asco.org/AbstView_74_41265.html, accessed July 22, 2010.
10. Kabbinavar FF, Hurwitz HI, Yi J, Sarkar S, Rosen O. Addition of bevacizumab to fluorouracil-based first-line treatment of metastatic colorectal cancer: pooled analysis of cohorts of older patients from two randomized clinical trials. J Clin Oncol. 2009;27(2):199–205.
11. Grothey A, Sugrue MM, Purdie DM, et al. Bevacizumab beyond first progression is associated with prolonged overall survival in metastatic colorectal cancer: results from a large observational cohort study (BRiTE). J Clin Oncol. 2008;26(33):5326–34.
12. Jimeno A, Messersmith WA, Hirsch FR, Franklin WA, Eckhardt SG. KRAS mutations and sensitivity to epidermal growth factor receptor inhibitors in colorectal cancer: practical application of patient selection. J Clin Oncol. 2009;27(7):1130–6.
13. Van Cutsem E, Peeters M, Siena S, Humblet Y, Hendlisz A, Neyns B, et al. Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. J Clin Oncol. 2007;25(13):1658.
14. Hecht JR, Mitchell E, Chidiac T, Scroggin C, Hagenstad C, Spigel D, et al. A randomized phase IIIB trial of chemotherapy, bevacizumab, and panitumumab compared with chemotherapy and bevacizumab alone for metastatic colorectal cancer. J Clin Oncol. 2009;27:672–80.
15. Dunn EF, Iida M, Myers RA, Campbell DA, Hintz KA, Armstrong EA, et al. Dasatinib sensitizes KRAS mutant colorectal tumors to cetuximab. Oncogene. 2011;30(5):561–74.
16. Loupakis F, Pollina L, Stasi I, Ruzzo A, Scartozzi M, Santini D, et al. PTEN expression and KRAS mutations on primary tumors and metastases in the prediction of benefit from cetuximab plus irinotecan for patients with metastatic colorectal cancer. J Clin Oncol. 2009;27(16):2622–9.
17. Yen LC, Uen YH, Wu DC, Lu CY, Yu FJ, Wu IC, et al. Activating KRAS mutations and overexpression of epidermal growth factor receptor as independent predictors in metastatic colorectal cancer patients treated with cetuximab. Ann Surg. 2010;251(2):254–60.
18. Prahallad A, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D, et al. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. Nature. 2012;483:100–3.
19. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69–90.
20. Center MM, Jemal A, Smith RA, Ward E. Worldwide variations in colorectal cancer. CA Cancer J Clin. 2009;59:366–78.

21. Migliore L, Migheli F, Spisni R, Coppedè F. Genetics, cytogenetics, and epigenetics of colorectal cancer. J Biomed Biotechnol. 2011;2011:792362.
22. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell. 1990;61: 759–67.
23. Forrester K, Almoguera C, Han K, Grizzle WE, Perucho M. Detection of high incidence of K-ras oncogenes during human colon tumorigenesis. Nature. 1987;327:298–303.
24. Bos JL, Fearon ER, Hamilton SR, et al. Nature. 1987;327:293–7.
25. Vogelstein B, Fearon ER, Hamilton SR, et al. N Engl J Med. 1988;319:525–32.
26. Ashkenazi A. Directing cancer cells to self-destruct with pro-apoptotic receptor agonists. Nat Rev Drug Discov. 2008;7:1001–12.
27. Johnstone RW, Frew AJ, Smyth MJ. The TRAIL apoptotic pathway in cancer onset, progression and therapy. Nat Rev Cancer. 2008;8:782–98.
28. Ullenhag GJ, Mukherjee A, Watson NF, et al. Overexpression of FLIPL is an independent marker of poor prognosis in CRC patients. Clin Cancer Res. 2007;13:5070–5.
29. Kaplan-Lefko PJ, Graves JD, Zoog SJ, 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;9:618–31.
30. Baron AD, O'Bryant CL, Cho Y. Phase Ib study of drozitumab combined with cetuximab (CET) plus irinotecan (IRI) or with FOLFIRI with or without bevacizumab (BV) in previously treated patients (pts) with mCRC (mCRC). J Clin Oncol. 2011;29 Suppl 4: abstr 532.
31. Sikic BI, Wakelee H, Von Mehren M, et al. A phase Ib study to assess the safety of lexatumumab, a human monoclonal antibody that activates TRAIL-R2, in combination with gemcitabine, pemetrexed, doxorubicin or FOLFIRI. J Clin Oncol. 2007; 25: abstr 14006.
32. Hotte SJ, Hirte HW, Chen EX, et al. A phase 1 study of mapatumumab (fully human monoclonal antibody to TRAIL-R1) in patients with advanced solid malignancies. Clin Cancer Res. 2008;14:3450–5.
33. Mita MM, Ochoa L, Rowinsky EK, et al. A phase I, pharmacokinetic and biologic correlative study of oblimersen sodium (GenasenseTM, G3139) and irinotecan in patients with mCRC. Ann Oncol. 2006;17:313–21.
34. van de Donk N, Kamphuis M, van Dijk M, et al. Evaluation of bcl-2 antisense oligonucleotide drugs in multiple myeloma. Blood. 2000;96:757.
35. Jansen B, Wacheck V, Heere-Ress E, et al. Chemosensitisation of malignant melanoma by BCL2 antisense therapy. Lancet. 2000;356:1728–33.
36. Rudin CM, Kosloff M, Hoffman PC, Edelman MJ, Vokes EE. Phase I study of G3139, a bcl-2 antisense oligonucleotide, combined with carboplatin and etoposide in patients with small cell lung cancer. J Clin Oncol. 2004;22:1110–7.
37. Combination Chemotherapy and Oblimersen in Treating Patients with Advanced CRC. NCT00055822.
38. Yarden Y, Sliwkovski MX. Untangling the ErB signaling network. Nat Rev Mol Cell Biol. 2001;2:127–37.
39. Engelman JA, Janne PA. Mechanisms of acquired resistance to epidermal growth factor receptor kinase inhibitors in non small cell lung cancer. Clin Cancer Res. 2008;14:2895–9.
40. Yuza Y, Glatt KA, Jiang J, et al. Allele-dependent variation in the relative cellular potency of distinct EGFR inhibitors. Cancer Biol Ther. 2007;6:661–7.
41. Li D, Ambrogio L, Shimamura T, et al. BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models. Oncogene. 2008;27:4702–11.
42. Janne PA, Boss DS, Camidge DR, et al. Phase I dose escalation study of the pan HER inhibitor PF299804 in patients with advanced malignant solid tumors. Clin Cancer Res. 2011;17:1131–9.
43. Ewing GP, Goff LW. The insulin-like growth factor signaling pathway as a target for treatment of colorectal carcinoma. Clin Colorectal Cancer. 2010;9:219–23.
44. Tricoli JV, Rall LB, Karakousis CP, et al. Enhanced levels of insulin-like growth factor messenger RNA in human colon carcinomas and liposarcomas. Cancer Res. 1986;46:6169–73.
45. Tolcher AW, Sarantopoulos J, Patnaik A, et al. Phase I, pharmacokinetic, and pharmacodynamic study of AMG 479, a fully human monoclonal antibody to insulin-like growth factor receptor 1. J Clin Oncol. 2009;27:5800–7.

46. Reidy DL, Vakiani E, Fakih MG, et al. Phase I, pharmacokinetic, and pharmacodynamic study of AMG 479, a fully human monoclonal antibody to insulin-like growth factor receptor 1. J Clin Oncol. 2010;28:4240–6.
47. Benvenuti S, Sartore-Bianchi A, Di Nicolantonio F, et al. Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. Cancer Res. 2007;15:2643–8.
48. Jiang WG, Lloyds D, Puntis MC, Nakamura T, Hallett MB. Regulation of spreading and growth of colon cancer cells by hepatocyte growth factor. Clin Exp Metastasis. 1993;11:235–42.
49. Rasola A, Fassetta M, De Bacco F, et al. A positive feedback loop between hepatocyte growth factor receptor and beta-catenin sustains colorectal cancer cell invasive growth. Oncogene. 2007;26:1078–87.
50. Rosen PJ, Sweeney CJ, Park DJ, et al. A Phase 1b study of AMG 102 in combination with bevacizumab or moteranib in patients with advanced solid tumors. Clin Cancer Res. 2010;16:2677–87.
51. Kolinsky KD, Su F, Bollag G, Lee R, et al. Efficacy of PLX4032, a selective V600EB-Raf inhibitor, as monotherapy or in combination with capecitabine ± bevacizumab in a CRC xenograft model. Gastrointestinal Cancers Symposium; San Francisco, CA; 2009; Abstract No:362.
52. Kopetz S, Desai J, Chan E, Hecht JR, et al. PLX4032 in mCRC patients with mutant BRAF tumors. J Clin Oncol. 2010; 28: abstr 3534.
53. Scartozzi M, Galizia E, Freddari F, Berardi R, Cellerino R, Cascinu S. Molecular biology of sporadic gastric cancer: prognostic indicators and novel therapeutic approaches. Cancer Treat Rev. 2004;30:451–9.
54. Yoon J, Koo KH, Choi KY. MEK1/2 inhibitors AS703026 and AZD6244 may be potential therapies for KRAS. Cancer Res. 2011 Jan 15;71(2):445–53.
55. Shannon AM, Telfer BA, Smith PD, et al. The mitogen-activated protein/extracellular signal-regulated kinase inase 1/2 inhibitor AZD6244 (ARRY-142886) enhances the radiation responsiveness of lung and colorectal tumor xenografts. Clin Cancer Res. 2009;15:6619–29.
56. Rinehart J, Adjei AA, Lorusso PM, et al. Multicenter phase II study of the oral MEK inhibitor, CI-1040, in patients with advanced non-small-cell lung, breast, colon, and pancreatic cancer. J Clin Oncol. 2004;22:4456–62.
57. Delord J, Houede N, Awada A, et al. First-in-human phase I safety, pharmacokinetic (PK), and pharmacodynamic (PD) analysis of the oral MEK-inhibitor AS703026 (two regimens [R]) in patients (pts) with advanced solid tumors. J Clin Oncol. 2010;28:15s.
58. Ryan DP. Novel therapies in CRC. Curr Colorectal Cancer Rep. 2006;2:116–9.
59. Oudard S, Medioni J, Aylllon J, et al. Everolimus (RAD001): an mTOR inhibitor for the treatment of metastatic renal cell carcinoma. Expert Rev Anticancer Ther. 2009;9:705–17.
60. Altomare I, Russell KB, Uronis HE, et al. Phase II trial of bevacizumab (B) plus everolimus (E) for refractory mCRC (mCRC). J Clin Oncol. 2010;28:15s (suppl; abstr 3535).
61. Shahda S, Yu M, Picus J, et al. Phase I study everolimus (RAD001) with irinotecan (Iri) and Cetuximab (C) in second-line mCRC. J Clin Oncol. 2011;29: (suppl; abstr 523).
62. Graff JR, McNulty AM, Hanna KR, et al. The protein kinase C beta-selective inhibitor, Enzastaurin (LY317615. HCl), suppresses signaling through the AKT pathway, induces apoptosis, and suppresses growth of human colon cancer and glioblastoma xenografts. Cancer Res. 2005;65:7462–9.
63. Chiappori A, Bepler G, Barlesi F, et al. Phase II, double-blinded, randomized study of enzastaurin plus pemetrexed as second-line therapy in patients with advanced non-small cell lung cancer. J Thorac Oncol. 2010;5:369–75.
64. Casey EM, Harb W, Bradford D, et al. Randomized, double blind, multicenter, phase II study of pemetrexed (PEM), carboplatin (CARBO), bevacizumab (BEV) with enzastaurin (ENZ) or placebo (PBO) in chemotherapy-naive patients with stage IIIB/IV non-small cell lung cancer (NSCLC). J Clin Oncol. 2009;27:15s (suppl; abstr 8035).
65. Kreisl TN, Kotliarova S, Butman JA, et al. A phase I/II trial of enzastaurin in patients with recurrent high-grade gliomas. Neuro Oncol. 2010;12:181–9.

66. Clemons M, Joy AA, Abdulnabi R, et al. Phase II, double-blind, randomized trial of capecitabine plus enzastaurin versus capecitabine plus placebo in patients with metastatic or recurrent breast cancer after prior anthracycline and taxane therapy. Breast Cancer Res Treat. 2010;124:177–86.
67. Wick W, Puduvalli VK, Chamberlain MC, et al. Phase III study of enzastaurin compared with lomustine in the treatment of recurrent intracranial glioblastoma. J Clin Oncol. 2010; 28:1168–74.
68. Morschhauser F, Seymour JF, Kluin-Nelemans HC, et al. A phase II study of enzastaurin, a protein kinase C beta inhibitor, in patients with relapsed or refractory mantle cell lymphoma. Ann Oncol. 2008;19:247–53.
69. Robertson MJ, Kahl BS, Vose JM, et al. Phase II study of enzastaurin, a protein kinase C beta inhibitor, in patients with relapsed or refractory diffuse large B-cell lymphoma. J Clin Oncol. 2007;25:1741–6.
70. Glimelius B, Lahn M, Gawande S, et al. A window of opportunity phase II study of enzastaurin in chemonaive patients with asymptomatic mCRC. Ann Oncol. 2010;21:1020–6.
71. Wolff RA, Schepp W, DiBartolomeo M, et al. A double-blind, randomized placebo-controlled, phase II study of maintenance enzastaurin (ENZ) with 5-FU/leucovorin (LV) plus bevacizumab (BV) following first-line therapy for mCRC (mCRC). J Clin Oncol. 2011; 29: (suppl 4; abstr 501).
72. Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. Nat Med. 2000;6:389–95.
73. Moroney JW, Sood AK, Coleman RL. Aflibercept in epithelial ovarian carcinoma. Future Oncol. 2009;5:591–600.
74. Lockhart AC, Rothenberg ML, Dupont J, et al. Phase I study of intravenous vascular endothelial growth factor trap, aflibercept, in patients with advanced solid tumors. J Clin Oncol. 2010;28:207–14.
75. William PT, Gordon M, Murren J, et al. Phase 1 study of aflibercept administered subcutaneously to patients with advanced solid tumors. Clin Cancer Res. 2010;16:358–66.
76. Rixe O, Verslype D, Khayat D, et al. A phase I dose escalation (DE) and pharmacokinetics (PK) study of intravenous aflibercept (VEGF Trap) plus irinotecan, 5-fluorouracil, and leucovorin (I-LV5FU2) in patients with advanced solid tumors (STs). J Clin Oncol 26: 2008 (May 20 suppl; abstr 3557).
77. Limentani S, Just R, Purdham A, et al. A phase I dose escalation and pharmacokinetic (PK) study of intravenous (iv) aflibercept (VEGF Trap) plus FOLFOX4 in patients (pts) with advanced solid tumors: Preliminary results. J Clin Oncol 26: 2008 (May 20 suppl; abstr 3556).
78. Tew WP, Colombo N, Ray-Coquard I, et al. VEGF-trap for patients (pts) with recurrent platinum-resistant epithelial ovarian cancer (EOC): preliminary results of a randomized, multicenter phase II study. J Clin Oncol. 2007 ASCO Annual Meeting Proceedings Part I. Vol 25, No. 18S (June 20 Supplement), 2007: 5508.
79. Colombo N, Mangili G, Mammoliti S, et al. Aflibercept (VEGF trap) for advanced epithelial ovarian cancer (EOC) patients (pts) with symptomatic malignant ascites: preliminary results of a pilot study. J Clin Oncol 26: 2008 (May 20 suppl; abstr 14598.
80. Massarelli E, Miller VA, Leighl B, et al. Phase II study of the efficacy and safety of intravenous (IV) AVE0005 (VEGF trap) given every 2 weeks in patients (pts) with platinum- and erlotinib- resistant adenocarcinoma of the lung (NSCLA). J Clin Oncol. 2007 ASCO Annual Meeting Proceedings Part I. Vol 25, No. 18S (June 20 Supplement), 2007:7627.
81. Townsley C, Hirte H, Hoskins R, et al. A phase II study of aflibercept (VEGF trap) in recurrent or metastatic gynecologic soft-tissue sarcomas: a study of the Princess Margaret Hospital Phase II consortium. J Clin Oncol. 2009 (suppl; abstr 5591).
82. Tarhini A, Christensen S, Frankel P, et al. Phase II study of aflibercept (VEGF trap) in recurrent inoperable stage III or stage IV melanoma of cutaneous or ocular origin. J Clin Oncol 27:15s, 2009 (suppl; abstr 9028).
83. De Groot JF, Wen PY, Lamborn K, et al. Phase II single arm trial of aflibercept in patients with recurrent temozolomide-resistant glioblastoma: NABTC 0601. J Clin Oncol 26: 2008 (May 20 suppl; abstr 2020).

84. Twardoski P, Sadler WM, Frankel P, et al. Phase II study of aflibercept (VEGF-trap) in patients with recurrent or metastatic urothelial cancer, a California cancer consortium trial. Urology. 2010;76:923–6.
85. Tang P, Cohen SJ, Bjarnason GA, et al. Phase II trial of aflibercept aflibercept (VEGF trap) in previously treated patients with mCRC (MCRC): a PMH phase II consortium trial. J Clin Oncol 26: 2008 (May 20 suppl; abstr 4027).
86. Yamazaki K, Yoshino K, Yamaguchi K, et al. Phase I dose escalation and pharmacokinetics study of intravenous aflibercept plus irinotecan, 5-fluorouracil, and folinic acid (FOLFIRI) in patients with mCRC. J Clin Oncol 29: 2011 (suppl 4; abstr 538).
87. De Luca A, Normanno N. Tivozanib, a pan-VEGFR tyrosine kinase inhibitor for the potential treatment of solid tumors. Drugs. 2010;13:636–45.
88. Bhargava P, Esteves B, Al-Adhami M, et al. Activity of tivozanib (AV-951) in patients (pts) with different histologic subtypes of renal cell carcinoma (RCC). J Clin Oncol. 2011; (suppl 7; abstr 327).
89. Bhargava P, Esteves B, Al-Adhami M, et al. Activity of tivozanib (AV-951) in patients with renal cell carcinoma (RCC): subgroup analysis from a phase II randomized discontinuation trial (RDT). J Clin Oncol. 2010 (suppl; abstr 4599).
90. Kabbinavar FF, Srinivas S, Kauke RJ, et al. A phase I trial of combined tivozanib (AV-951) and temsirolimus therapy in patients (pts) with renal cell carcinoma (RCC). J Clin Oncol. 2011; (suppl 7; abstr 330).
91. Mayer EL, Scheulen ME, Beckman J, et al. Combination of tivozanib, an oral inhibitor of vascular endothelial growth factor receptors (VEGFRs), with weekly paclitaxel for metastatic breast cancer: preliminary results of an ongoing phase 1 study. 33rd Annual San Antonio Breast Cancer Symposium; San Francisco, CA; 2010.
92. Motzer RJ, Bhargava P, Esteves B, et al. A phase III, randomized, controlled study to compare tivozanib with sorafenib in patients (pts) with advanced renal cell carcinoma (RCC). J Clin Oncol. 2011; (suppl 7; abstr 310).
93. Eskens F, Oldenhuis CN, Bhargava P, et al. A phase Ib, open-label, dose-escalation study of tivozanib and FOLFOX6 in patients (pts) with advanced gastrointestinal (GI) tumors. J Clin Oncol. 2011; (suppl 4; abstr 549).
94. A trial of tivozanib (AV-951) in combination with capecitabine (Xeloda®) in subjects with advanced solid tumors. 2010; http://clinicaltrials.gov/ct2/show/NCT01306630.
95. Hu-Lowe DD, Zou HY, Grazzini ML, et al. Nonclinical antiangiogenesis and antitumor activities of axitinib (AG-013736), an oral, potent, and selective inhibitor of vascular endothelial growth factor receptor tyrosine kinases 1, 2, 3. Clin Cancer Res. 2008;14:7272–83.
96. Cohen EE, Rosen LS, Vokes EE, et al. Axitinib is an active treatment for all histologic subtypes of advanced thyroid cancer: results from a phase II study. J Clin Oncol. 2008; 26:4708–13.
97. Rugo HS, Herbst RS, Liu G, et al. Phase I trial of the oral antiangiogenesis agent AG-013736 in patients with advanced solid tumors: pharmacokinetics and clinical results. J Clin Oncol. 2005;23:5474–83.
98. Fruehauf JP, Lutzky J, McDermott DF, et al. Axitinib (AG-013736) in patients with metastatic melanoma: a phase II study. J Clin Oncol. 2008;26:484s.
99. Schiller JH, Larson T, Ignatius Ou SH, et al. Efficacy and safety of axitinib in patients with advanced non-small-cell lung cancer: results from a phase II study. J Clin Oncol. 2009;25:7507.
100. Rixe O, Bukowski R, Michaelson MD, et al. Axitinib treatment in patients with cytokine-refractory metastatic renal-cell cancer: a phase II study. Lancet Oncol. 2007;8:975–84.
101. Spano J-P, Chodkiewicz C, Maurel J, et al. Efficacy of gemcitabine plus axitinib compared with gemcitabine alone in patients with advanced pancreatic cancer: an open-label randomised phase II study. Lancet. 2008;371:2101–8.
102. Wilmes L, Pallavicini MG, Fleming LM, et al. AG-013736, a novel inhibitor of VEGF receptor tyrosine kinases, inhibits breast cancer growth and decreases vascular permeability as detected by dynamic contrast-enhanced magnetic resonance imaging. Magn Reson Imaging. 2007;25:319–27.

103. Bendell JC, Tournigand C, Bednarzyk M, et al. Axitinib or bevacizumab (bev) plus FOLFOX or FOLFIRI as second-line therapy in patients (pts) with mCRC (mCRC). J Clin Oncol. 2011;29:478s.
104. Fernando NT, Kock M, Rothrock C, et al. Tumor escape from endogenous extracellular matrix associated angiogenesis inhibitors by up regulation of multiple proangiogenic factors. Clin Cancer Res. 2008;14:1529–39.
105. Mross K, Stefanic M, Gmehling D, et al. Phase I study of the angiogenesis inhibitor BIBF 1120 in patients with advanced solid tumors. Clin Cancer Res. 2010;16:311–9.
106. Prenen H, D'Haens G, Capdevila A, et al. A phase I dose escalation study of BIBF 1120 combined with FOLFOX in MCRC patients. J Clin Oncol. 2010;28:14054.
107. Okamoto I, Kaneda H, Satoh T, et al. Phase I safety, pharmacokinetic, and biomarker study of BIBF 1120, an oral triple tyrosine kinase inhibitor in patients with advanced solid tumors. Mol Cancer Ther. 2010;9:2825–33.
108. Bouche O, Ducreux M, Lledo G, et al. A phase II trial of weekly alternating sequential administration of BIBF1120 and BIBW2992 in patients with advanced CRC. J Clin Oncol. 2008;26:15001.
109. Carlomagno F, Anaganti S, Guida T, et al. BAY 43-9006 inhibition of oncogenic RET mutants. J Natl Cancer Inst. 2006;98:326–34.
110. Wilhelm SM, Carter C, Tang L, et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res. 2004;64:7099–109.
111. Escudier B, Eisen T, Stadler WM. Sorafenib in advanced clear-cell renal-cell carcinoma. N Engl J Med. 2007;356:123–34.
112. Galal KM, Khaled Z, Mourad AM. Role of cetuximab and sorafenib in treatment of mCRC. Indian J Cancer. 2011;48:47–54.
113. Suen AW, Galoforo S, Marples B, et al. Sorafenib and radiation: a promising combination in CRC. Int J Radiat Oncol Biol Phys. 2010;78:213–20.
114. External-beam radiation therapy, capecitabine, and sorafenib in treating patients with locally advanced rectal cancer. 2011; http://clinicaltrials.gov/ct2/show/NCT00869570.
115. Grothey A, Lafky JM, Morlan BW, et al. Dual VEGF inhibition with sorafenib and Bevacizumab (BEV) as salvage therapy in mCRC (mCRC): results of the phase II north central cancer treatment group study N054C. J Clin Oncol. 2010;28:15s.
116. Sorafenib and bevacizumab in treating patients with MCRC. 2011; NCT00826540.
117. Dasari A, Rudek MA, Arcaroli J, et al. Tolerance of full-dose sorafenib(S) combined with irinotecan (I; weekly, two on, on off) and cetuximab (C) in previously treated patients with advanced CRC. J Clin Oncol 29: 2011 (suppl 4; abstr 522).
118. Sorafenib and FOLFIRI Regimen in 2nd CRC (CRC) after failure of oxaliplatin treatment. 2010; http://clinicaltrials.gov/ct2/show/NCT00839111.
119. Ychou M, François E, Thézenas S, et al. Sorafenib (S) in combination with Irinotecan (I) as a treatment in mCRC (mCRC) patients (pts) with KRAS mutation (mt) as second-line or later: interim analysis results of multicenter phase II part trial (NEXIRI). J Clin Oncol. 2010;28:14022.
120. Sorafenib with irinotecan in MCRC (mCRC) and K-RAS mutation (NEXIRI). 2010; NCT00989469.
121. Sorafenib plus capecitabine efficacy assessment in patients with advanced pre-treated CRC (SoMore). 2011; NCT01290926.
122. Study to evaluate the effects of sorafenib if combined with chemotherapy (FOLFOX6 or FOLFIRI) in the second-line treatment of CRC (FOSCO). 2011; NCT0088934.
123. Study of modified FOLFOX6 plus or minus sorafenib in stage IV metastatic colorectal carcinoma (mCRC) subjects. 2011; NCT00865709.
124. Faivre S, Demetri G, Sargent W, Raymond E. Molecular basis for sunitinib efficacy and future clinical development. Nat Rev Drug Discov. 2007;6:734–45.
125. Ding W, Cai T, Zhu H, et al. Synergistic antitumor effect of TRAIL in combination with sunitinib in vitro and in vivo. Cancer Lett. 2010;293:158–66.

126. Starling N, Vázquez F, Cunningham D, et al. Phase I study to evaluate the safety and efficacy of sunitinib in combination with FOLFIRI in treatment-naïve mCRC (mCRC). J Clin Oncol 26: 2008 (May 20 suppl; abstr 3563).
127. Study of sunitinib in combination with Folfox in patients with CRC. 2011; NCT00631410.
128. Randomized study of sunitinib plus FOLFOX versus bevacizumab plus FOLFOX in MCRC. 2011; NCT00609622.
129. Samson B, Latreille J, Nguyen NT, et al. SUNCAP, a phase II study with sunitinib and capecitabine in patients with mCRC (MCRC) refractory to previous treatment with 5FU/irinotecan/oxaliplatin. J Clin Oncol 29: 2011 (suppl 4; abstr 545).
130. Johnson FM, Saigal B, Talpaz M, Donato NJ. Dasatinib (BMS-354825) tyrosine kinase inhibitor suppresses invasion and induces cell cycle arrest and apoptosis of head and neck squamous cell carcinoma and non -small cell lung cancer cells. Clin Cancer Res. 2005;11:6924–32.
131. Starodub A, Cohn AL, Arrowood C, et al. Phase I study of dasatinib in combination with capecitabine, oxaliplatin, and bevacizumab followed by an expanded cohort in previously untreated mCRC. J Clin Oncol 29: 2011 (suppl 4; abstr 513).
132. Sharma M, Wroblewski K, Kozloff M, et al. Dasatinib (D) in previously treated mCRC (mCRC) patients: a pahse II trial of the Univrsity of Chicago phase II consortium. J Clin Oncol 29: 2011 (suppl 4; abstr 506).
133. Dunn EF, Iida M, Myers RA, et al. Dasatinib sensitizes KRAS mutant colorectal tumors to cetuximab. Oncogene. 2011;30:561–74.
134. Cetuximab and/or dasatinib in treating patients with CRC and liver metastases that can be removed by surgery. 2010; NCT00835679.
135. Heiss MM, Murawa P, Koralewski P, 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;127:2209–21.
136. Ruf P, Lindhofer H. Induction of a long-lasting antitumor immunity by a trifunctional bispecific antibody. Blood. 2001;98:2526–34.
137. Zeidler R, Mysliwietz J, Csánady M, 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;83:261–6.
138. Heiss MM, Ströhlein MA, Jäger M, et al. Immunotherapy of malignant ascites with trifunctional antibodies. Int J Cancer. 2005;117:435–43.
139. Burges A, Wimberger P, Kümper C, et al. Effective relief of malignant ascites in patients with advanced ovarian cancer by a trifunctional anti-EpCAM x anti-CD3 antibody: a phase I/II study. Clin Cancer Res. 2007;13:3899–905.
140. Strohlein MA, Heiss MM. Catumaxomab therapy in peritoneal carcinomatosis from colon cancer: clinical benefit in comparison to systemic chemotherapy alone and cytoreductive surgery/hyperthermic chemoperfusion J Clin Oncol 29: 2011 (suppl 4; abstr 488).
141. Ströhlein MA, Essing MM, Hennig M, et al. Effect of catumaxomab treatment in patients with peritoneal carcinomatosis and malignant ascites due to gastrointestinal cancers on survival. J Clin Oncol 29: 2011 (suppl 4; abstr 490).
142. Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu Rev Immunol. 2003;21:335–76.
143. Hemmi H, Takeuchi O, Kawai T, et al. A toll-like receptor recognizes bacterial DNA. Nature. 2000;408:740–5.
144. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. Nat Med. 2004;10:909–15.
145. Pulendran B, Ahmed R. Translating innate immunity into immunological memory: implications for vaccine development. Cell. 2006;124:849–63.
146. Rayburn ER, Wang W, Zhang R, Wang H. Experimental therapy for colon cancer: anti-cancer effects of TLR9 agonism, combination with other therapeutic modalities, and dependence upon p53. Int J Oncol. 2007;30:1511–9.
147. Damiano V, Caputo R, Bianco R, et al. Novel toll-like receptor 9 agonist induces epidermal growth factor receptor (EGFR) inhibition and synergistic antitumor activity with EGFR inhibitors. Clin Cancer Res. 2006;12:577–83.

148. Garin-Chesa P, Sakamoto J, Welt S, et al. Organ specific expression of the colon cancer antigen A33, a cell surface target for antibody-based therapy. Int J Oncol. 1996;9:465–71.
149. Daghighian F, Barenswaard E, Welt S, et al. Enhancement of radiation dose to the nucleus by vesicular internalization of iodine 125 labeled A33. J Nucl Med. 1996;37:1052–7.
150. King DJ, Antoniw P, Owens R, et al. Preparation and preclinical evaluation of humanised A33 immunoconjugates for radioimmunotherapy. Br J Cancer. 1995;72:1364–72.
151. Welt S, Ritter G, Williams Jr C, et al. Phase I study of anticolon cancer humanized antibody A33. Clin Cancer Res. 2003;9:1338–46.
152. Taipale J, Beachy PA. The hedgehog and Wnt signaling pathways in cancer. Nature. 2001;411:349–54.
153. Qualtrough D, Buda A, Gaffield W, Williams AC, Paraskeva C. Hedgehog signaling in colorectal tumor cells: induction of apoptosis with cyclopamine treatment. Int J Cancer. 2004;110:831–7.
154. NCT 00636610. A study of GDC-0449 (Hedgehog pathway inhibitor) with concurrent chemotherapy and bevacizumab as first-line therapy for mCRC. 2008; NCT00636610.
155. Sade A, Tunçay S, Cimen I, Severcan F, Banerjee S. Celecoxib reduces fluidity and decreases metastatic potential of colon cancer cell lines irrespective of COX-2 expression. Biosci Rep. 2012;32:35–44.
156. Galamb O, Spisák S, Sipos F, et al. Reversal of gene expression changes in the colorectal normal-adenoma pathway by NS398 selective COX2 inhibitor. Br J Cancer. 2010;102:765–73.
157. Carothers AM, Davids JS, Damas BC, Bertagnolli MM. Persistent cyclooxygenase-2 inhibition downregulates NF-κB, resulting in chronic intestinal inflammation in the Min/+ mouse model of colon tumorigenesis. Cancer Res. 2010;70:4433–42.
158. Clarke PA, Hostein I, Banerji U, et al. Gene expression profiling of human colon cancer cells following inhibition of signal transduction by 17-allylamino-17 demethoxygeldnamycin, an inhibitor of the hsp90 molecular chaperone. Oncogene. 2000;19:4125–33.
159. Vasilevskaya IA, O'Dwyer PJ. 17-allylamino-17-demethoxygeldnamycin overcomes TRAIL resistance in colon cancer cell lines. Biochem Pharmacol. 2005;70:580–9.
160. Hwang M, Moretti L, Lu B. HSP90 inhibitors: multi-targeted antitumor effects and novel combinatorial therapeutic approaches in cancer therapy. Curr Med Chem. 2009;16: 3081–92.
161. Li Q-X, Liu G, Wong-Staal F. Oncolytic virotherapy as a personalised cancer vaccine. Int J Cancer. 2008;123:493–9.
162. Park BH, Hwang T, Liu TC, et al. Use of a targeted oncolytic poxvirus, JX-594 in patients with a refractory primary or metastatic cancer, a phase 1 trial. Lancet Oncol. 2008;9:533–42.
163. Breitbach CJ, Burke J, Jonker D, et al. Intravenous delivery of a multi-mechanistic cancer-targeted oncolytic poxvirus in humans. Nature. 2011;477:99–102.
164. Clevers H. The cancer stem cell: premises, promises and challenges. Nat Med. 2011;17(3): 313–9.
165. Shipitsin M, Polyak K. The cancer stem cell hypothesis: in search of definitions, markers, and relevance. Lab Invest. 2008;88(5):459–63.
166. Abdul Khalek FJ, Gallicano GI, Mishra L. Colon cancer stem cells. Gastrointest Cancer Res, 2010; Supplement 1:S16–23.
167. Thenappan A, Li Y, Shetty K, et al. New therapeutics targeting colon cancer stem cells. Curr Colorectal Cancer Rep. 2009;5(4):209.
168. Todaro M, Alea MP, Di Stefano AB, et al. Colon cancer stem cells dictate tumor growth and resist cell death by production of interleukin-4. Cell Stem Cell. 2007;1(4):389–402.
169. Yu XF, Zou J, Bao ZJ, Dong J, et al. miR-93 suppresses proliferation and colony formation of human colon cancer stem cells. World J Gastroenterol. 2011;17(42):4711–7.
170. Kemper K, Rodermond H, Colak S, Grandela C, Medema JP, et al. Targeting colorectal cancer stem cells with inducible caspase-9. Apoptosis. 2012;17(5):528–37.
171. Gallant JN, Allen JE, Smith CD, Dicker DT, Wang W, Dolloff NG, et al. Quinacrine synergizes with 5-fluorouracil and other therapies in colorectal cancer. Cancer Biol Ther. 2011;12(3):239–51.

172. Huang C, Zhang XM, Tavaluc RT, Hart LS, Dicker DT, Wang W, et al. The combination of 5-fluorouracil plus p53 pathway restoration is associated with depletion of p53-deficient or mutant p53-expressing putative colon cancer stem cells. Cancer Biol Ther. 2009;8(22):2186–93.
173. Lin L, Liu Y, Li H, Li PK, Fuchs J, Shibata H, et al. Targeting colon cancer stem cells using a new curcumin analogue, GO-Y030. Br J Cancer. 2011;105(2):212–20.
174. Saif MW, Chu E. Biology of colorectal cancer. Cancer J. 2010;16(3):196–201.
175. Katoh Y, Katoh M. Hedgehog signaling pathway and gastrointestinal stem cell signaling network (review). Int J Mol Med. 2006;18(6):1019–23.
176. Markowitz SD, Bertagnolli MM. Molecular origins of cancer: molecular basis of colorectal cancer. N Engl J Med. 2009;361(25):2449–60.
177. Spike BT, Wahl GM. p53, stem cells, and reprogramming: tumor suppression beyond guarding the genome. Genes Cancer. 2011;2(4):404–19.
178. Allen JE, Hart LS, Dicker DT, Wang W, El-Deiry WS. Visualization and enrichment of live putative cancer stem cell populations following p53 inactivation or Bax deletion using non-toxic fluorescent dyes. Cancer Biol Ther. 2009;8(22):2194–205.
179. Takebe N, Harris PJ, Warren RQ, Ivy SP. Targeting cancer stem cells by inhibiting Wnt, Notch, and Hedgehog pathways. Nat Rev Clin Oncol. 2011;8(2):97–106.
180. Garber K. Companies waver in efforts to target transforming growth factor beta in cancer. J Natl Cancer Inst. 2009;101(24):1664–7.
181. Garber K. Drugging the Wnt pathway: problems and progress. J Natl Cancer Inst. 2009;101(8):548–50.
182. Low JA, de Sauvage FJ. Clinical experience with Hedgehog pathway inhibitors. J Clin Oncol. 2010;28(36):5321–6.
183. Pannuti A, Foreman K, Rizzo P, Osipo C, Golde T, Osborne B, et al. Targeting Notch to target cancer stem cells. Clin Cancer Res. 2010;16(12):3141–52.
184. Essmann F, Schulze-Osthoff K. Translational approaches targeting the p53 pathway for anticancer therapy. Br J Pharmacol. 2012;165(2):328–44.
185. Medema JP, Vermeulen L. Microenvironmental regulation of stem cells in intestinal homeostasis and cancer. Nature. 2011;474(7351):318–26.
186. Shaker A, Rubin DC. Intestinal stem cells and epithelial-mesenchymal interactions in the crypt and stem cell niche. Transl Res. 2010;156(3):180–7.
187. van der Flier LG, Clevers H. Stem cells, self-renewal, and differentiation in the intestinal epithelium. Annu Rev Physiol. 2009;71:241–60.
188. Vermeulen L, De Sousa E, Melo F, van der Heijden M, et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. Nat Cell Biol. 2010;12(5):468–76.

Update on Clinical Trials: Genetic Targets in Breast Cancer

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Abstract Breast cancer is the most commonly diagnosed cancer in women in United States. From data of American Cancer Society from 2007 reported total of 178,480 women diagnosed with breast cancer. The death rate from breast cancer has decreased in North America over time, but still accounts for second highest cancer death, following lung cancer. Breast cancer is staged based on tumor size, nodal involvement, and distant metastasis like any other solid tumors. However clinical staging is not the only important factor in management of breast cancer. Various molecular features divides breast cancer into many subgroups – that act differently, and respond differently from therapy. Thus the focus of breast cancer treatment has evolved focusing on specific targets. The most important biologic markers in subtyping of breast cancer so far are hormone receptor positivity and HER2/neu protein expression. Five molecular subtypes using intrinsic gene set include Basal mRNA, HER2 + mRNA, Luminal AmRNA, Luminal B mRNA, and Normal-like mRNA. In addition, better understanding of genetic target of breast cancer has given us arsenal of personalized, and more effective treatment approach.

This review will focus on examples that highlight several mechanism of tumorigenesis, giving us not just understanding of gene pathways and the molecular biology, that could lead us to therapeutic target. Several important molecular targets have been investigated in preclinical and clinical trials, others are yet to be explored. We will also describe genetic mechanisms discovery related to overcoming resistance to current targeted therapies in breast cancer, including hormone receptor expression and HER 2- neu amplification. We will also review other exciting

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W.S. El-Deiry (ed.), *Impact of Genetic Targets on Cancer Therapy*, Advances in Experimental Medicine and Biology 779, DOI 10.1007/978-1-4614-6176-0_2,

developments in understanding of breast cancer, the tumor microenvironment and cancer stem cells, and targeting agents in that area.

Keywords Breast cancer • Her2 • Growth factor • Tyrosine kinase • Herceptin • Trastuzumab • PI3K • mTOR • PTEN • c-Met • IGF-1R • TKI • TDM1 • Fulvestrant • Tamoxifen • Cancer stem cells • Bevacizumab • Ras • EGF • Tipifarnib • Letrozole • Sorafenib • HDAC Inhibitor • Sir2 • HIF • Vorinostat • miRNA • Telomerase • Olaparib

Cell Membrane Signaling

Growth Factor Receptors EGFR and HER2- neu as Molecular Targets in Breast Cancer

About 15–20% of breast cancers have over-expression of HER2 [1]. HER2(ErbB2) is a transmembrane glycoprotein with an intracellular receptor tyrosine kinase(TK) domain, and extracellular ligand binding domain. The HER family consists of four family members – HER1(ErbB1 = EGFR), 2, 3, and 4. Each different subtype of HER protein shares similar intracellular TK domains, but expresses distinct ligand binding extracellular domains [2]. The HER receptor acts via dimerization of receptors, either homodimerization, or heterodimerization between different proteins [3, 4]. HER2 overexpression is also found in other types of cancers, for example, gastric cancer. However especially in breast cancer, HER2 overexpression is one of the most important carcinogenic features, as well as being a prognostic and predictive marker for treatment response [5].

The first HER2 targeting agent approved by the FDA in 1998 was trastuzumab (brand name Herceptin®). Trastuzumab is a monoclonal antibody that targets the juxtamembrane domain of HER2 [6]. Since trastuzumab was approved by FDA, it has become the cornerstone of treating HER2 overexpressing breast cancer patients in the neoadjuvant, adjuvant and metastatic settings. Combining trastuzumab with chemotherapy in HER2 overexpressing breast cancer increases the median survival and disease free survival by 25% [7, 8]. Trastuzumab not only inhibits HER2 by binding at the extracellular domain, but it also induces the activity of p21 or p27 which then cause transcription inhibition [9]. Other mechanisms of actions of this antibody including an immunologic basis are also possible.

However, just like with other biologic agents, HER2 positive breast cancers either develop resistance, or are natively resistant to trastuzumab (Fig. 1). There are two major mechanisms of resistance. One is activation of HER2 downstream pathways (PI3K-AKT-mTOR) either by existence of a HER2 form that lacks the trastuzumab binding site, or via a mutated pathways controlled not by HER2 but by intracellular activation that does require HER2 for its activation [10]. A second mechanism of resistance is activation of alternative signaling pathways via HER2

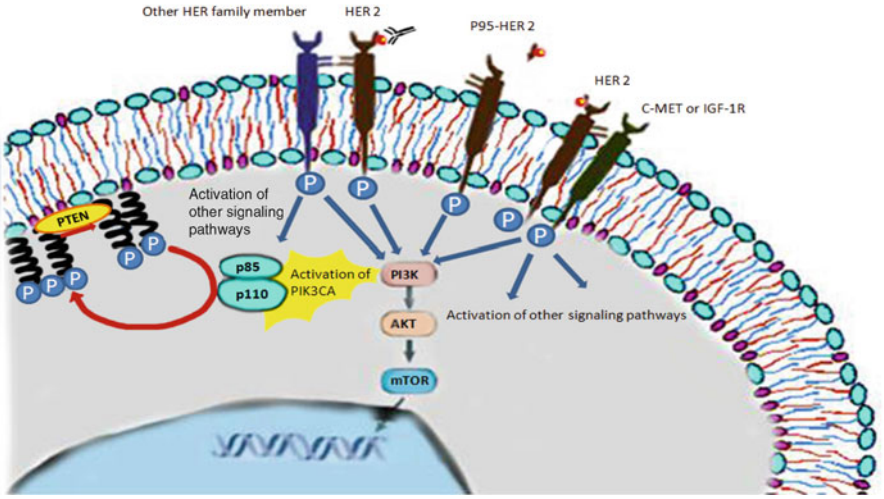

Fig. 1 Suggested trastuzumab resistance mechanisms. From the left: (1) Loss of PTEN, or mutations in PI3KCA result in constitutive activation of PI3K pathway (via activation of PI3K via dephosphorylation of PIP3 to PIP2. (2) Activation of PI3K pathway by HER2, can be activated also by other HER family members including HER3. (3) P95-HER2 can constitutively activate downstream, and escape trastuzumab. (4) Dimerization with other receptors, c-MET, IGF-1R

heterodimerization with other HER family members or non-HER family member dimerization partner proteins, (C-MET, IGF-1R for example) [10–12].

Existence of P95-HER2 is part of the first resistance mechanism. This is a form of truncated HER2, which lacks the binding domain of trastuzumab, thus it stays constitutively active. When there are a large numbers of p95-HER2 proteins, they will send downstream signals to activate PI3K-AKT-mTOR pathway despite presence of trastuzumab [13–15]. Also, if there is a PIK3CA mutation that does not require HER2 to be activated, but can be activated by PTEN intracellular activator, blocking HER2 by trastuzumab wi1ll not affect ultimate cell growth and proliferation. Improving the inhibition of HER2 using combinations of more than one biologic agents, is currently the direction of HER2 therapy [16].

HER2 activation of other HER family members by heterodimerization with other HER receptors also signal downstream activation so the cells can continue growth and proliferation. Thus, blocking other HER family members has been recognized as an important mechanism of overcoming resistance in HER2 overexpressing breast cancers. HER2 itself lacks a ligand binding domain, however other HER proteins have extracellular ligand binding domain, can form a dimer with HER2 and are activated by ligand binding. HER2 gains stable dimer activity and then activates intracellular signaling: Other receptor proteins that form dimer with HER2 also can send downstream signaling independently from HER2 [3, 17, 18]. HER3 for example, which is thought to be a signaling expert by having six different domains that can activate PI3K(p85 SH2 adaptor domain) HER3 can send signals to activate PI3K

pathway – via para/auto/intracrine expression even after HER2 is blocked [19, 20]. Thus, using inhibitors of HER2/3 coupling is emerging as a method of overcoming this form of resistance. Pertuzumab in a novel agent which inhibits this type of dimerization [21]. HER1 which can also form dimers with HER2, activates the MAPK downstream pathway, which is not affected by inhibition of HER2 [3].

Small molecule TKIs(tyrosine kinase inhibitors) have activity against HER2 overexpressing breast cancers. These small TKIs can bind to HER2 in different domains than trastuzumab; and some also interfere with HER2/3 coupling, which can escape from the resistance mechanism described above. Examples of these small molecules include lapatinib, pelitinb, erlotinib, gefitinib, afatinib, neratinib, and canertinib [22–24]. So far the most promising small molecules that showed activity is lapatinib. Lapatinib is an oral, small molecule TKI that has dual activity to HER1 and HER2. It also seems to inhibit insulin-like growth factor 1 signaling, another mechanism of trastuzumab resistance [25–27]. In the metastatic setting, lapatinib as first line therapy has similar efficacy to first line trastuzumab therapy in trastuzumab naïve patients [25–27]. Combinations of lapatinib and trastuzumab are also being investigated in early stage breast cancer, since these two agents target different sites of HER2. The ALTTO (Adjuvant Lapatinib Trastuzumab Treatment Optimization) study (NCT00490139) trial is looking at chemotherapy combined with Trastuzumab, compared with combination with Lapatinib arm, and chemotherapy combined with both Trastuzumab and Lapatinib [28]. Cleopatra trial (trastuzumab and pertuzumab with docetaxel) showed 6 months improvement in progression free survival in HER2 positive metastatic breast cancer. Based on promising data of this dual therapy combination, more dual targeted approaches using other agents are underway.

Another approach to HER2 targeted therapy, is the antibody-drug conjugate approach. This approach uses a targeted antibody not just for blocking the tumor regulating protein, but also uses them as carrier of toxin to targeted cells. T-DM1 is HER2 specific antibody-drug conjugate, that consists of trastuzumab and emtansine(DM1) [29]. Emtansine is cytoxin mertansine, an anti microtubule maytansinoid derivative. This agent has shown activity in heavily pretreated and trastuzumab resistant patients [30]. The toxicity profile of this agent is more favorable than standard chemotherapy since the cytotoxic effect is limited to malignant HER2 overexpressing cells. The MARIANNE trial is a phase III registration trial currently accruing patients in the first line metastatic setting which will address the comparative efficacy of standard trastuzumab based therapy, versus T-DM1 and T-DM1 in combination with pertuzumab [31]. With the many new therapeutic options in her two therapy, the main issue will be how to optimally sequence and combine these therapies.

IGF in Breast Cancer

In vitro studies suggest that proliferation, migration and cell survival of breast cancer cell lines depends on activation of the insulin growth factor (IGF) type 1 receptor (IGF-1R). The IGF signaling pathway contributes to both cellular proliferation and apoptosis.

IGF receptor has been functionally linked with estrogen receptor signaling in breast cancer. Multiple polymorphisms of the IGF-1 pathway have been analyzed and studies have revealed certain functional polymorphisms as independent prognostic markers for tumor recurrence [32–35].

A U.K. study analyzed tissue samples of 222 patients with ER positive primary invasive breast cancer who had undergone surgery and adjuvant hormone treatment between 1981 and 2003. Six functional IGF-1 pathway polymorphisms were analyzed. Patients carrying one of these polymorphisms, IGF-1_rs2016347, had a significantly better disease free survival than patients without this polymorphism (5.3 years vs 7.6 years; p-0.02) [36].

IGF pathway signaling has also been linked to tamoxifen resistance. In a recent study, ligand stimulation of IGF-1R in ER-positive MCF7 human breast cancer cells rendered MCF7/IGF-1R cells highly resistant to the antiestrogens tamoxifen and fulvestrant. The downstream pathways of IGF-1R were found to act independently of ER signaling suggesting that the IGF-1/IGF signaling axis may play a crucial role in antiestrogen resistance despite continuous suppression of ER transcription by antiestrogens [37].

IGF insulin receptor kinase inhibitors are currently undergoing clinical trials. One such IGF-IR kinase inhibitor BMS-754807 shows promising results in preclinical evaluation. It showed synergistic in vitro anti-proliferative activity in combination with tamoxifen, letrozole or fulvestrant in the aromatase expressing breast cancer model, MCF-7/AC-1. Currently it is being evaluated in a phase II study in patients with HR positive breast cancer who have progressed with prior non-steroidal aromatase inhibitor treatment [38].

Anti-IGF-IR/InsR therapy has also being tested in triple-negative breast cancer. Litzenburger and colleagues tested the sensitivity of triple negative cell lines with IGF gene expression. These cell lines showed tumor growth inhibition and, in combination with docetaxel, resulted in tumor regression. Regression was associated with reduced proliferation, increased apoptosis, and mitotic catastrophe. This suggests rationale for further testing of IGF inhibitors in combination with chemotherapy in patients with triple negative breast cancer [39].

Impact of the Tumor Microenvironment and Cancer Stem Cells

Cancer Stem Cells and Stem Cell Niche in Breast Cancer

Stem cells are the cells with a capacity to self - renewal and to generate daughter cells that can differentiate into different cell lineages to form multiple differentiated cell types and mature tissue [40]. Stem cell biology has been the area of hematologic disease due to easy access to liquid biopsy of cancer cells. However recently, the importance of stem cell biology in solid cancers started to be elucidated [36]. Whether there is true ‘stem cells’ in solid cancer is ongoing debate. Also, the exact mechanism of stem cell activity and identification has been a challenge.

In solid cancers including breast, there are two main models suggested. In a transitory, or an induced model, a stem cell behavior can be gained or lost, and mammary cells can interact with microenvironment, or stem cell niche. On the other hand, in a permanent stem cell model, a fixed group of cells are the only ones with ability to function as stem cells. We know from the animal experiments, that mammary tree stem cell, or alveolar bud-only cells, or duct-only stem cells showed different level of ability to form structures, when the cells were engrafted in same clean fat pad. This could support the model of pre-existing selected stem cell population.

Whether stem cell in solid cancer is transitory, or permanent, many studies showed the importance of stem cell niche, or microenvironment in function of cancer stem cell. Stem cell niches in breast cancer are specialized locations within a tissue that have the ability to support stem cell function. In mice experiments, cancer cells injection only fail to generate full grown cancer, unless human fat pad microenvironment with proper vascular environment. There is also arguable evidence that when stem cells are depleted, nearby daughter cells can be induced to transform as stem cells [33, 41].

Whether mammary stem cells are related to actual breast cancer tumorigenesis, is different question. The cells expressing CD44/CD24, lacking expression of epithelial markers were suggested as putative breast cancer stem cells. These population of cells showed up to 50 fold increased ability to form tumor in xenograft model. Assuming these stem cell population will always grow regardless of targeting bulk of tumor cells by conventional therapy, a search for the method to better recognize this group of cells, and development of targeting method is highly mandated. Detection of putative genes, or epigenetic mechanism that cause the 'stemness' of breast cells, can lead us to anti-stem-cell therapy by silencing those targets. However this stem cell targeting therapy is at its beginning step, and long years of researches are to come.

Angiogenic Targets

Tumorigenesis is a complicated, well orchestrated, multi-step process. As researchers reach a better understanding of tumorigenesis, focus has turned to the microenvironment of the tumor. Tumors are analogous to small organisms that grow and survive, based on constant interaction s with their microenvironment. A key component of this process is blood vessel transport function of crucial nutrients and waste products. For tumors to grow bigger than 1–2 mm, new vessel formation is mandatory [42–44]. Without a new viable vasculature supply, tumors can't grow further, and face cell death. Angiogenesis can happen in various steps of tumorigenesis – during maintenance of survival, and later during invasion and metastasis [45–47]. This vessel formation and activity can be measured as microvascular density(MVD), and considered as important prognostic factor to assess a cancer patient's prognosis [48–50].

An important factor involved in angiogenesis is hypoxia of tumor cells, either via VEGF or via direct increases of HIF(Hypoxia induced factor). When the tumor cells

get crowded enough to be in hypoxic environment, they secrete lymphangiogenic factors, like VEGF, PDGF, bFGF. Among these factors, VEGF is the most well studied, and known to be the key activator of angiogenesis [51–53]. Hypoxia upregulates VEGF levels by stabilizing messenger RNA, and increases VEGF expression by facilitating transcription of genes mediated by hypoxia inducible transcription factors(HIF) [54]. VEGF is heparin binding glycoprotein family, consisting of six isoforms. Isoforms include VEGF-A to E, and placental growth factor [55]. Normally, VEGF is produced by endothelial cells, however in tumors, it is produced by tumor cells or stromal cells via paracrine signaling. VEGF is essential in the induction, growth and maintenance of vascular endothelial cells by direct and indirect actions. Direct actions are via stimulation of endothelial mitogenesis, This Akt dependent pathway a promotes endothelial cell survival, and endothelial cell migration by degradation of the extracellular matrix and synthesis of erythropoietin [56–59]. In actively growing and metastasizing tumors, VEGF levels are elevated. Up regulation of VEGF factors have been observed in many different tumor types, including breast, colorectal, gastric, cervical cancers [60–66]. Based on these findings, it has been suggested that VEGF is prognostic in various cancers, including breast cancer [67–72].

The other mechanism of enhancing angiogenesis is via mutation of oncogenes that increase growth factor signaling and which can result in the activation of MAPK, PI3K, or PKC. Activation of these pathways can then increase HIF-1a activity or synthesis, even in a normoxic environment [51, 52]. However angiogenesis is not a simple straightforward process, and it requires interactions with the extracellular matrix, and involvement of pericytes, etc. [73–76].

Since angiogenesis is a key mechanism of tumor survival and growth, targeting angiogenesis in cancers stimulated active investigation. However, studies have shown that antiangiogenic drug alone is not sufficient, and sometimes can cause accelerated tumor growth. For instance, when a VEGF inhibitor was used as a single agent, growth of tumor itself is inhibited, but the ability of cells to become more invasive and metastatic can be enhanced. This can be explained by ‘paradoxical normalization of tumor vasculature’, which improves blood supply to the tumor, by selecting for healthy vessels. Since VEGF also acts as vascular permeability factor, inhibiting VEGF caused an increase in tumor interstitial pressure, and limited penetration of drugs [77]. Thus, these agents are currently being used in combination with other agents, and more often in the setting of treatment failure or metastasis, rather than as first line.

The agent which has been most widely tested in breast cancer is thus far bevacizumab. . It is monoclonal antibody that binds and inactivates all isoforms of VEGF [78, 79]. In 2007, bevacizumab was given accelerated approval by the FDA for use in combination with paclitaxel for first line therapy in locally recurrent or metastatic HER2 negative breast cancer. This approval was based on improved progression free survival rather than overall survival data. After initial approval, the FDA requested further study to define clinical benefit. Three years later, the follow up study (NCT00028990) still did show significant progression free survival (11.3 months vs 5.8 months), but no overall survival benefit emerged. The marginal benefit of progression free survival was not felt to outweigh treatment related adverse

events. After review of these results, ODAC (oncologic drugs advisory committee) recommended withdrawal of the approval of bevacizumab for use in breast cancer [80]. The decision has been appealed, and bevacizumab is currently still under FDA consideration for use in metastatic disease. The agent continues to be investigated in trials for treatment of early breast cancer.

Other studies using bevacizumab are AVADO, RIBBON-1, and RIBBON-2, which also showed improved progression free survival, as well as increased tumor response rate; however the differences were less significant than those seen in the pivotal E2100 study. Progression free survival may be a more meaningful endpoint in metastatic breast cancer, (because most patients receive therapy following progression), and ma replace overall survival in this setting. However more data is needed regarding quality of life to answer whether or not this increased progression free survival is with a quality of life compared to standard therapy. Other anti-angiogenesic agents include small molecule tyrosine kinase inhibitors, like Sorafenib. Sorafenib has multiple target activities towards VEGFR2, VEGFR3, Raf, PDGFR and KIT, RET [81]. TKIs are of interest in both as anti-ras/raf agents in the breast cancer setting, as well as for their anti-angiogenic effect. These agents will be reviewed in next section.

Intracellular Signaling and Genetic, Epigenetic Targets

Intracellular Signaling; Ras, Raf, mTOR

Elucidating intracellular signaling pathways in breast cancer has proved challenging. Not only is there is extensive crosstalk between pathways; but there is also overlapping function of these pathways. This makes single targets, or pathways, difficult to block. A particularly important pathway in breast cancer is the ras/raf/MAPK pathway. Ras is a GTPase which has downstream effects on proliferation and cell regulation. Ras is activated extracellularly by estrogen, IGF and EGF ligands [82]. Ras mutations occur in 2% of breast cancers, but ras hyperactivation is frequent [83]. In HER2 -neu overexpressing and estrogen receptor positive breast cancers, ras signaling is dysregulated. In addition, ras has been found to be over expressed in other breast cancers [84].

One strategy for inhibiting ras signaling is the use of farnestyl transferase inhibitors (FTI). Ras has to be prenylated which renders it hydrophobic and causes the protein to be localized near the cell membrane. FTIs block this process which decreases ras. Several studies of a farnestyltransferase inhibitor, tipifarnib have been reported. A phase II study of 32 patients treated with tipifarnib, in addition to standard cytotoxic neoadjuvant therapy, has been reported. Interestingly complete pathologic responses were seen in hormone positive tumors, which is unusual in hormone positive tumors treated with neoadjuvant cytotoxic therapy. In metastatic breast cancer, tipifarnib, in combination with fulvestrant, had a 51% clinical

benefit rate [85]. A small study with tipifarnib and letrozole in patients unresponsive to one hormonal therapy did not show any improvement in response rate [86].

Sorafenib is a multikinase inhibitor which blocks angiogenic and ras pathways. A phase II trial of sorafenib was conducted in 23 patients, but was stopped when there were no responses. Only two patients achieved stable disease. Sorafenib was well tolerated. The investigators concluded that sorafenib was ineffective as a single agent but may be effective in combinations [82].

The PI3K-AKT-mTOR pathway is an important regulator of cell survival. This pathway has multiple stimulating and inhibiting molecules and is responsible for transition through the cell cycle, and transcription and response to angiogenic signals [81]. There is aberrant function of this pathway in breast cancer. Thus, mTOR inhibitors have been actively investigated in clinical trials - and showed improved survival in metastatic setting in combination with examestane (Bolero trial).

Metformin, an insulin lowering medication, blocks multiple kinases. Cell death has been observed in vitro in breast cancer cell lines treated with metformin. Metformin can block the mTOR pathway indirectly. In addition, metformin also modifies IGF expression and binding. Retrospective data have shown higher neoadjuvant response rates in patients who are concurrently on metformin. At least two clinical trials are ongoing to address the efficacy of metformin. The NSABP is conducting a large adjuvant trial analyzing the effect of metformin on breast cancer recurrence. Others are prospectively adding metformin to neoadjuvant therapy in breast cancer [87, 88].

Urokinase plasminogen activator is a poor prognostic factor in breast cancer. This protease is important in disrupting the extracellular membrane which facilitates migration and invasion of breast cancer cells. Disabling this pathway may be a target for breast tumors which over-express urokinase plasminogen activator [89].

Post-Translational Modification: Histone Deacytelase Inhibitors (HDACIs)

HDAC (histone deacetylases) are enzymes whose principal role is regulating gene transcription by counter activity of histone acetyl transferases (HATs). While HATs acetylate histone and non histone proteins, HDACs work by removing acetyl groups from lysine residues of histone tails of chromatin, and from non histone proteins. HDACs were initially found to contribute to tumorigenesis by regulating DNA transcription, as well in addition to another mechanisms [90, 91]. There are different groups of HDAC. There are total 18 HDACs that form four groups. Class I consists of HDAC 1,2,3, 8 and this class of HDAC family members are located in the nucleus. Class II families are located both in the nucleus and in the cytoplasm, and includes 4,5,6,7, and 9. Class III family is class of Sir2 homologues (sirtuin), and class IV includes HDAC 11. Class IV HDAC, 11 shows features of class I and II HDAC family, and arebalso structurally similar. Mainly class I, II, and IV are the focus of developing targeting agents, and class III has t distinct activity from the others [57, 58].

Each HDAC members shows expression in different types of cancers [92–95]. In breast cancer cells, HDAC 4,6,11s were found to be overexpressed [96]. HDACs play important role in epigenetic mechanism in cancers, both in steps of initial tumorigenesis and metastasis [97].

Interestingly, HDACs are involved in tumorigenesis not only through regulation of transcription, but also by several other mechanisms. When tumor cells activate angiogenesis responding to hypoxia, HDACs functionality is also affected. First, cells in a hypoxic environment express increased level of HDAC mRNA and proteins, which increase HDAC expression. The increased number of HDACs can interact directly with HIF-1a via ODD domain, regulate stability and transcriptional activity of HIF-1a. Different types of HDAC are related to different mechanisms of activity, and different cells are affected distinctively [92–94, 98]. In renal cell carcinoma, inhibition of HDAC4 and 6 showed that it induced HIF-1a protein stability, via proteosome dependent pathway [99]. HDAC mediates also angiogenesis by indirect mechanism, by regulation of p53, pVHL expression. Both in vitro and in vivo, overexpression of HDAC1 reduced expression of p53 and pVHL, and subsequent overexpression of HIF-1a, VEGF [100].

Another mechanism by which HDACs are involved in cancer biology is, via indirect and direct regulation of apoptosis [101]. Apoptosis is programmed cell death, a well regulated process that is important in tissue homeostasis and development. During this apoptosis, cells undergo typical morphologic changes. The cell's membrane forms a bleb, chromatin gets condensed, DNA gets degraded, and the cell fractionates into smaller vesicles. These fractionated vesicles get engulfed by phagocytes. Increased HDACs prevent DNA from degradation, and give cells a way to escape from apoptotic process [102–105].

HDACIs (HDAC inhibitors) were first found as antitumor agents by inhibiting HDAC function on the histone tail, however further studies found that they also affect gene transcription by inhibiting the interaction of HDACs with other proteins. Moreover, HDAC mutation or change in the level of expression was found to be correlated with HDACIs activity. HDACI seems to work both by transcription dependent and independent mechanisms, as suggested by studies about HDACs involvement in tumorigenesis [106–108]. It is not surprising to see that many HDAC inhibitors are under evaluation by researchers. There are many HDAC inhibitors undergoing phase I and II trials. HDAC inhibitors showed some benefit in animal and some early human data, however they primarily have more promising results when they are used in combination with other chemotherapy, or targeted therapies [109–111]. There are number of medications under way, however the main ones that has been and further studied include vorinostat(SAHA), romidepsin(FK228, FR901228), etinostat(SNDX-275, MS-275), and panobinostat(LBH-589).

Vorinostat, (brand name Zolinza) is Suberoylanilide hydroxamic acid (SAHA), is a member of a larger HDAC inhibitor compounds (HDACI). It is one of the most studied compounds among HDACI. Currently vorinostat is approved by the FDA for use in cutaneous T cell lymphoma (CTCL). This agent is undergoing phase I and II trials in many solid cancers including colon, lung, renal, and breast cancers. In breast cancer, vorinostat showed restored sensitivity of aromatase inhibition after primary or secondary resistance when it was used in combination for estrogen

receptor positive breast cancer patient [112]. Also, it is currently studied as part of advanced solid tumor studies including breast cancer, in combination with doxorubicin, docetaxel [113], and carboplatin-paclitaxel combination. Romidepsin, the other agent approved by FDA to treat lymphoma, is also studied in advanced solid cancers in phase I trial including breast cancer [114].

Entinostat currently studied in combination with the aromatase inhibitor, letrozole in phase II study in ER positive breast cancer patients [90]. This is based on mouse xenograft models with ER-negative breast cancer cells. Entinostat was found to upregulate ERα receptors and aromatase expression and subsequently sensitized ER-negative MDA-MB-231 cells to estrogen and letrozole [115]. Panobinostat and letrozole in aromatase-positive breast cancer inhibition. Panobinostat is 40 times more potent than SAHA in its ability to suppress aromatase expression. It selectively inhibits promoters I.3/II of the human aromatase gene. Panobinostat, the required concentration of letrozole was only one-fifth of that used in the absence of panobinostat, to achieve the same degree of aromatase inhibition [116].

MicroRNAs and the Molecular Pathogenesis of Breast Cancer

In 1993, micro RNA (miRNA), small non coding sequences, was recognized as being responsible for degradation and silencing of messenger RNA. Multiple downstream affects of micro RNAs, via repression of transcription; on proliferation, apoptosis and differentiation have now been described in breast cancer. Thus far, almost 500 different microRNAs have been identified in human tissue, and more will likely be identified [117]. Of these known miRNAs, high through put sequencing has established differences in profiles between normal and malignant breast tissue. Using microarray and northern blot analysis of 76 human breast cancer samples, and ten normal breast tissue samples; Iorio et al., showed aberrant expression *of miR-10b*, *miR-125b*, *miR-145*, *miR-155* and *miR-21* in human breast cancer. Using, these miRNA expression profiles the investigators could predict, ER/PR status, tumor stage and proliferative index of breast tumors. The investigators also showed that down-regulation of *MiR-9-3* was associated with either high vascular invasion and metastatic potential [118].

Other miRNAs, such as *miR-21*, has been shown to have prognostic importance. Wang and colleagues revealed that upregulation of *miR-21* in doxorubicin resistant MCF-7 breast cancer cells was associated with downregulation of tumor suppressor gene (PTEN) protein. The half maximal inhibitory concentration (IC50) of doxorubicin was 16.5 ± 0.08 μmol/L for resistant MCF-7 cells with over-expression of PTEN versus 0.21 ± μmol/L for parental MCF-7 cells [119]. Micro RNA 27a was investigated as a potential oncogene. Mertens-Talcott et al., showed an oncogenic effect of *miR-27a* by increasing expression of specificity proteins which in turn promoted cell survival and angiogenesis. This same micro RNA can suppress genes that cause G2-M arrest, thus promoting cell proliferation [120].

Micro RNAs are intriguing targets for development of novel therapy. Liang et al., reported on whether down regulation of microRNA would affect a tumor's metastatic potential via the CXCR4 pathway. They used artificial microRNA in transfected cell

lines to show an in vitro decrease of invasion and migration; and an in vivo decrease of lung tumor metastasis. Potentially, anti sense compounds could inactivate transcription, which could decrease tumor proliferation and enhance chemo-sensitivity of breast cancer cells. Alternatively, liposomal or viral delivery of other microRNA could slow tumor growth by preferential effects of apoptosis [121].

Telomerase Inhibitors in Breast Cancer

The telomerase enzyme in cancer cells provides an attractive new therapeutic target. Telomeres are repeating DNA sequences that cap the ends of chromosomes. Repeated cell division results in loss of telomere length and progressive shortening of chromosomes. Critical shortening of chromosomes triggers apoptosis. Telomerase (telomere terminal transferase) is an enzyme complex that elongates chromosomes by adding TTAGGG sequences to the ends of chromosomes. Activation of telomerase enables increased proliferation in cells. Since telomerase is several fold more active in malignant cells than in normal cells, effective inhibition of this activity has become a logical basis for investigation [122, 123]. Promising results from preclinical investigation [124] are leading to Phase I and Phase II clinical trials. Intriguing data suggest that in the appropriate setting, these agents might target cancer stem cells [125]. For example, imetelstat is a telomerase inhibitor that is now entering a trial in breast cancer where this agent will be combined with paclitaxel and possibly with bevacizumab as first or second line treatment for metastatic breast cancer [126].

PARP in Breast Cancer

PARP (poly ADP-ribose polymerase) is nuclear enzyme that catalyses the polymerization of ADP-ribose moieties into target proteins using NAD+. It was first found in 1963 by Chambon et al. Out of 17 members of the PARP superfamily [127, 128], PARP-1 and 2 are the ones well studied PARP-1 is recognized for its critical role in DNA repair. It is mainly integrated in base excision repair(BER). BER is a repair mechanism for single strand breaks (SSB) of DNA during cell growth and division. PARP-1 recognizes this single strand DNA breaks and catalyzes itself and activates PAR protein to repair this breakage [129–131]. PARP-1 also recruits other key BER proteins like XRCC1, and affects scaffolding by recruiting histones H1, H2B. Methylating agents and tomoisomerase I inhibitors attack tumor cells by causing SSB DNA damages [132]. Thus, by inhibiting PARP-1 activity, the damage to DNA caused by chemotherapy, or radiation will no longer be repaired, and the tumor cell will die more effectively by adding PARP-1 inhibitor [133].

Moreover, PARP-1 is involved not only in the repair of SSB, but also in that of double strand breaks(DSB). If there is deficient in PARP-1 activity, SSB repair failure (BER failure) leads to a collapsed replication fork. This collapsed fork now requires DSB repair by homologous repair(HR) by BRCA protein [134]. However if

there is BRCA mutation, defect, or down-regulation in the pathway, the cell can't have successful repair of its defective DNA, and undergo augmented cell death. This phenomenon, a double defect causing cell death, while only one deficiency does not, is called 'synthetic lethality'. The observation of synthetic lethality brought attention to PARP inhibitor as a single agent therapy for specific types of breast or ovarian cancer.

One of the biggest challenges in the treatment of breast cancer, has been developing a novel agent targeting 'basal like breast cancer'. This type of cancer expresses cytokeratin 5/6, similar to basal cells, and has low or nonexistent expression of hormonal receptors and HER2 protein. Basal like breast cancer is not the same as triple negative breast cancer, however there is large overlap. Basal like breast cancers,or triple negative cancers, share many characteristics of hereditary BRCA mutation related breast cancer. They both down regulate BRCA, not by mutations but other mechanisms. Many researchers paid attention to 'BRCAness' of this type of cancer. PARP inhibitors harboring the ability of 'synthetic lethality' in targeting this basal like breast cancer has shown some promising early results [135–137], and lead to development of various PARP inhibitor agents by many pharmaceutical companies.

Iniparib (BSI-201) by Sanofi-Aventis, showed a survival benefit in triple negative breast cancer (TNBC) in a phase 2 trial presented at 2009 ASCO. Unfortunately, the first phase III trial of a PARP inhibitor, reported by Joyce O'Shaunessy and colleagues, failed to show a benefit in progression free survival. Some postulate that this failure of efficacy may be because these patients were unselected. Biomarkers of PARP responsive tumors are needed.

Olaparib(AZD-2281) by AstroZeneca in TNBC showed PR as a s single agent. Although some of phase 2 and 3 trial data were disappointing, there are still many other PARP inhibitors to come. At present, Veliparib(ABT-888) by Abbott, is in phase 2 trials in BRCA 1 and BRCA 2 deficient breast cancer and advanced breast cancer, as well as melanoma, colorectal cancer and hepatocellular carcinoma. PF-01367338(AG-014699) is investigated by Pfizer, undergoing a phase 2 trial in triple negative breast cancer or BRCA1- and BRCA 2- deficient breast or ovarian cancer.

We do not yet fully understand the role of PARP inhibitors in this breast cancer phenotype. We need to elucidate the role as single agent, and in combination with DNA damaging cytotoxins, in BRCA mutated tumors. Never the less, PARP inhibitors still hold promise and the possibility to open doors to the treatment of one of the most challenging subtypes of breast cancer.

Hormone Receptor Positive but Resistant to Hormone Therapy

Mechanisms of Hormone Resistance

The majority of breast cancers express estrogen receptor alpha. Unfortunately, while most metastatic breast carcinomas initially are responsive to endocrine therapies, most eventually develop resistance. Thus, there is great interest in restoring

endocrine sensitivity. A MCF-7 cell line which models acquired endocrine resistance was found to show differences in genomic expression in these cells compared to the cell line which was estrogen sensitive [138]. There was amplification of ESR1, a gene which upregulates ER alpha expression; suggesting that these cells require much less estrogen than they initially did prior to being deprived of estrogen. 11–20% of breast cancers show ESR1 amplification [139]. In addition, through signaling crosstalk, ER alpha can activate growth factor receptor pathways in a ligand independent manner. Tamoxifen resistance can also be facilitated by PI3K/AKT/mTOR and MAPK phosphorylation of ER alpha, in a hormone independent AF-1 area of ER alpha. Molecular activation of EGFR/ERBB/AKT in cells with acquired estrogen resistance supports the theory of growth factor signaling crosstalk as well. Unfortunately, to date, clinical trials aimed at blocking growth factor and ER alpha pathways have been unsuccessful. Subsets of AKT expression profiles by immunohistochemistry, in 402 breast carcinoma samples was able to predict prognosis. High pAKT and low AKT2 expression profiles were most likely to relapse. During tamoxifen treatment, AKT activation was associated with her two and ER alpha activation linking AKT to tamoxifen resistance [140]. Another mechanism of tamoxifen resistance is overexpression of FGFR1 (FGFR2 is overexpressed in triple negative breast tumors) [141]. FGFR is regulated by E2F1.

Conclusion

Although we have made substantial headway in understanding genetic targets in breast cancer therapy, many questions remain unanswered. There is little doubt, that successful treatment of breast cancer will require identification and manipulation of genetic targets.

References

1. Sjogren S, et al. Prognostic and predictive value of c-erbB-2 overexpression in primary breast cancer, alone and in combination with other prognostic markers. J Clin Oncol. 1998;16(2):462–9.
2. Slamon DJ, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science. 1989;244(4905):707–12.
3. Graus-Porta D, et al. ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling. EMBO J. 1997;16(7):1647–55.
4. Dankort D, et al. Grb2 and Shc adapter proteins play distinct roles in Neu (ErbB-2)-induced mammary tumorigenesis: implications for human breast cancer. Mol Cell Biol. 2001;21(5):1540–51.
5. Slamon DJ, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med. 2001;344(11):783–92.
6. Gajria D, Chandarlapaty S. HER2-Amplified breast cancer: mechanisms of trastuzumab resistance and novel targeted therapies. Expert Rev Anticancer Ther. 2011;11(2):263–75.

7. Romond EH, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med. 2005;353(16):1673–84.
8. Piccart-Gebhart MJ, et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med. 2005;353(16):1659–72.
9. Bange J, Zwick E, Ullrich A. Molecular targets for breast cancer therapy and prevention. Nat Med. 2001;7(5):548–52.
10. Ritter CA, et al. Mechanisms of resistance development against trastuzumab (Herceptin) in an in vivo breast cancer model. Int J Clin Pharmacol Ther. 2004;42(11):642–3.
11. Lu Y, et al. Insulin-like growth factor-I receptor signaling and resistance to trastuzumab (Herceptin). J Natl Cancer Inst. 2001;93(24):1852–7.
12. Shattuck DL, et al. Met receptor contributes to trastuzumab resistance of Her2-overexpressing breast cancer cells. Cancer Res. 2008;68(5):1471–7.
13. Serra V, et al. PI3K Inhibition results in enhanced HER signaling and acquired ERK dependency in HER2-overexpressing breast cancer. Oncogene. 2011;30(22):2547–57.
14. Scaltriti M, et al. Expression of p95HER2, a truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast cancer. J Natl Cancer Inst. 2007;99(8):628–38.
15. Berns K, et al. A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell. 2007;12(4):395–402.
16. Nagata Y, et al. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. Cancer Cell. 2004;6(2):117–27.
17. Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer. 2005;5(5):341–54.
18. Hynes NE, MacDonald G. ErbB receptors and signaling pathways in cancer. Curr Opin Cell Biol. 2009;21(2):177–84.
19. Lee-Hoeflich ST, et al. A central role for HER3 in HER2-amplified breast cancer: implications for targeted therapy. Cancer Res. 2008;68(14):5878–87.
20. Sergina NV, et al. Escape from HER-family tyrosine kinase inhibitor therapy by the kinase-inactive HER3. Nature. 2007;445(7126):437–41.
21. Yao E, et al. Suppression of HER2/HER3-mediated growth of breast cancer cells with combinations of GDC-0941 PI3K inhibitor, trastuzumab, and pertuzumab. Clin Cancer Res. 2009;15(12):4147–56.
22. Sachdev JC, Jahanzeb M. Blockade of the HER family of receptors in the treatment of HER2-positive metastatic breast cancer. Clin Breast Cancer. 2011;12:19–29.
23. Sanchez-Martin M, Pandiella A. Differential action of small molecule HER kinase inhibitors on receptor heterodimerization: therapeutic implications. Int J Cancer. 2011;131:244–52.
24. Noonberg SB, Benz CC. Tyrosine kinase inhibitors targeted to the epidermal growth factor receptor subfamily: role as anticancer agents. Drugs. 2000;59(4):753–67.
25. Blackwell KL, et al. Randomized study of lapatinib alone or in combination with trastuzumab in women with ErbB2-positive, trastuzumab-refractory metastatic breast cancer. J Clin Oncol. 2010;28(7):1124–30.
26. Blackwell KL, et al. Single-agent lapatinib for HER2-overexpressing advanced or metastatic breast cancer that progressed on first- or second-line trastuzumab-containing regimens. Ann Oncol. 2009;20(6):1026–31.
27. Kaufman B, et al. Lapatinib monotherapy in patients with HER2-overexpressing relapsed or refractory inflammatory breast cancer: final results and survival of the expanded HER2+ cohort in EGF103009, a phase II study. Lancet Oncol. 2009;10(6):581–8.
28. ALTTO (Adjuvant Lapatinib and/or Trastuzumab Treatment Optimisation) study. BIG 2-06/N063D. NCT00490139.
29. Gupta M, et al. Clinical implications of pathophysiological and demographic covariates on the population pharmacokinetics of trastuzumab emtansine, a HER2-targeted antibody-drug conjugate, in patients with HER2-positive metastatic breast cancer. J Clin Pharmacol. 2012;52:691–703.
30. Baselga J, Swain SM. CLEOPATRA: a phase III evaluation of pertuzumab and trastuzumab for HER2-positive metastatic breast cancer. Clin Breast Cancer. 2010;10(6):489–91.

31. Verma S, et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med 367:1783-1791, doi:10.1056/NEJMoa1209124 (2012).
32. Kai S, Hara H. Allogeneic hematopoietic stem cell transplantation. Ther Apher Dial. 2003;7(3):285–91.
33. Kai T, Spradling A. An empty drosophila stem cell niche reactivates the proliferation of ectopic cells. Proc Natl Acad Sci USA. 2003;100(8):4633–8.
34. Tokuzawa Y, et al. Fbx15 is a novel target of Oct3/4 but is dispensable for embryonic stem cell self-renewal and mouse development. Mol Cell Biol. 2003;23(8):2699–708.
35. Fiebiger W, et al. Occurrence of a pulmonary carcinoid following allogeneic stem cell transplantation for chronic myelogenous leukemia: a case report. Ann Hematol. 2003;82(6):374–6.
36. Smalley M, Ashworth A. Stem cells and breast cancer: a field in transit. Nat Rev Cancer. 2003;3(11):832–44.
37. Zhang Y, et al. Elevated insulin-like growth factor 1 receptor signaling induces antiestrogen resistance through the MAPK/ERK and PI3K/Akt signaling routes. Breast Cancer Res. 2011;13(3):R52.
38. Study of BMS-754807 combined with letrozole or BMS-754807 alone in patients with hormone receptor-positive breast cancer and resistance to non-steroidal aromatase inhibitors. Clinical Trials.gov # NCT01225172.
39. Litzenburger BC, et al. High IGF-IR activity in triple-negative breast cancer cell lines and tumorgrafts correlates with sensitivity to anti-IGF-IR therapy. Clin Cancer Res. 2011;17(8):2314–27.
40. Kleinsmith LJ, Pierce Jr GB. Multipotentiality of single embryonal carcinoma cells. Cancer Res. 1964;24:1544–51.
41. Nishimura EK, et al. Dominant role of the niche in melanocyte stem-cell fate determination. Nature. 2002;416(6883):854–60.
42. Folkman J. Tumor angiogenesis in women with node-positive breast cancer. Cancer J Sci Am. 1995;1(2):106–8.
43. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med. 1995;1(1):27–31.
44. Dayan F, et al. A dialogue between the hypoxia-inducible factor and the tumor microenvironment. Cancer Microenviron. 2008;1(1):53–68.
45. Fujita Y, Abe R, Shimizu H. Clinical approaches toward tumor angiogenesis: past, present and future. Curr Pharm Des. 2008;14(36):3820–34.
46. Poon RT, Fan ST, Wong J. Clinical implications of circulating angiogenic factors in cancer patients. J Clin Oncol. 2001;19(4):1207–25.
47. Baeriswyl V, Christofori G. The angiogenic switch in carcinogenesis. Semin Cancer Biol. 2009;19(5):329–37.
48. Zhao J, et al. Correlation between serum vascular endothelial growth factor and endostatin levels in patients with breast cancer. Cancer Lett. 2004;204(1):87–95.
49. Weidner N, et al. Tumor angiogenesis and metastasis–correlation in invasive breast carcinoma. N Engl J Med. 1991;324(1):1–8.
50. Weidner N, et al. Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. Am J Pathol. 1993;143(2):401–9.
51. Semenza GL. Involvement of oxygen-sensing pathways in physiologic and pathologic erythropoiesis. Blood. 2009;114(10):2015–9.
52. Semenza GL. Regulation of oxygen homeostasis by hypoxia-inducible factor 1. Physiology (Bethesda). 2009;24:97–106.
53. Simon MC, Keith B. The role of oxygen availability in embryonic development and stem cell function. Nat Rev Mol Cell Biol. 2008;9(4):285–96.
54. Tang N, et al. Loss of HIF-1alpha in endothelial cells disrupts a hypoxia-driven VEGF autocrine loop necessary for tumorigenesis. Cancer Cell. 2004;6(5):485–95.
55. Rosen LS. Clinical experience with angiogenesis signaling inhibitors: focus on vascular endothelial growth factor (VEGF) blockers. Cancer Control. 2002;9(2 Suppl):36–44.

56. LeCouter J, et al. Identification of an angiogenic mitogen selective for endocrine gland endothelium. Nature. 2001;412(6850):877–84.
57. LeCouter J, Ferrara N. EG-VEGF and the concept of tissue-specific angiogenic growth factors. Semin Cell Dev Biol. 2002;13(1):3–8.
58. Ferrara N, et al. EG-VEGF and Bv8: a novel family of tissue-restricted angiogenic factors. Biochim Biophys Acta. 2004;1654(1):69–78.
59. Ferrara N, et al. Differential expression of the angiogenic factor genes vascular endothelial growth factor (VEGF) and endocrine gland-derived VEGF in normal and polycystic human ovaries. Am J Pathol. 2003;162(6):1881–93.
60. Kamiyama M, et al. VEGF receptor antisense therapy inhibits angiogenesis and peritoneal dissemination of human gastric cancer in nude mice. Cancer Gene Ther. 2002;9(2):197–201.
61. Mathur SP, et al. Serum vascular endothelial growth factor C (VEGF-C) as a specific biomarker for advanced cervical cancer: relationship to insulin-like growth factor II (IGF-II), IGF binding protein 3 (IGF-BP3) and VEGF-A [corrected]. Gynecol Oncol. 2005;98(3):467–83.
62. Mathur RS, Mathur SP. Vascular endothelial growth factor (VEGF) up-regulates epidermal growth factor receptor (EGF-R) in cervical cancer in vitro: this action is mediated through HPV-E6 in HPV-positive cancers. Gynecol Oncol. 2005;97(1):206–13.
63. Bachtiary B, et al. Serum VEGF levels in patients undergoing primary radiotherapy for cervical cancer: impact on progression-free survival. Cancer Lett. 2002;179(2):197–203.
64. Cao X, et al. Upregulation of VEGF-A and CD24 gene expression by the tGLI1 transcription factor contributes to the aggressive behavior of breast cancer cells. Oncogene. 2012;31(1):104–15.
65. Liu L, et al. VEGF 936C>T polymorphism and breast cancer risk: evidence needed further clarification. Breast Cancer Res Treat. 2011;127(2):569–71.
66. Liu L, et al. Expression of connective tissue growth factor is in agreement with the expression of VEGF, VEGF-C, -D and associated with shorter survival in gastric cancer. Pathol Int. 2007;57(11):712–8.
67. Nicolini A, et al. Vascular endothelial growth factor (VEGF) and other common tissue prognostic indicators in breast cancer: a case-control study. Int J Biol Markers. 2004;19(4):275–81.
68. Kostopoulos I, et al. Evaluation of the prognostic value of HER-2 and VEGF in breast cancer patients participating in a randomized study with dose-dense sequential adjuvant chemotherapy. Breast Cancer Res Treat. 2006;96(3):251–61.
69. Gadducci A, et al. Serum preoperative vascular endothelial growth factor (VEGF) in epithelial ovarian cancer: relationship with prognostic variables and clinical outcome. Anticancer Res. 1999;19(2B):1401–5.
70. Des Guetz G, et al. Microvessel density and VEGF expression are prognostic factors in colorectal cancer. Meta-analysis of the literature. Br J Cancer. 2006;94(12):1823–32.
71. Bremnes RM, Camps C, Sirera R. Angiogenesis in non-small cell lung cancer: the prognostic impact of neoangiogenesis and the cytokines VEGF and bFGF in tumours and blood. Lung Cancer. 2006;51(2):143–58.
72. Balbi G, et al. Vascular endothelial growth factor (VEGF): can we use it as prognostic factor in endometrial cancer? Minerva Ginecol. 2006;58(5):411–5.
73. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science. 2005;307(5706):58–62.
74. Hughes CC. Endothelial-stromal interactions in angiogenesis. Curr Opin Hematol. 2008;15(3):204–9.
75. Xian X, et al. Pericytes limit tumor cell metastasis. J Clin Invest. 2006;116(3):642–51.
76. Lindblom P, et al. Endothelial PDGF-B retention is required for proper investment of pericytes in the microvessel wall. Genes Dev. 2003;17(15):1835–40.
77. Augustin HG. Translating angiogenesis research into the clinic: the challenges ahead. Br J Radiol. 2003;76(Spec No 1):S3–10.
78. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med. 2003;9(6):669–76.
79. Gerber HP, Ferrara N. The role of VEGF in normal and neoplastic hematopoiesis. J Mol Med (Berl). 2003;81(1):20–31.

80. D'Agostino Sr RB. Changing end points in breast-cancer drug approval – the avastin story. N Engl J Med. 2011;365(2):e2.
81. Baselga J. Targeting the phosphoinositide-3 (PI3) kinase pathway in breast cancer. Oncologist. 2011;16(Suppl 1):12–9.
82. Alvarez RH, Valero V, Hortobagyi GN. Emerging targeted therapies for breast cancer. J Clin Oncol. 2010;28(20):3366–79.
83. O'Regan RM, Khuri FR. Farnesyl transferase inhibitors: the next targeted therapies for breast cancer? Endocr Relat Cancer. 2004;11(2):191–205.
84. Eckert LB, et al. Involvement of Ras activation in human breast cancer cell signaling, invasion, and anoikis. Cancer Res. 2004;64(13):4585–92.
85. Li T, et al. Phase II trial of the farnesyltransferase inhibitor tipifarnib plus fulvestrant in hormone receptor-positive metastatic breast cancer: New York Cancer Consortium Trial P6205. Ann Oncol. 2009;20(4):642–7.
86. Johnston SR, et al. A phase II, randomized, blinded study of the farnesyltransferase inhibitor tipifarnib combined with letrozole in the treatment of advanced breast cancer after antiestrogen therapy. Breast Cancer Res Treat. 2008;110(2):327–35.
87. Stambolic V, et al. Utility of metformin in breast cancer treatment, is neoangiogenesis a risk factor? Breast Cancer Res Treat. 2009;114(2):387–9.
88. Dowling RJ, et al. Metformin inhibits mammalian target of rapamycin-dependent translation initiation in breast cancer cells. Cancer Res. 2007;67(22):10804–12.
89. Huang HY, et al. Inhibition of human breast cancer cell invasion by siRNA against urokinase-type plasminogen activator. Cancer Invest. 2010;28(7):689–97.
90. Choudhary C, et al. Lysine acetylation targets protein complexes and co-regulates major cellular functions. Science. 2009;325(5942):834–40.
91. Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. Nat Rev Drug Discov. 2006;5(9):769–84.
92. Weichert W, et al. Expression of class I histone deacetylases indicates poor prognosis in endometrioid subtypes of ovarian and endometrial carcinomas. Neoplasia. 2008;10(9):1021–7.
93. Weichert W, et al. Association of patterns of class I histone deacetylase expression with patient prognosis in gastric cancer: a retrospective analysis. Lancet Oncol. 2008;9(2):139–48.
94. Weichert W, et al. Histone deacetylases 1, 2 and 3 are highly expressed in prostate cancer and HDAC2 expression is associated with shorter PSA relapse time after radical prostatectomy. Br J Cancer. 2008;98(3):604–10.
95. Weichert W, et al. Class I histone deacetylase expression has independent prognostic impact in human colorectal cancer: specific role of class I histone deacetylases in vitro and in vivo. Clin Cancer Res. 2008;14(6):1669–77.
96. Cebrian A, et al. Genetic variants in epigenetic genes and breast cancer risk. Carcinogenesis. 2006;27(8):1661–9.
97. Cress WD, Seto E. Histone deacetylases, transcriptional control, and cancer. J Cell Physiol. 2000;184(1):1–16.
98. Ozdag H, et al. Differential expression of selected histone modifier genes in human solid cancers. BMC Genomics. 2006;7:90.
99. Qian DZ, et al. Class II histone deacetylases are associated with VHL-independent regulation of hypoxia-inducible factor 1 alpha. Cancer Res. 2006;66(17):8814–21.
100. Kim SH, et al. Regulation of the HIF-1alpha stability by histone deacetylases. Oncol Rep. 2007;17(3):647–51.
101. Peart MJ, et al. Novel mechanisms of apoptosis induced by histone deacetylase inhibitors. Cancer Res. 2003;63(15):4460–71.
102. Rodriguez-Gonzalez A, et al. Role of the aggresome pathway in cancer: targeting histone deacetylase 6-dependent protein degradation. Cancer Res. 2008;68(8):2557–60.
103. Vousden KH, Lane DP. p53 in health and disease. Nat Rev Mol Cell Biol. 2007;8(4):275–83.
104. Vervoorts J, Luscher-Firzlaff J, Luscher B. The ins and outs of MYC regulation by posttranslational mechanisms. J Biol Chem. 2006;281(46):34725–9.

105. Spange S, et al. Acetylation of non-histone proteins modulates cellular signalling at multiple levels. Int J Biochem Cell Biol. 2009;41(1):185–98.
106. Crabb SJ, et al. Characterisation of the in vitro activity of the depsipeptide histone deacetylase inhibitor spiruchostatin A. Biochem Pharmacol. 2008;76(4):463–75.
107. Moore PS, et al. Gene expression profiling after treatment with the histone deacetylase inhibitor trichostatin A reveals altered expression of both pro- and anti-apoptotic genes in pancreatic adenocarcinoma cells. Biochim Biophys Acta. 2004;1693(3):167–76.
108. Glaser KB, et al. Differential protein acetylation induced by novel histone deacetylase inhibitors. Biochem Biophys Res Commun. 2004;325(3):683–90.
109. Frew AJ, et al. Combination therapy of established cancer using a histone deacetylase inhibitor and a TRAIL receptor agonist. Proc Natl Acad Sci USA. 2008;105(32):11317–22.
110. Lindemann RK, Gabrielli B, Johnstone RW. Histone-deacetylase inhibitors for the treatment of cancer. Cell Cycle. 2004;3(6):779–88.
111. Minucci S, Pelicci PG. Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. Nat Rev Cancer. 2006;6(1):38–51.
112. Munster PN, et al. A phase II study of the histone deacetylase inhibitor vorinostat combined with tamoxifen for the treatment of patients with hormone therapy-resistant breast cancer. Br J Cancer. 2011;104(12):1828–35.
113. Schneider BJ, et al. Phase I study of vorinostat (suberoylanilide hydroxamic acid, NSC 701852) in combination with docetaxel in patients with advanced and relapsed solid malignancies. Invest New Drugs. 2012;30:249–57.
114. Grant C, et al. Romidepsin: a new therapy for cutaneous T-cell lymphoma and a potential therapy for solid tumors. Expert Rev Anticancer Ther. 2010;10(7):997–1008.
115. Chumsri S, et al. Aromatase inhibitors and xenograft studies. Steroids. 2011;76(8):730–5.
116. Chen S, et al. The HDAC inhibitor LBH589 (panobinostat) is an inhibitory modulator of aromatase gene expression. Proc Natl Acad Sci USA. 2010;107(24):11032–7.
117. Wang Y, et al. MicroRNA: past and present. Front Biosci. 2007;12:2316–29.
118. Iorio MV, et al. MicroRNA gene expression deregulation in human breast cancer. Cancer Res. 2005;65(16):7065–70.
119. Wang ZX, et al. MicroRNA-21 modulates chemosensitivity of breast cancer cells to doxorubicin by targeting PTEN. Arch Med Res. 2011;42(4):281–90.
120. Mertens-Talcott SU, et al. The oncogenic microRNA-27a targets genes that regulate specificity protein transcription factors and the G2-M checkpoint in MDA-MB-231 breast cancer cells. Cancer Res. 2007;67(22):11001–11.
121. Liang Z, et al. Blockade of invasion and metastasis of breast cancer cells via targeting CXCR4 with an artificial microRNA. Biochem Biophys Res Commun. 2007;363(3):542–6.
122. Shay JW. Telomerase in human development and cancer. J Cell Physiol. 1997;173(2):266–70.
123. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. Eur J Cancer. 1997;33(5):787–91.
124. Harley CB. Telomerase and cancer therapeutics. Nat Rev Cancer. 2008;8(3):167–79.
125. Joseph I, et al. The telomerase inhibitor imetelstat depletes cancer stem cells in breast and pancreatic cancer cell lines. Cancer Res. 2010;70(22):9494–504.
126. Imetelstat in combination with paclitaxel (with or without bevacizumab) in patients with locally recurrent or metastatic breast cancer. Clinical Trials.gov NCT01256762.
127. Gagne JP, et al. The expanding role of poly(ADP-ribose) metabolism: current challenges and new perspectives. Curr Opin Cell Biol. 2006;18(2):145–51.
128. Ame JC, Spenlehauer C, de Murcia G. The PARP superfamily. Bioessays. 2004;26(8):882–93.
129. Hartman AR, Ford JM. BRCA1 and p53: compensatory roles in DNA repair. J Mol Med (Berl). 2003;81(11):700–7.
130. Hartman AR, Ford JM. BRCA1 Induces DNA damage recognition factors and enhances nucleotide excision repair. Nat Genet. 2002;32(1):180–4.
131. Ford JM. Regulation of DNA damage recognition and nucleotide excision repair: another role for p53. Mutat Res. 2005;577(1–2):195–202.

132. Boulton S, Kyle S, Durkacz BW. Interactive effects of inhibitors of poly(ADP-ribose) polymerase and DNA-dependent protein kinase on cellular responses to DNA damage. Carcinogenesis. 1999;20(2):199–203.
133. McCabe N, et al. Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition. Cancer Res. 2006;66(16):8109–15.
134. Gudmundsdottir K, Ashworth A. The roles of BRCA1 and BRCA2 and associated proteins in the maintenance of genomic stability. Oncogene. 2006;25(43):5864–74.
135. Alli E, et al. Defective repair of oxidative DNA damage in triple-negative breast cancer confers sensitivity to inhibition of poly(ADP-ribose) polymerase. Cancer Res. 2009;69(8):3589–96.
136. Fong PC, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med. 2009;361(2):123–34.
137. Fong PC, et al. Poly(ADP)-ribose polymerase inhibition: frequent durable responses in BRCA carrier ovarian cancer correlating with platinum-free interval. J Clin Oncol. 2010;28(15):2512–9.
138. Aguilar H, et al. Biological reprogramming in acquired resistance to endocrine therapy of breast cancer. Oncogene. 2010;29(45):6071–83.
139. Holst F, et al. Estrogen receptor alpha (ESR1) gene amplification is frequent in breast cancer. Nat Genet. 2007;39(5):655–60.
140. Kirkegaard T, et al. AKT activation predicts outcome in breast cancer patients treated with tamoxifen. J Pathol. 2005;207(2):139–46.
141. Turner N, et al. FGFR1 Amplification drives endocrine therapy resistance and is a therapeutic target in breast cancer. Cancer Res. 2010;70(5):2085–94.

Impact of Genetic Targets on Cancer Therapy in Esophagogastric Cancer

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Abstract The treatment of esophagogastric cancer has been rapidly evolving in the past decade. New cytotoxic drugs and targeted agents have been integrated in the therapeutic paradigm. To better understand the tumor biology and to better utilize targeted agents, genetic alterations in esophagogastric cancer have been actively explored. For example, Her2/Neu amplification and expression were observed in gastric and gastroesophageal (GE) junction cancers. Combination of trastuzumab with cytotoxic chemotherapy has demonstrated a survival advantage in patients with Her2/Neu positive gastric cancer. However, the prognosis of advanced esophagogastric cancer remains poor. This is largely attributed to the tumor heterogeneity and poorly understood tumor biology. This article provides a summary of potential genetic targets and the role of novel targeted agents in the treatment of esophagogastric cancer.

Keywords Esphageal cancer • Gastric cancer • EGFR • Her2/Neu • Angiogenesis • Cetuximab • Trastuzumab • Bevacizumab

Introduction

Esophagogastric cancer is a heterogeneous disease. Histologically, it includes squamous cell carcinoma and adenocarcinoma. Traditionally, these two histologies are treated very similarly. Most clinical protocols include both histologies. However, the etiology and prognosis are very different between squamous cell carcinoma and

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W.S. El-Deiry (ed.), *Impact of Genetic Targets on Cancer Therapy*, Advances in Experimental Medicine and Biology 779, DOI 10.1007/978-1-4614-6176-0_3,

adenocarcinoma. Anatomically, adenocarcinoma is usually located at the distal one-third of the esophagus and frequently involves GE junction with extending into the stomach. The disease is mainly caused by acid reflux, Barrett's esophagus, high fat diet and obesity [1–3]. Squamous cell carcinoma, however, involves the upper third of the esophagus and is usually related to tobacco and alcohol consumption. Epidemiologically, adenocarcinoma is more prevalent in Western world [4, 5]. The incidence of squamous cell carcinoma is declining while the incidence of adenocarcinoma is rising [6]. Thus, these two histologies should be considered two distinct disease entities. With new targeted agents available on the market, better understanding the molecular pathologenesis of squamous cell carcinoma and adenocarcinoma of the esophagus is at urge. Incorporating targeted agents to the therapeutic paradigm would potentially allow us to have better patient selection and to tailor therapy based on tumor genetics.

Targeting Epidermal Growth Factor Receptor (EGFR) Family Pathway

Epidermal growth factor receptor family consists of EGFR (ErbB1), Her2/Neu (ErbB2), ErbB3 and ErbB4 [7–9]. Upon binding of the ligand, the receptor undergoes dimerization and activation of its intrinsic tyrosine kinase activity resulting in initiation of down stream signaling cascade [10]. The activation of EGFRs ultimately leads to cell proliferation, differentiation or even transformation. Targeting EGFR with monoclonal antibody or small typrosine kinase inhibitors has been demonstrated clinically efficacious in solid tumors such as non-small cell lung cancer, head and neck cancer and colorectal cancers either alone or in combination with chemotherapy or radiotherapy. In head and neck cancers, cetuximab potentiates the effect of radiotherapy with a significant survival benefit [11]. More importantly, targeted agents allow us better understanding the tumor biology and tailoring therapy to individual patient. For example, non-small cell lung cancer harboring specific EGFR mutations are particularly sensitive to gefitinib therapy [12]. Patients with such mutations usually demonstrated a higher response rate and longer survival. Like conventional chemotherapy, drug resistance remains a problem. Such resistance to gefitinib was identified [13].

Expression or overexpression of EGFR family members has been described in esophageal and gastric cancers. Overexpression of EGFR has been detected in majority of esophagogastric cancers ranging from 18 to 90% [14–16]. This overexpression is generally associated with a more clinically aggressive disease and a poor prognosis [17–20]. In addition, the EGFR overexpression may cause treatment resistance such as radio resistance in esophageal cancer [21]. Cetuximab, a chimeric monoclonal antibody against the extracellular domain of EGFR, was evaluated in esophageal and gastric cancers. Single agent cetuximab has minimal activity in patients with metastatic esophageal and gastric cancers [22, 23]. However, addition cetuximab

to chemotherapy may augment the efficacy of cytotoxic drugs. Pinto and colleagues investigated the activity of cetuximab in combination with cisplatin and decetaxel in patients with advanced GE junction or gastric cancers for first line therapy [24]. The authors showed that a 41.2% response rate was achieved. The median overall survival was 9 months and median time to progression was 5 months. Comparing to the historically data, the addition of cetuximab may have a small improvement of response rate but not overall survival.

Several studies have assessed cetuximab in the preoperative chemoradiation setting and demonstrated feasibility and safety. Ruhstaller et al. published a phase IB/II study (SAKK75/06) using cetuximab with chemotherapy and chemoradiation in both squamous cell carcinoma and adenocarcinoma of the esophagus [25]. A total of 28 patients entered the study. All patients were treated with 2 cycles of induction chemotherapy with cisplatin and docetaxel. Subsequently, patients were allocated into two cohorts. One cohort received weekly cisplatin and cetuximab with radiation while the other cohort received weekly decetaxel, cisplatin and cetuximab with radiation. R0 resection was performed in 25 patients. The study demonstrated that addition of cetuximab with chemoradiation is feasible. An impressive 86% (95% CI, 57–98%) pathological complete response (pCR) or near complete response were achieved in patients with adenocarcinoma. A 64% (95% CI, 31–89%) pCR or near pCR were observed in squamous cell carcinoma. De Vita and colleagues reported another approach using oxaliplatin and cetuximab in a single arm phase II study [26]. Patients in the study were treated with induction chemotherapy of FOLFOX4 and cetuximab followed by cetuximab concurrent with radiotherapy (50 Gy). A 27% pCR was reached. RTOG phase III study is under the way to evaluate cisplatin, paclitaxel and radiation with or without cetuximab in adenocarinoma or squamous cell carcinoma of the esophagus (www.clinicaltrials.gov). The role of cetuximab in the preoperative chemoradiation will be further delineated.

Erlotinib is a small molecule inhibiting EGFR function. Ilson et al. reported a phase II study of erlotinib in patients with advanced previously treated adenocarcinoma and squamous cell carcinoma of the esophagus [27]. Eighty percent of patients in the study had tumors overexpressing EGFR. The partial response rate is low (8%). Time-to-progression was longer in squamous cell histology than that of adenocarcinoma. Thus, it is worthwhile to further evaluate this agent in squamous histology.

Her2/neu is a receptor tyrosine kinase that belongs to EGFR family. Her2/neu gene amplification (FISH) and protein overexpression are found in approximately 20–25% of breast cancers and are predictive of poor prognosis [28]. Trastuzumab, a humanized IgG1 monoclonal antibody to Her2/neu, showed clinical activity when used as a single agent or in combination with chemotherapy in Her2/neu positive breast cancers. Similar to breast cancer, roughly 19–22% of gastric or GE junction cancers have amplification and overexpression of Her2/neu [29–31]. Unlike in breast cancers, Her2/neu as a prognostic marker is less consistent. For example, some studies showed that the expression and amplification were frequently associated with nodal metastasis, advanced stages, distant metastasis and intestinal histology [31, 32]. On the other hand, Shah and co-workers found that Her2/neu expression is

a favorable prognostic factor in an univariate analysis but not in the multivariate analysis [33]. Hence, Her2/neu is not an independent prognostic biomarker.

ToGA study is by far the largest phase III study evaluating the activity of trastuzumab in advanced gastric and GE junction cancer [30]. A total of 3,803 patients were screened for the study. The study tested 3,665 patients for Her2/neu expression by both fluorescence in-situ hybridization (FISH) and immunohistochemistry staining (IHC). Eight hundred and ten patients were positive for either FISH or IHC. Finally, a total of 594 patients were randomized 1:1 to receive either chemotherapy (cisplatin, 5FU) alone or trastuzumab with chemotherapy. The median overall survival (OS) was 13.8 months in the trastuzumab arm and 11.1 months in the chemotherapy arm (HR 0.74; 95% CI 0.6–0.91; p=0.0046). Progression-free survival was also superior in the trastuzumab arm (6.7 months vs. 5.5 months; HR 0.71; 95% CI 0.59–0.85; p=0.0002). Further analysis showed that nearly all patients with FISH positive/IHC 1+ above tumors benefited from trastuzumab. However, a small proportion of patients that are FISH positive but IHC negative had minimal benefit from trastuzumab (HR 0.92; 95% CI 0.48–1.76). About 15 patients whose tumors were IHC 3+ but FISH negative had no benefit from trastuzumab. The discrepancies of these results could be due to testing error or bias. In addition, these two groups contained very small number of patients that did not have sufficient statistical power to draw further conclusions. The ToGA study is the first phase III study to incorporate a targeted agent in treating gastric and esophageal cancers. Assessing Her2/neu expression in patients with metastatic gastric or GE junction cancers became a new standard practice. Transtuzumab in combination with different chemotherapy regimens such as oxaliplatin and capecitabine (CAPOX) is also under investigation. Currently, RTOG has launched a phase III study using carboplatin, paclitaxel with or without trastuzumab concurrent with radiotherapy in the preoperative setting (www.clinicatrial.gov). Disease-free survival and pCR will be assessed. The results of these clinical studies are awaited.

Lapatinib, a small molecular inhibitor to Her2/neu and EGFR, has demonstrated good clinical activity in breast cancer. Recently, Iqbal et al. published a Southwest Oncology Group (SWOG) study using lapatinib as a single agent in unselected metastatic gastric cancer patients [34]. The study enrolled a total of 47 patients. A 11% partial response rate and 23% stable disease were reported. Combination of lapatinib with various chemotherapies is being explored.

Targeting Angiogenesis Pathway

Anti-angiogenesis therapy has been proven to be effective in many solid tumors. Bevacizumab, a humanized monoclonal antibody against vascular endothelial growth factor (VEGF), has been used to treat many solid tumors including colorectal cancer, breast cancer, renal cell carcinoma and hepatocellular carcinoma [35–39]. In colorectal cancer, addition of bevacizumab to chemotherapy results in a better response rate, progression-free survival and overall survival [36].

Like in other solid tumors, a higher level of VEGF was also found in the surgical specimen of esophagogastric cancer specimens [40–43]. The higher level of VEGF expression is associated with a more aggressive clinical course, nodal metastasis, higher TNM staging and poor prognosis. Based on these preclinical findings and clinical success in other tumors, bevacizumab was first evaluated in several small single arm studies. Shah and co-workers showed that combination of bevaizumab, irinotecan and cisplatin resulted in 65% response rate and 12.3 overall median survival rate [44]. Recently, Shah and colleagues reported another single arm phase II study using bevacizumab with modified DCF (decetaxel, cisplatin and 5FU) [45]. The study demonstrated a median overall survival of 16.8 months. During the subgroup analysis, a 85% response rate was achieved in GE junction and proximal gastric cancers. A 56% response rate was reached for distal and body gastric cancers. However, diffuse type gastric cancer has much lower response rate (38%). Despite these encouraging data, the efficacy of bevacizumab was disappointing in the phase III study. AVAGAST (avastin for advanced gastric cancer) study is a double-blind, randomized, placebo-controlled phase III study of cisplatin and capecitabine with or without bevacizumab as first line therapy in advanced gastric or GE junction cancers [46]. A total of 774 patients were enrolled in the study. The study subjects were randomized to chemotherapy with placebo or chemotherapy with bevacizumab. The overall response rate was significantly higher with the addition of bevacizumab (38% vs. 29.5%, $p=0.0121$). The progression-free survival is significantly longer in the bevacizumab arm (6.7 months in bevacizumab arm vs. 5.3 months in the placebo arm; $p=0.0037$). However, there was no difference in overall survival between the two study arms (HR = 0.87, $p=0.1002$).

Bevacizumab was also evaluated in preoperative chemoradiation to explore the feasibility, safety and preliminary efficacy. Ilson et al. presented their study of bevacizumab, irinotecan and cisplatin with radiotherapy in localized gastroesophageal cancer [47]. Preliminary analysis showed that it is safe and feasible. Delayed wound-healing was not observed. Other approaches such as using bevacizumab in the perioperative chemotherapy are under evaluation. MAGIC study offered preioperative chemotherapy ECF in operable gastric and GE junction cancer. The study demonstrated survival benefit for this approach [48]. Because of the success of MAGIC trial, integrating bevacizumab in perioperative chemotherapy is currently being evaluated in a phase III study (ST03). Preliminary data showed reasonable safety without increasing surgical risks significantly [49]. Other anti-angiogenesis agents (ramucuryumab and apatinib) in the second or third line therapy are under the way (www.clinicaltrials.gov). Multi-tyrosine kinase inhibitors with anti-angiogenic effects including sorafenib and sunitinib are being actively evaluated [50–52]. To better select patients and to stratify patients in future clinical trials, several biomarkers have been explored to predict the efficacy of bevacizumab. These markers include VEGF level, circulating endothelial progenitor cells or circulating endothelial cells [38, 53–58]. However, the results are rather disappointing. More researches are needed to better understand the role angiogenesis in tumor development and progression. Furthermore, the mechanism of these anti-angiogenic agents needs to be further defined.

Other Potential Targets

In addition to these two major pathways, other potential molecular targets were assessed. Cyclooxygenase-2 (COX-2) pathway has been the center of attention in cancer development and progression several years ago. It has been shown that COX-2 inhibitors can reduce the risks for esophageal, gastric and colorectal cancers [59–61]. COX-2 is frequently upper regulated in gastrointestinal tumors [62, 63]. Through cross talk with other signaling pathways, COX-2 has been shown to activate NFκB and EGFR or to inactive tumor suppressor gene, APC [64–67]. Celecoxib, a COX-2 inhibitor, has been assessed with chemotherapy in both advanced and localized disease [68, 69]. However, it is difficult to obtain conclusive results in these small studies. Because of the recently recognized cardiac complications of COX-2 inhibitors, further utilization of celecoxib in gastroesophageal cancers and other tumor types has been placed on hold.

PI3 kinase/AKT/mTOR pathways are important in regulating cell proliferation and transformation. These pathways frequently cross talk with other receptor tyrosine kinase mediated signal cascade. Hilebrandt and co-workers from M.D. Anderson examined the genetic variations of PI3 kinase/PTEN/AKT/mTOR pathway in 210 patients with respectable esophageal cancer [70]. The authors demonstrated that certain single nucleotide polymorphism (SNP) is associated with higher risk of recurrence and resistance to chemoradiation. For example, patients with SNP AKT1:rs892119 variation has much poor prognosis comparing to wild type AKT (median survival of 12 months for one or two AKT variant; median survival of 42 months for the wild type). The poor survival for the AKT1:rs892119 was not affected by different chemotherapy agents such as 5FU or cisplatin. Clinically, everolimus, a mTOR inhibitor, is being actively assessed in advanced gastric cancer [71].

Perhaps, the most intriguing target is "cancer stem cells" that was first explored in hematological malignancies. The esophageal stem cells have similar characteristics to other cancer stem cells. They are slow growing, self-renewal and a high proliferative potential triggered by wound healing process [72]. Characterization of esophageal stem cells, however, is rather inconsistent in the literature. Kalabis et al. described that the esophageal basal epithelial cells with self-renewal properties are $CD34^{+}$ [73]. Other markers including CD133, adenosine triphosphate-binding cassette superfamily G 2 (ABCG2), CD44 and Musashi-1 have been described in the literature [74–76]. It is not clear what the clinical implications are when these markers are detected in clinical specimens or cell lines. Although there is a great interest to target stem cells in cancer therapy, such approach is largely hindered by poorly defined biology of these cells.

Conclusions

Esophagogastric cancer remains a public health problem worldwide. Over the past decade, the treatment of esophagogastric cancer has been evolving rapidly. These include surgical techniques and development of new cytotoxic agents as well as

targeted therapy. Recent introduction of trastuzumab in advanced disease brought a new era of personalized medicine in managing esophagogastric cancers. However, more researches are required to better understand the tumor biology and the mechanism of action of targeted agents. Only through these mechanistic explorations, potential predictive and prognostic biomarkers could be identified. These biomarkers will allow us to have better patient selection, better stratification and better trial designs.

References

1. Solaymani-Dodaran M, Logan RF, West J, Card T, Coupland C. Risk of oesophageal cancer in Barrett's oesophagus and gastro-oesophageal reflux. Gut. 2004;53(8):1070–4.
2. Altorki NK, Oliveria S, Schrump DS. Epidemiology and molecular biology of Barrett's adenocarcinoma. Semin Surg Oncol. 1997;13(4):270–80.
3. Spechler SJ, Robbins AH, Rubins HB, Vincent ME, Heeren T, Doos WG, et al. Adenocarcinoma and Barrett's esophagus. An overrated risk. Gastroenterology. 1984;87(4):927–33.
4. Holmes RS, Vaughan TL. Epidemiology and pathogenesis of esophageal cancer. Semin Radiat Oncol. 2007;17(1):2–9. Epub 2006/12/23.
5. Siewert JR, Ott K. Are squamous and adenocarcinomas of the esophagus the same disease. Semin Radiat Oncol. 2007;17(1):38–44. Epub 2006/12/23.
6. American Cancer Society: estimated new cases by sex for all sites, U.S. 2007.
7. Schlessinger J. Ligand-induced, receptor-mediated dimerization and activation of EGF receptor. Cell. 2002;110(6):669–72.
8. Burgess AW, Cho HS, Eigenbrot C, Ferguson KM, Garrett TP, Leahy DJ, et al. An open-and-shut case? Recent insights into the activation of EGF/ErbB receptors. Mol Cell. 2003;12(3):541–52. Epub 2003/10/07.
9. 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. Epub 2004/04/20.
10. Yarden Y, Ullrich A. Growth factor receptor tyrosine kinases. Annu Rev Biochem. 1988;57:443–78. Epub 1988/01/01.
11. Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med. 2006;354(6):567–78. Epub 2006/02/10.
12. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med. 2004;350(21):2129–39. Epub 2004/05/01.
13. Kobayashi S, Boggon TJ, Dayaram T, Janne PA, Kocher O, Meyerson M, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med. 2005;352(8):786–92. Epub 2005/02/25.
14. Takehana T, Kunitomo K, Suzuki S, Kono K, Fujii H, Matsumoto Y, et al. Expression of epidermal growth factor receptor in gastric carcinomas. Clin Gastroenterol Hepatol. 2003;1(6):438–45. Epub 2004/03/16.
15. Tokunaga A, Onda M, Okuda T, Teramoto T, Fujita I, Mizutani T, et al. Clinical significance of epidermal growth factor (EGF), EGF receptor, and c-erbB-2 in human gastric cancer. Cancer. 1995;75(Suppl 6):1418–25. Epub 1995/03/15.
16. Albanell J, Rojo F, Baselga J. Pharmacodynamic studies with the epidermal growth factor receptor tyrosine kinase inhibitor ZD1839. Semin Oncol. 2001;28(5 Suppl 16):56–66. Epub 2001/11/14.

17. 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. Epub 1996/05/01.
18. 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. Epub 2005/06/30.
19. Wilkinson NW, Black JD, Roukhadze E, Driscoll D, Smiley S, Hoshi H, et al. Epidermal growth factor receptor expression correlates with histologic grade in resected esophageal adenocarcinoma. J Gastrointest Surg. 2004;8(4):448–53. Epub 2004/05/04.
20. Gibson MK, Abraham SC, Wu TT, Burtness B, Heitmiller RF, Heath E, et al. Epidermal growth factor receptor, p53 mutation, and pathological response predict survival in patients with locally advanced esophageal cancer treated with preoperative chemoradiotherapy. Clin Cancer Res. 2003;9(17):6461–8. Epub 2003/12/26.
21. Liang K, Ang KK, Milas L, Hunter N, Fan Z. The epidermal growth factor receptor mediates radioresistance. Int J Radiat Oncol Biol Phys. 2003;57(1):246–54. Epub 2003/08/12.
22. 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. Epub 2010/07/16.
23. 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. Epub 2011/01/11.
24. 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. Epub 2009/09/24.
25. 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. Epub 2011/01/06.
26. De Vita F, Orditura M, Martinelli E, Vecchione L, Innocenti R, Sileni VC, et al. A multicenter phase II study of induction chemotherapy with FOLFOX-4 and cetuximab followed by radiation and cetuximab in locally advanced oesophageal cancer. Br J Cancer. 2011;104(3):427–32. Epub 2011/01/20.
27. Ilson DH, Kelsen D, Shah M, Schwartz G, Levine DA, Boyd J, et al. A phase 2 trial of erlotinib in patients with previously treated squamous cell and adenocarcinoma of the esophagus. Cancer. 2011;117(7):1409–14. Epub 2011/03/23.
28. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science. 1987;235(4785):177–82. Epub 1987/01/09.
29. Sato-Kuwabara Y, Neves JI, Fregnani JH, Sallum RA, Soares FA. Evaluation of gene amplification and protein expression of HER-2/neu in esophageal squamous cell carcinoma using Fluorescence In Situ Hybridization (FISH) and immunohistochemistry. BMC Cancer. 2009;9:6. Epub 2009/01/09.
30. 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. Epub 2010/08/24.
31. Yan SY, Hu Y, Fan JG, Tao GQ, Lu YM, Cai X, et al. Clinicopathologic significance of HER-2/neu protein expression and gene amplification in gastric carcinoma. World J Gastroenterol. 2011;17(11):1501–6. Epub 2011/04/08.
32. Begnami MD, Fukuda E, Fregnani JH, Nonogaki S, Montagnini AL, Da Costa Jr WL, et al. Prognostic implications of altered human epidermal growth factor receptors (HERs) in gastric carcinomas: HER2 and HER3 Are predictors of poor outcome. J Clin Oncol. 2011;29:3030–6.

33. Shah MA, Janjigian YY, Pauligk C, et al. Prognostic significance of human epidermal growth factor-2 (HER2) in advanced gastric cancer: a U.S. and European international collaborative analysis. J Clin Oncol. 2011;29:(suppl; abstr 4014).
34. Iqbal S, Goldman B, Fenoglio-Preiser CM, Lenz HJ, Zhang W, Danenberg KD, et al. Southwest oncology group study S0413: a phase II trial of lapatinib (GW572016) as first-line therapy in patients with advanced or metastatic gastric cancer. Ann Oncol. 2011;22:2610–5.
35. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med. 2006;355(24):2542–50. Epub 2006/12/15.
36. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med. 2004;350(23):2335–42. Epub 2004/06/04.
37. Miller K, Wang M, Gralow J, Dickler M, Cobleigh M, Perez EA, et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med. 2007;357(26):2666–76. Epub 2007/12/28.
38. Yang JC, Haworth L, Sherry RM, Hwu P, Schwartzentruber DJ, Topalian SL, et al. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. N Engl J Med. 2003;349(5):427–34. Epub 2003/08/02.
39. Bukowski RM, Kabbinavar FF, Figlin RA, Flaherty K, Srinivas S, Vaishampayan U, et al. Randomized phase II study of erlotinib combined with bevacizumab compared with bevacizumab alone in metastatic renal cell cancer. J Clin Oncol. 2007;25(29):4536–41. Epub 2007/09/19.
40. Tanaka T, Ishiguro H, Kuwabara Y, Kimura M, Mitsui A, Katada T, et al. Vascular endothelial growth factor C (VEGF-C) in esophageal cancer correlates with lymph node metastasis and poor patient prognosis. J Exp Clin Cancer Res. 2010;29:83. Epub 2010/06/30.
41. Kozlowski M, Naumnik W, Niklinski J, Milewski R, Dziegielewski P, Laudanski J. Vascular endothelial growth factor C and D expression correlates with lymph node metastasis and poor prognosis in patients with resected esophageal cancer. Neoplasma. 2011;58(4):311–9. Epub 2011/04/28.
42. Maeda K, Chung YS, Takatsuka S, Ogawa Y, Onoda N, Sawada T, et al. Tumour angiogenesis and tumour cell proliferation as prognostic indicators in gastric carcinoma. Br J Cancer. 1995;72(2):319–23. Epub 1995/08/01.
43. Juttner S, Wissmann C, Jons T, Vieth M, Hertel J, Gretschel S, et al. Vascular endothelial growth factor-D and its receptor VEGFR-3: two novel independent prognostic markers in gastric adenocarcinoma. J Clin Oncol. 2006;24(2):228–40. Epub 2005/12/14.
44. 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. Epub 2006/11/23.
45. 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. Epub 2010/12/30.
46. Kang Y, Ohtsu A, Van Cutsem E, et al. AVAGAST: a randomized, double-blind, placebo-controlled, phase III study of first-line capecitabine and cisplatin plus bevacizumab or placebo in patients with advanced gastric cancer (AGC). J Clin Oncol. 2010;28:18s (suppl; abstr LBA4007).
47. Ilson D, Bains M, Rizk N, et al. Phase II trial of preoperative bevacizumab (Bev), irinotecan (I), cisplatin (C), and radiation (RT) in esophageal adenocarcinoma: preliminary safety analysis. J Clin Oncol. 2009;27(15s). suppl; abstr 4573 2009.
48. Cunningham D, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M, et al. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. N Engl J Med. 2006;355(1):11–20.
49. Okines AF, Langley R, Cafferty FH, et al. Preliminary safety data from a randomized trial of perioperative epirubicin, cisplatin plus capecitabine (ECX) with or without bevacizumab (B) in patients (pts) with gastric or oesophagogastric junction (OGJ) adenocarcinoma. J Clin Oncol. 2010;28(15s) (suppl; abstr 4019).

50. Bang YJ, Kang YK, Kang WK, Boku N, Chung HC, Chen JS, et al. Phase II study of sunitinib as second-line treatment for advanced gastric cancer. Invest New Drugs. 2011;29:1449–58. Epub 2010/05/13.
51. Sun W, Powell M, O'Dwyer PJ, Catalano P, Ansari RH, Benson III AB. Phase II study of sorafenib in combination with docetaxel and cisplatin in the treatment of metastatic or advanced gastric and gastroesophageal junction adenocarcinoma: ECOG 5203. J Clin Oncol. 2010;28(18):2947–51.
52. Kim C, Lee JL, Choi YH, Kang BW, Ryu MH, Chang HM, et al. Phase I dose-finding study of sorafenib in combination with capecitabine and cisplatin as a first-line treatment in patients with advanced gastric cancer. Invest New Drugs. 2012;30:306–15. Epub 2010/09/15.
53. Dowlati A, Gray R, Sandler AB, Schiller JH, Johnson DH. Cell adhesion molecules, vascular endothelial growth factor, and basic fibroblast growth factor in patients with non-small cell lung cancer treated with chemotherapy with or without bevacizumab—an Eastern cooperative oncology group study. Clin Cancer Res. 2008;14(5):1407–12. Epub 2008/03/05.
54. Kopetz S, Hoff PM, Morris JS, Wolff RA, Eng C, Glover KY, et al. Phase II trial of infusional fluorouracil, irinotecan, and bevacizumab for metastatic colorectal cancer: efficacy and circulating angiogenic biomarkers associated with therapeutic resistance. J Clin Oncol. 2010;28(9):453. Epub 2009/12/17.
55. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science. 1997;275(5302):964–7. Epub 1997/02/14.
56. Lyden D, Hattori K, Dias S, Costa C, Blaikie P, Butros L, et al. Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. Nat Med. 2001;7(11):1194–201. Epub 2001/11/02.
57. Gao D, Nolan DJ, Mellick AS, Bambino K, McDonnell K, Mittal V. Endothelial progenitor cells control the angiogenic switch in mouse lung metastasis. Science. 2008;319(5860):195–8. Epub 2008/01/12.
58. Furstenberger G, von Moos R, Lucas R, Thurlimann B, Senn HJ, Hamacher J, et al. Circulating endothelial cells and angiogenic serum factors during neoadjuvant chemotherapy of primary breast cancer. Br J Cancer. 2006;94(4):524–31. Epub 2006/02/02.
59. Abnet CC, Freedman ND, Kamangar F, Leitzmann MF, Hollenbeck AR, Schatzkin A. Non-steroidal anti-inflammatory drugs and risk of gastric and oesophageal adenocarcinomas: results from a cohort study and a meta-analysis. Br J Cancer. 2009;100(3):551–7. Epub 2009/01/22.
60. Chan AT, Ogino S, Fuchs CS. Aspirin and the risk of colorectal cancer in relation to the expression of COX-2. N Engl J Med. 2007;356(21):2131–42. Epub 2007/05/25.
61. Cuzick J, Otto F, Baron JA, Brown PH, Burn J, Greenwald P, et al. Aspirin and non-steroidal anti-inflammatory drugs for cancer prevention: an international consensus statement. Lancet Oncol. 2009;10(5):501–7. Epub 2009/05/05.
62. Rigas B, Goldman IS, Levine L. Altered eicosanoid levels in human colon cancer. J Lab Clin Med. 1993;122(5):518–23. Epub 1993/11/01.
63. Wu WK, Sung JJ, Yu L, Li ZJ, Chu KM, Cho CH. Constitutive hypophosphorylation of extracellular signal-regulated kinases-1/2 and down-regulation of c-Jun in human gastric adenocarcinoma. Biochem Biophys Res Commun. 2008;373(2):330–4. Epub 2008/06/24.
64. Wu WK, Sung JJ, Lee CW, Yu J, Cho CH. Cyclooxygenase-2 in tumorigenesis of gastrointestinal cancers: an update on the molecular mechanisms. Cancer Lett. 2010;295(1):7–16. Epub 2010/04/13.
65. Pai R, Soreghan B, Szabo IL, Pavelka M, Baatar D, Tarnawski AS. Prostaglandin E2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. Nat Med. 2002;8(3):289–93. Epub 2002/03/05.
66. Pai R, Nakamura T, Moon WS, Tarnawski AS. Prostaglandins promote colon cancer cell invasion; signaling by cross-talk between two distinct growth factor receptors. FASEB J. 2003;17(12):1640–7. Epub 2003/09/06.

67. Boolbol SK, Dannenberg AJ, Chadburn A, Martucci C, Guo XJ, Ramonetti JT, et al. Cyclooxygenase-2 overexpression and tumor formation are blocked by sulindac in a murine model of familial adenomatous polyposis. Cancer Res. 1996;56(11):2556–60. Epub 1996/06/01.
68. Govindan R, McLeod H, Mantravadi P, Fineberg N, Helft P, Kesler K, et al. Cisplatin, fluorouracil, celecoxib, and RT in resectable esophageal cancer: preliminary results. Oncology (Williston Park). 2004;18(14 Suppl 14):18–21. Epub 2005/02/03.
69. Enzinger PC, Mamon H, Choi N, et al. Phase II cisplatin, irinotecan, celecoxib and concurrent radiation therapy followed by surgery for locally advanced esophageal cancer. 2004 Gastrointestinal Cancer Symposium, abstract 35.
70. Hildebrandt MA, Yang H, Hung MC, Izzo JG, Huang M, Lin J, et al. Genetic variations in the PI3K/PTEN/AKT/mTOR pathway are associated with clinical outcomes in esophageal cancer patients treated with chemoradiotherapy. J Clin Oncol. 2009;27(6):857–71. Epub 2009/01/24.
71. Yamada Y, Doi T, Muro K, et al. Multicenter phase II study of everolimus in patients with previously treated metastatic gastric cancer: main results. Gastrointestinal cancers symposium, San Francisco January 15–17, 2009.
72. Lavker RM, Sun TT. Epidermal stem cells: properties, markers, and location. Proc Natl Acad Sci U S A. 2000;97(25):13473–5. Epub 2000/11/23.
73. Kalabis J, Oyama K, Okawa T, Nakagawa H, Michaylira CZ, Stairs DB, et al. A subpopulation of mouse esophageal basal cells has properties of stem cells with the capacity for self-renewal and lineage specification. J Clin Invest. 2008;118(12):3860–9. Epub 2008/11/27.
74. Hang D, Dong HC, Ning T, Dong B, Hou DL, Xu WG. Prognostic value of the stem cell markers CD133 and ABCG2 expression in esophageal squamous cell carcinoma. Dis Esophagus. 2012;25:638–44. Epub 2012/01/13.
75. Zhao JS, Li WJ, Ge D, Zhang PJ, Li JJ, Lu CL, et al. Tumor initiating cells in esophageal squamous cell carcinomas express high levels of CD44. PLoS One. 2011;6(6):e21419. Epub 2011/07/07.
76. Bobryshev YV, Freeman AK, Botelho NK, Tran D, Levert-Mignon AJ, Lord RV. Expression of the putative stem cell marker musashi-1 in Barrett's esophagus and esophageal adenocarcinoma. Dis Esophagus. 2010;23(7):580–9. Epub 2010/05/13.

Impact of Genetic Targets on Cancer Therapy: Hepatocellular Cancer

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Abstract Understanding cancer at the genetic level had gained significant attention over the last decade since the human genome was first sequenced in 2001. For hepatocellular carcinoma (HCC) a number of genome-wide profiling studies have been published. These studies have provided us with gene sets, based on which we can now classify tumors and have an idea about the likely clinical outcomes. In addition to that, genomic profiling for HCC has provided us a better understanding of the carcinogenesis process and identifies key steps at multiple levels (i.e. Genetics, molecular pathways) that can be potential targets for treatment and prevention. Although still an incurable disease, unresectable HCC has one proven systemic therapy, sorafenib, and many under active investigation. With advancement in technology and understanding of hepatocarcinogenesis, scientists hope to provide true personalized treatment for this disease in the near future. In this review article we discuss advances in understanding genetics and pathogenesis of HCC and the currently available and ongoing trials for targeted therapies. These emerging therapies may guide the development of more effective treatments or possibly a cure for HCC.

Keywords Hepatocellular carcinoma • Monoclonal antibodies • Sorafenib • Targeted therapy • Tyrosine kinase inhibitors

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W.S. El-Deiry (ed.), *Impact of Genetic Targets on Cancer Therapy*, Advances in Experimental Medicine and Biology 779, DOI 10.1007/978-1-4614-6176-0_4,

Abbreviations

AASLD	American Asssociation for the Study of Liver Disease
BCLC	Barcelona clinic liver cancer
BRAF	V-raf murine sarcoma viral oncogene homolog B1
CRC	Colorectal cancer
FDA	Food and Drug Administration
EGF	Epidermal growth factor
ERK	Extracellular signal-regulated kinases
FGF	Fibroblast growth factor
FRAP1	FK506 binding protein 12-rapamycin associated protein 1
GEMOX	Gemcitabine/ Oxaliplatin
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HCC	Hepatocellular carcinoma
HGFR	Hepatocyte growth factor receptor
IFN	Interferon
IGF	Insulin like growth factor
IL6	Interleukin 6
JAK	Janus kinase
KIT	V-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
mAb	Monoclonal antibody
MAPK	Mitogen activated protein kinase
mTOR	Mammalian target of rapamycin
NASH	Nonalcoholic steatohepatitis
PI3K	Phosphatidylinositol 3-kinase-related kinase
PDGF	Platelet-derived growth factor
PDGFR	Platelet-derived growth factor receptor
PR	Partial remission
RCT	Randomized controlled trial
RECIST	Response evaluation criteria in solid tumors
SCFR	Stem cell factor receptor
SD	Stable disease
SHARP	Sorafenib hepatocellular carcinoma assessment randomized protocol
STAT	Signal transducer and activator of transcription
STORM	Sorafenib as adjuvant treatment in the prevention of recurrence of hepatocellular carcinoma
TAE	Transarterial chemoembolization
TACE	Transarterial chemoembolization
TKI	Tyrosine kinase inhibitor
TLR	Toll like receptors
TNFα	Tumor necrosis factor α
ToGA	Trastuzumab for gastric adenocarcinoma
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor

Introduction

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer; it is the sixth most common malignancy and the third leading cause of cancer-related death worldwide [1]. Although HCC has very high incidence and prevalence in Asia and Sub-Saharan regions, its incidence and prevalence have been on the rise over the last decade in Westernized countries (United States and Western Europe) [2, 3]. Despite the significant decrease in mortality in most malignancies over the last two decades, mortality from HCC has increased by more than 50% [2]. This is not only due to the lack of effective systemic therapy, but also because of a poor understanding of the disease process and associated liver disease. This overall increase in incidence and mortality have resulted in significant interest to better understand HCC at multiple levels and intensified the work for the development of more effective systemic therapy.

Chronic liver disease is the etiology of most cases of HCC. In 80% of cases HCC affects patients with liver cirrhosis [4], which is most typically a result of chronic Hepatitis B and C infections, alcoholic liver disease and nonalcoholic steatohepatitis (NASH) [4, 5]. Measures to eliminate Hepatitis B related liver disease in parts of the world where it is endemic with the use of effective antiviral therapies and vaccinations have resulted in decrease in the incidence of HCC [6, 7]. Besides these prophylactic measures, there are no other available strategies to limit the development of HCC in patients with liver cirrhosis other than the intensive screening programs for early detection, as recommended by the American Association for the Study of Liver Diseases (AASLD) practice guidelines [8]. Despite these guidelines, which have been available since 2005, less than 20% of patients in United States with HCC received regular surveillance [9] and the majority of patients with HCC present with advanced stage disease with no possible curative therapies available [10, 11].

Prognosis of HCC depends not only on the stage of the tumor but also on the underlying liver disease and the degree of liver dysfunction; the most commonly used classification system for HCC is the Barcelona Clinic Liver Cancer (BCLC) classification [8]. It includes variables related to tumor stage, liver functional status, physical status and cancer related symptoms. The main advantage of the BCLC staging system is that it links staging with treatment modalities and with an estimation of life expectancy that is based on published response rates to various available treatment options [8].

The BCLC staging classification for HCC classifies patients as having stages of disease from 0 to D (Fig. 1). Stage 0 is very early disease, defined as the presence of a solitary liver tumor that measures <2 cm without tumor invasion into surrounding tissues. Patients rarely present at this stage unless they are enrolled in screening programs. Stage A is early disease and includes patients who exhibit preserved liver function with a solitary HCC <5 cm in size, or up to three tumors each of which is <3 cm in size. Patients with stage 0 or stage A disease can be effectively treated with curative therapies, such as surgical resection, liver transplantation or ablation methods [8]. With these treatments it is possible to obtain complete responses with

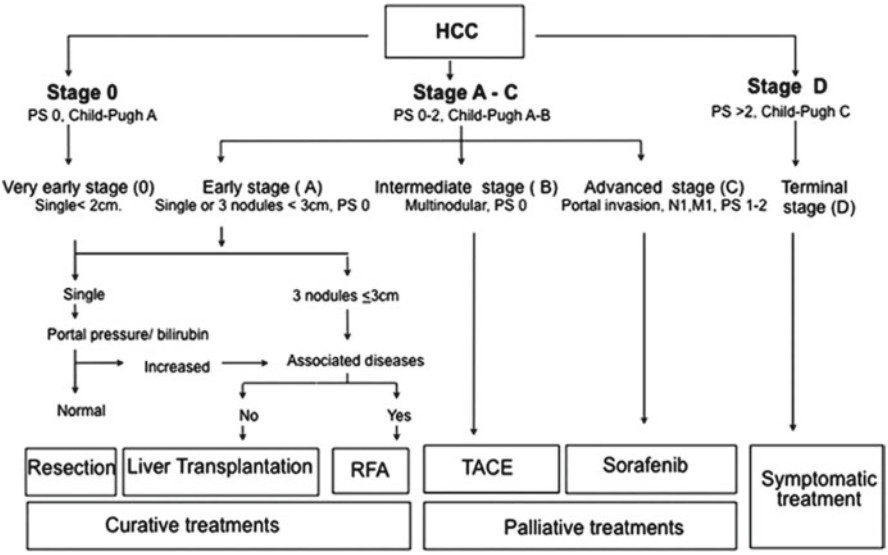

Fig. 1 The Barcelona clinic liver cancer staging system and treatment allocation. (Copyright © 2010, American Association for the study of Liver Diseases. Adapted with permission from Bruix J. Management of hepatocellular carcinoma: an update. Hepatology 2011 Mar;53(3):1,020–2. doi: 10.1002/hep.24199 Available from: http://www.aasld.org/practiceguidelines/Documents/Bookmarked%20practice%20Guidelines/Hccupdate2010.pdf. *CLT* cadaveric liver transplantation, *HCC* hepatocellular carcinoma, *LDLT* living donor liver transplantation, *PEI* percutaneous ethanol injection, *RF* radiofrequency

potential long-term cure, as reflected by a 5-year survival of 50–70% [8]. The BCLC intermediate stage (stage B) consists of asymptomatic patients with well-preserved liver function, and multinodular or large tumor extension, without macrovascular invasion or extrahepatic spread. Stage B disease patients usually treated with transarterial embolization (TAE) or transarterial chemoembolization (TACE). These treatments have demonstrated a significant increase in survival compared with best supportive care (median survival, 20 months vs 16 months) [8]. BCLC stage C (advanced) patients are those with mild related symptoms and/or macrovascular invasion or extrahepatic spread. Previously, no standard systemic therapy existed for the treatment of patients at this stage; and multiple traditional chemotherapy regimens have failed to improve survival. In 2007 sorafenib, a multiknase inhibitor became the first targeted therapy for unresectable HCC approved by FDA [8]. Patients with cancer symptoms, related to progressive liver failure, tumor growth with vascular involvement, extrahepatic metastasis or physical impairment (performance status >2) are classified as stage D (end stage) disease; they do not benefit from any treatment and should receive only the best available supportive care [8].

Although the BCLC represent the most commonly used classification system in most clinical trials, it has significant limitations and drawbacks, for example in a recent meta-analysis looking at the spontaneous course of the unresectable HCC

which analyzed the survival rates of the placebo and untreated arms of several RCTs, the 1- and 2-year survival was extremely heterogeneous and inconsistent for the same stage [12]. This meta-analysis confirms the heterogeneity of behavior of HCC and provides a sound basis for stratifying patients with HCC by parameters that consider genetics and molecular basis of the tumor.

Pathogenesis and Genetics for HCC

Liver cirrhosis is considered a precursor for HCC [13]. HCC rarely develops in a de novo fashion without antecedent chronic liver injury [4]. As with other solid tumors, HCC typically follows a multistep progression from small hyperplastic nodule in cirrhotic liver to a nodule with some degree of dysplasia, initially low grade dysplasia then progressing to high grade dysplasia. This is followed by the development of early HCC, which is typically small with indistinct margins, and finally to moderately or poorly differentiated HCC [14, 15].

On macroscopic level, throughout the course of HCC progression, nodules acquire evident changes in vascular supply with development of unpaired arteries that progressively replace portal vessels giving the nodule the typical hypervascular appearance on a triphasic CT scan with delayed images (i.e., presence of a hypervascular pattern in arterial phase with a washout of contrast during the venous and late phase) [16].

Studies have analyzed the genetic features associated with each stage HCC development, particularly the transition from high-grade dysplastic nodules to early stage HCC. These studies have identified MYC and TLRs (Toll-like Receptors) as important mediators of this transition [17]. In addition to that a large number of genetic and epigenetic alterations were found to be involved in HCC carcinogenesis leading to mutations in genes that control cell cycle, proliferation, and apoptosis. Principal mutations observed in HCC carcinogenesis are point mutations, deletions or gains of segments, and epigenetic changes such as hypermethylation of gene promoters [18].

Knowing that carcinogenesis represent a complex dynamic process of a large number of single gene mutations, the classic single-gene approach to study and understand malignancies has been outdated by the advancements in gene expression profiling and microarray technology. These efforts have led to the identification of gene-sets that help classify tumors and predict clinical outcomes. Recently, the application of genomic profiling has been extended to identify the molecular pathways involved in the carcinogenesis that can act as potential therapeutic targets for the tumors. In a recent study, Woo et al. [19] had described a new classification for the currently available genetic signatures to minimize overlap and decrease confusion; he categorized them into Prediction, Phenotype, and Functional signatures.

Prediction signatures represent the most common gene signatures studied so far [19], it has been found to be most useful in estimating the potential for survival, recurrence, metastasis, and other clinical features. It includes the signatures generated from

gene expression [20–24], epigenetic regulation [25], and microRNA analysis [26]. Most of these prediction signatures do not have functional roles in the process of carcinogenesis [19], and therefore may not be appropriate candidates for therapeutic targets. By identifying gene sets associated with an improved response to a specific treatment option (e.g. surgical resection, liver transplantation, or systemic therapy) [27], prediction signatures have enormous potential to further advance personalized treatment of HCC. Gene expression profiling studies also have been performed on cirrhotic tissue adjacent to a HCC, to identify patterns that might be used in the prediction of clinical outcomes [28, 29]. The expression of 186 genes from adjacent cirrhotic tissue, including genes that encoded epidermal growth factor (EGF), interleukin-6, and components of the nuclear factor tor-κ correlated with survival for patients with early stage HCC who were treated by surgical resection [28]. Therefore accurate prognosis for patients with HCC will require a combination of clinical variables (the BCLC algorithm), histological variables, genetic and molecular data not only from the tumor itself but also from the adjacent cirrhotic tissue [30]. Villanueva et al. [31] in a recent study showed that an integrated approach, combining clinical, pathology, and gene expression data, increased the accuracy of prognosis, compared with just considering clinical and/or pathologic variables [31].

Phenotype signatures are related to the clinical and pathological characteristics of the HCC [19], including factors related to its pathogenesis (including HBV and HCV) [32], clinical stage [33], and the tumor grade [34]. Phenotype signatures do not necessarily have a relationship to clinical outcomes, but rather are indicative of the functional characteristics of a certain tumor subtype [19]. Some phenotypic signatures have been associated with certain drug response, as is seen in HCC with chromosome 17q amplification which is linked to a good response to rapamycin [35]. Integrated utilization of both phenotype signatures with the prediction signatures will help provide more accurate way of classification and advance the era of personalized cancer treatment in the future [36].

Functional genetic signatures encode particular cellular processes or molecular pathways involved in the carcinogenesis of HCC [19]. Because of the functional roles these signatures and their molecular pathways represent the most suitable targets for therapeutic interventions for HCC. As previously mentioned, the pathogenesis of HCC follows a multi-step progression of worsening pathologic states, starting with hyperplastic nodules in cirrhotic liver, to dysplastic nodules, and finally progression to HCC [14, 15]. Careful genetic and molecular analysis of this step-by-step progression of the tumor identified key genetic events and their molecular pathways in this complex process. For example by performing pathway analysis, Wurmbach et al. [33] have revealed the dysregulation of the Notch and Toll-like receptor TLR pathways in cirrhosis, this is followed by the dysregulation of Jak/STAT receptor in early carcinogenesis of HCV associated HCC. Kaposi-Novak et al. [17] have identified a central role of MYC gene signature and its pathway during malignant transformation from high grade dysplasia to early HCC.

In a recent review Hoshida et al. [37] categorized HCCs into three subgroups, based on altered gene expression and the predominant molecular pathways involved. The first group includes altered expression of genes that regulate cellular

proliferation, involving pathways such as insulin-like growth factor (IGF) [38], mammalian target of rapamycin (mTOR) [39], and epidermal growth factor (EGF) [40]. The second group includes genes involved in hepatocyte differentiation and the related pathways of the WNT signaling and the Hedgehog pathways [41]. The third group includes altered expression of genes involved in interferon signaling and inflammation (e.g., IL6, IFN, and TNFα) [40]. These pathways and others that are frequently deregulated in the hepatocarcinogenesis process are mediated by multiple molecules (e.g., VEGF, FGF, PDGF, and angiopoietin) [42], and represent potential targets for therapeutic intervention and multiple targeted therapies are currently under investigation [40]. Despite our growing understanding of the genetics and the different pathways altered in HCC, the specific sequence of genetic events and the exact molecular mechanisms that lead to malignant transformation of hepatocytes and give rise to a HCC are still poorly understood [40].

Hepatitis Virus Infections and HCC

Hepatitis virus infections are a leading cause of HCC, they are responsible for 50–70% cases of HCC worldwide [43]. Hepatitis viruses can lead to HCC in a direct way through viral induced genomic alterations in hepatocytes, or indirectly through chronic inflammation and cirrhosis.

Hepatitis B virus is a member of Hepadnaviridae, a group of closely related DNA viruses; it is classified as para-retrovirus because its replication depends on reverse transcription of host genomic RNA [44]. Hepatitis B virus induces HCC by acting as a direct carcinogen in the absence of chronic inflammation and cirrhosis. Hepatitis B viral protein X (protein of uncertain function X) has been implicated in the direct stimulation of intracellular protein kinases involved in cellular proliferation. It stimulates multiple transcription factors such as NF-κB, Nuclear Factor of Activated T Cells, and cAMP Responsive Element Binding protein, which in turns stimulates the transcription of host genes involved in cellular proliferation (i.e. c-myc and c-fos) [45], this results in the up regulation of growth factor receptors such as EGF-R. Protein X also inhibits p53, tumor suppressor gene, which is frequently inactivated in patients with HCC [46].

Hepatitis C virus (HCV) is a member of Flaviviridea, a family of positive strand RNA viruses [47]. Chronic infection with HCV leads to cirrhosis in 10–30% of patients. Cirrhosis is almost always present in patients who develop HCC. The development of HCC in HCV infected patients typically occurs 10 years from the onset of cirrhosis and 30 years from the initial exposure to HCV [48]. Both cirrhosis and HCV may act on different pathways to induce hepatocarcinogenesis, while cirrhosis can leads to many changes, including oxidative stress, and elevated levels of many growth factors (IGF-1and 2, TGF-a, and IL-6) due to chronic inflammation, HCV can act as a WNT ligand, which up-regulate cell signaling pathways (MAP kinases) and stimulate cellular proliferation [49, 50].

Sorafenib and Targeted Therapy for HCC

The discovery of imatinib, a tyrosine kinase inhibitor (TKI), as an effective therapy for chronic myeloid leukemia (CML) represents the landmark step in the era of targeted therapies in oncology. In 2001, the FDA approved imatinib for treatment of CML after it had been shown to induce remission in most patients [51]. Shortly after, imatinib was approved as a targeted therapy for multiple solid tumors including gastrointestinal stromal tumor (GIST) [52] and dermatofibrosarcoma [53]. Since then multiple TKIs have been discovered and applied to multiple malignancies including lung, renal and recently liver cancer.

In 2007, a phase 3 randomized controlled trial (Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol (SHARP) Trial) [54] showed that sorafenib, a non-specific inhibitor of tyrosine kinases including BRAF, VEGFR, and PDGFR, significantly increased survival of patients with advanced HCC. Although sorafenib prolonged survival only 3 months compared to placebo, this was the first time a systemic therapy provided a survival advantage for HCC. The multicenter SHARP trial [54] randomly assigned 602 patients with inoperable HCC and Child–Pugh A cirrhosis to sorafenib (400 mg twice daily) or placebo. Overall survival, the primary end-point, was significantly longer in the sorafenib-treated patients (10.7 vs 7.9 months), as was time to radiologic progression (5.5 vs 2.8 months). This magnitude of benefit was markedly less in a second trial [55] in the Asia-Pacific region. Of note, the treated group in the Asian-Pacific trial had a shorter survival duration than the control group in the SHARP trial (6.5 vs 7.9 months), this was related to the fact that patients accrued to the Asian-Pacific study were more ill at the start of therapy than those in the SHARP trial, with a worse performance status and more advanced stage of HCC.

Sorafenib was well tolerated and the overall incidence of serious adverse events in the sorafenib and placebo groups was comparable, 52–54%, respectively. Grade 1 or 2 drug-related adverse events (e.g. diarrhea, hand–foot skin syndrome, anorexia, weight loss, asthenia, alopecia, and voice changes) were observed more frequently in the sorafenib group than in the placebo group (80% vs 52%). The two most common grade 3 adverse reactions with sorafenib were hand–foot skin syndrome (8%) and diarrhea (8%). There were no significant differences in discontinuation rates between the sorafenib and the placebo group (38% and 37% respectively). The safety profile of sorafenib was comparable in the subsequent Asia–Pacific Phase 3 trial that enrolled patients of Eastern countries with similar eligibility criteria.

After this landmark development in the history of HCC, researchers have concentrated efforts to discover key molecular pathways in hepatocarcinogenesis and target them with novel agents, currently there are more than 220 clinical trials in multiple phases, testing around 60 targeted agents for HCC (www.clinicaltrials.gov accessed August, 2011) [56].

More than 20 of these trials have been reported so far, the majority in abstract form. Carefully analyzing the results one will notice that the patient populations included in most of these trials are heterogeneous in terms of underlying liver disease

and staging systems used for classification; the studies also vary in measures used to check for tumor response (RECIST or World Health Organization criteria) and primary end points (response rate, progression free survival, overall survival). These variations make it a challenge to compare findings with previous studies and to determine efficacy. For this reason a set of guidelines for trial design has been proposed to provide the scientific community with a consensus framework [57]. These recommendations took into consideration that most patients with HCC have significant underlying liver disease; therefore, the cause of patient mortality should be carefully analyzed to differentiate between HCC-related death and cirrhosis-related death. Also the low objective response, based on RECIST criteria, achieved in the SHARP trial indicates the need for better methods to evaluate tumor responses to therapy.

Targeted therapies can be classified according to the types of targeted therapies used or the molecular pathway and the Cellular process targeted (Tables 1, 2, 3 and 4), (Fig. 2).

Types of Targeted Therapies:

1. Tyrosine Kinase inhibitors (Table 1)
2. Monoclonal Antibodies (Table 2)
3. Enzyme Inhibitors (Table 3)
4. Miscellaneous (Table 4)

Molecular pathways and cellular processes targeted: (Fig. 2)

1. Angiogenesis
2. Cellular proliferation and growth factors
3. Apoptosis and programmed cell death
4. Immune modulators

Types of Targeted Therapies

Tyrosine Kinase Inhibitors TKIs

Tyrosine kinase receptors are cell-surface receptors with high affinities for specific ligands. They comprise an extracellular, N-terminal region that binds ligands and a conserved C-terminal region that autophosphorylates to create binding sites for phosphotyrosine-binding proteins, such as Src. These proteins recruit additional adaptors that propagate signals. In cancer cells, the C-terminal domains of some receptor tyrosine kinases contain mutations that allow their constitutive activation (even in the absence of ligand) and signaling, TKIs prevent autophosphorylation of receptor tyrosine kinase, through either competitive binding with adenosine triphosphate or allosteric inhibition, to interrupt signal transduction [40].

Table 1 Tyrosine kinase inhibitors

#	Name	Phases	Trials	Targets
Non-specific				
1	Sorafenib	1, 1–2, 2, 3, 4	65	BRAF, VEGFR, PDGFR
2	Erlotinib	1, 1–2, 2, 3	15	EGFR
3	Brivanib	1, 2, 3	6	FGFR, VEGFR, PDGFR
4	Sunitinib	2, 3	6	VEGFR, PDGFR, KIT
5	Linifanib	2, 3	2	VEGF, PDGFR
6	Cediranib	1, 2	3	VEGFR
7	BIBF1120	2	2	VEGFR, PDGFR, FGFR
8	Dasatinib	2	2	BCR-ABL
9	Gefitinib	2	2	EGFR
10	Lapatinib	2	2	EGFR, HER2/neu
11	Pazopanib	2	2	VEGFR, PDGFR, KIT
12	Alvocidib	1, 2	2	Cyclin-dependent kinase
13	Regorafenib	2	1	VEGFR, TIE-2
14	Lenvatinib	1–2	1	VEGFR, FGFR, SCFR
15	Vandetanib	2	1	EGFR, VEGFR, RET
16	Orantinib	1–2	1	VEGFR, FGFR, PDGFR
Selective TKI				
17	OSI-906	2	1	IGF-1R, IR
MEK Inhibitors				
18	AZD6244/Selumetinib	1–2, 2	4	MEK inhibitor
C Met inhibitors				
19	ARQ197	1, 2	2	MET
20	Foretinib	1	1	MET
mTOR inhibitors				
21	Temsirolimus	1, 2	3	MTORC1
22	Everolimus	1, 1–2, 2, 3	7	MTORC1
23	Rapamycin/ Sirolimus	1, 2–3, 3	5	MTORC1
24	AZD8055	1–2	1	MTORC1

Sorafenib is considered the prototype of TKI available for HCC due to the results of the the SHARP trial, and is considered the standard of care against which other targeted therapies should be evaluated and tested. Currently, there are now more than 84 registered clinical trials testing sorafenib for HCC alone or in combination with other modalities and agents in numerous phases. For more information please visit ClinicalTrials.gov at the following website: (http://www.clinicaltrials.gov/ct2/results?term=%22sorafenib%22+AND+%22hepatocellular+carcinoma%22&recr=Open&rslt=Without&type=Intr&show_flds=Y, Accessed August 2011).

As a potential adjuvant treatment option to prevent recurrence and prolong disease free survival, The Sorafenib as Adjuvant Treatment in the Prevention of Recurrence of Hepatocellular Carcinoma (STORM) RCT has been established. It is an international study that is evaluating effects of sorafenib versus placebo after resection or local ablation. The primary end-point of this RCT is recurrence-free survival [56]. Recently, although in the absence of solid evidence, panels of experts

Table 2 Monoclonal antibody

#	Name	Phases	Trials	Targets
1	Bevacizumab	1, 1–2, 2	20	VEGF
2	Ramucirumab	2,3	2	VEGFR2
3	Cetuximab	1, 2	3	EGFR
4	Cixutumumab	1, 2	3	IGF-1R
5	GC33	1	2	GPC3
6	Licartin (Metuximab)	2, 4	2	HAb18G/CD147
7	AVE1642	1, 2	1	IGF-1R
8	BIIB022	1–2	1	IGF-1R
9	CS-1008 (Tigatuzumab)	2	1	TRAIL2
10	CT-011	1–2	1	PD-1 (Programmed Death-1)
11	IMC-1121B	2	1	VEGFR2
12	IMC-A12	2	1	IGF-1R
13	Mapatumumab	1–2	1	TRAIL 1
14	Tremelimumab	2	1	B7-CD28

Table 3 Enzyme inhibitors

#	Name	Phases	Trials	Targets
1	PI-88	2,3	2	Endo-β-D-glucuronidase heparinase
2	Bortezomib	1,2	4	Proteasome
3	Belinostat	1–2	1	Histone deacetylase
4	IDN-6,556	2	1	Caspase
5	LBH589	1	1	Histone deacetylase
6	Lonafarnib	2	1	Farnesyl-OH-transferase
7	Panobinostat	1	1	Histone deacetylase
8	Resminostat	2	1	Histone deacetylase
9	Vorinostat	1	1	Histone deacetylase
10	Talabostat	1	1	Dipeptidyl peptidases
11	MLN8237	2	1	Aurora kinase

Table 4 Miscellaneous

#	Name	Phases	Trials	Targets
1	TAC-101	1–2,2	4	RAR-α
2	56 Z-208	1–2	1	RAR
3	AEG35156	1–2	1	XIAP mRNA
4	AMG386	2	1	Angiopoietin
5	Ispinesib	2	1	Kinesin spindel protein
6	LY2181308	1–2	1	Survivin
7	Oblimersen	2	1	BCL2

proposed shifting therapy to sorafenib in patients in intermediate stage if they showed poor tolerance or disease progression after the first or second TACE treatment [58], and suggested sorafenib therapy, even if patients previously treated showed disease progression during treatment [59].

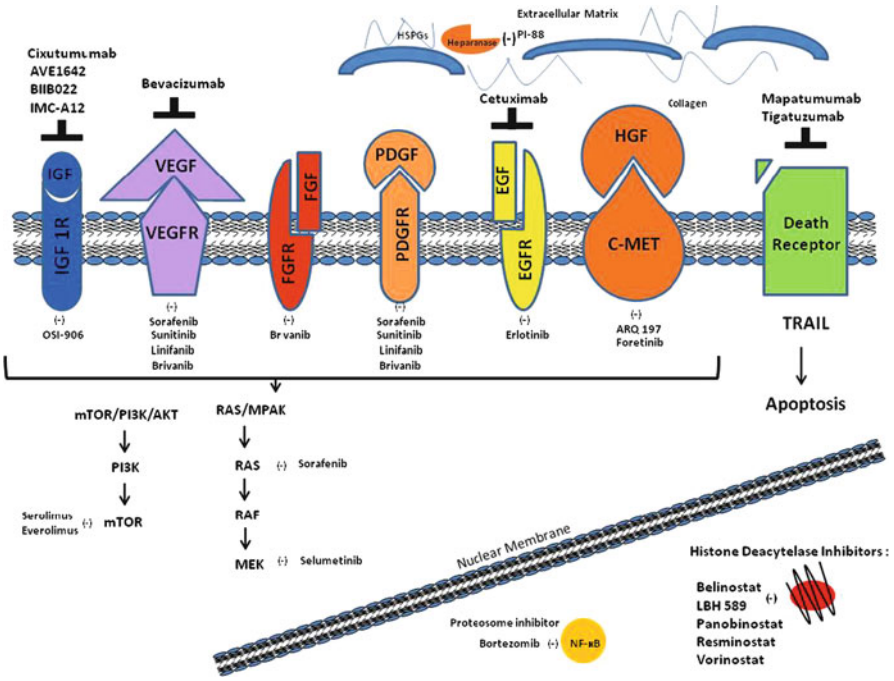

Fig. 2 Targeted therapy in HCC

Sorafenib is a non specific TKI that blocks different signaling pathways, including those that regulate cell proliferation through the RAS–MAPK pathway and angiogenesis through the VEGFR and PDGFR pathways (Fig. 2). It is therefore a challenge to determine which specific effects of sorafenib slow HCC progression [40]. In a phase 2 trial of sorafenib, patients with tumors that demonstrated high levels of phosphorylated ERK, a downstream molecule of RAS, had a significantly greater rate of survival than those with low levels of phosphorylated ERK [60]. However, these findings were not validated in the phase 3 trial. In the SHARP study there was a non-significant trend in the association between response to sorafenib and levels of the kinase v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (KIT) and hepatocyte growth factor, but there were no strong biomarkers of response [40].

Currently there are more than 16 non-specific TKIs, which are similar to sorafenib, being tested for the treatment of HCC (Table 1). Most of these TKIs target cellular proliferation and/or angiogenesis by acting on multiple receptors (e.g. BRAF, VEGFR, PDGFR, EGFR, FGFR, SCFR). OSI-906 (Table 1) is the first orally active and selective TKI of the insulin-like growth factor-1 receptor (IGF-1R), it is currently being evaluated in phase 2 trial [56].

Erlotinib (a TKI of EGFR) is the second most commonly studied non-selective TKIs in HCC after sorafenib. In two single-arm studies of erlotinib in unresectable HCC [61, 62] median survival times were 13 and 6.2 months, with response rates of less than 10%. A phase 3 trial of erlotinib in combination with sorafenib versus sorafenib alone in advanced HCC is currently ongoing [56].

Sunitinib is another oral tyrosine kinase inhibitor that blocks several receptors, including VEGFR1, VEGFR2, VEGFR3, PDGFRa, PDGFRb, and stem cell factor receptor (KIT) (Fig. 2). Its clinical spectrum overlaps that of sorafenib. After two phase 2 trials [63, 64] which analyzed efficacy and tolerability of sunitinib in advanced HCC, a phase 3 trial comparing sunitinib and sorafenib was conducted and it was recently discontinued because of the high incidence of serious adverse effects in the sunitinib treated group (5–10% of patients died of treatment-related causes) [56].

Linifanib (ABT-869) is an oral agent that acts as an inhibitor of VEGF and PDGF tyrosine kinase receptors, it was recently evaluated in a phase 2 trial on Child–Pugh A or B cirrhotic patients, the reported median time to progression and progression-free survival was 112 days with a median overall survival of 295 days [65]. Preliminary pharmacokinetics analysis in Child Pugh A and B patients showed that degree of hepatic impairment do not influence linifanib pharmacokinetics [66]. Its safety profile was acceptable and therefore a new phase 3 study comparing linifanib with sorafenib in patients with advanced HCC is ongoing [56].

Brivanib is another non specific TKIs against FGFR, VEGFR and PDGFR was studied as a first-line therapy for advanced HC, the median survival time was 10 months [67]. The safety profile of the drug was manageable, and it is now under evaluation in phase 3 trials as a second line therapy in patients who failed sorafenib [56]. Lapatinib, a TKI of EGFR and Her2, had marginal efficacy in patients with HCC—their median survival time was 6.3 months [68]; patients who developed a rash, an effect attributable to EGFR blockade, had longer survival times.

MEK inhibitor (AZD6244/Selumetinib) (Table 1) is another selective group of TKIs. MEK is a key protein kinase in the RAS/RAF/MEK/ERK pathway, which signals for cancer cell proliferation and survival. MEK also regulates the biosynthesis of the inflammatory cytokines TNF, IL-6 and IL-1, which can act as growth and survival factors in cancer. Preclinical data show that MEK inhibitors are additive or synergistic in combination with other agents [69]. In a phase 2 trial of selumetinib in advanced HCC with no prior systemic therapy [70], patients had no measurable radiographic response with short time for disease progression (8 weeks), the study was stopped at the interim analysis. Currently there is a phase 2 trial testing AZD6244 in combination with sorafenib for advanced HCC [56].

Another promising group of TKIs are the **c-MET inhibitors**; c-MET is a unique member of the RTK family, c-Met encodes the high-affinity receptor for hepatocyte growth factor (HGF) or scatter factor (SF). c-Met and HGF are each required for normal mammalian development and have been shown to be particularly important in cell migration, morphogenic differentiation, and organization of three-dimensional tubular structures as well as cell growth and angiogenesis. Both c-Met and HGF have been shown to be deregulated in HCC and to correlate with poor prognosis and metastasis [71]. In phase 1 study ARQ 197 [72], a selective inhibitor of c-Met in patients with advanced HCC reached the recommended phase 2 dose, ARQ 197 appears well tolerated in this patient population and there were no observed drug-related worsening of liver function. Currently ARQ 197 is tested in a phase 2 study for patients with HCC who failed sorafenib or other systemic therapy. Two additional phase 1 trials are

looking at ARQ 197 in combination with sorafenib for advanced HCC and as a single agent in cirrhotics with HCC [56]. Another c-Met inhibitor, foretinib, is being studied in a phase 1 trial treating patients with advanced HCC [73] with a daily dose of 30 mg. The early promising activity observed in this phase I trial, as determined by excellent radiologic response, needs to be confirmed in a phase 2 trial.

Mamalian Target Of Rapamycin Inhibitors (mTOR Inhibitors) are non selective TKIs (Table 1). mTOR, also known as mechanistic target of rapamycin or FK506 binding protein 12-Rapamycin associated protein 1 (FRAP1), is encoded by the FRAP1 gene. mTOR is a serine/threonine protein kinase that regulates cell growth, cell proliferation, cell motility, and cell survival that belongs to the phosphatidylinositol 3-kinase-related kinase (PI3K) protein family [74]. The mTOR pathway is activated in 40–50% of patients with HCC [75]. Currently, there are four mTOR inhibitors (Table 1) (temsirolimus, everolimus, rapamycin (sirolimus), and AZD8055) that are under active investigation in multiple clinical trials [56].

The prototype mTOR inhibitor, rapamycin, has immunosuppressive properties and is used in solid organ transplantation. Rapamycin recently has been demonstrated to have anti-tumor properties via inhibition of mTOR signaling pathway in HCC cell lines and after liver transplantation for HCC [76, 77]. In a retrospective study of 73 patients who underwent liver transplantation for HCC outside the Milan criteria, patients who received rapamycin had better survival than those who were given tacrolimus-based immunosuppression, This is thought to be the result of fewer tumor recurrences in the rapamycin-treated group [78]. In a small pilot study looking at rapamycin as a primary therapy in 21 patients with unresectable HCC [79], one patient had a partial response and five patients had stable disease at 3 months. This pilot study indicates that rapamycin might be a promising drug for HCC, but further clinical studies needed. Currently, there are more than five clinical trials looking at rapamycin for HCC in different phases as a single therapy or in combination with other agents [56].

Everolimus is another mTOR inhibitor that has been shown to have activity against HCC in xenografts [80]. It has also been studied in conjunction with sorafenib in a mouse model for HCC with promising early results [81]. In a recently published single arm phase1/2 trial for patients with advanced HCC who failed prior systemic therapy, everolimus was well tolerated. Although the study did not proceed to the second stage of phase 2, 4% of patient had a partial response, and the median PFS and overall survival were 3.8 months and 8.4 months respectively [82]. This suggestion of an antitumor activity for everolimus in patients with advanced HCC is being actively evaluated in multiple ongoing trials [56].

Monoclonal Antibodies (mABs)

The second type of targeted molecular therapy commonly used in HCC are monoclonal antibodies (mAbs). mAbs usually are directed against specific cell surface receptors or growth factors that are involved in the carcinogenesis.

mAbs are large molecules that cannot cross the blood-brain barrier and require intravenous administration, their large size makes them unable to pass through the cell membrane, which limits their potential targets to cell surface receptors and/ or secreted molecules. Their half-life is longer than that of a TKI, which allows for less frequent dosing. TKIs are much smaller molecules and can easily cross cell membranes; they are less specific than mAb which may result in increased toxicities [83, 84]. Currently, there are more than 14 mABs under investigation for HCC in variable phases (Table 2) [56] that have an individually specific molecular target that mediates essential cellular processes in carcinogenesis.

Bevacizumab is a recombinant humanized monoclonal antibody against VEGF-A. It is the second-most frequently tested targeted therapy in patients with HCC after sorafenib. It is approved for the treatment of several malignancies in the Unites States, including non–small cell lung cancer, breast cancer, renal cell carcinoma, and colon cancer. By selectively binding to VEGF-A, bevacizumab inhibits angiogenesis, a key process in hepatic carcinogenesis [85]. Bevacizumab has been studied as a single agent in the treatment of HCC and in combination with standard chemotherapeutic agents. In a phase 2 study of 46 patients with compensated liver disease (Child-Pugh A or B) and unresectable HCC, the 6-month progression- free survival was achieved in 65% of the patients, and 13% of the patients experienced a partial response to treatment based on RECIST criteria (a 30% decrease in the sum of the longest diameters of the lesions) [86]. This response rate compares favorably to that seen in the SHARP trial in which only 2–3% of patients had objective radiological responses to sorafenib therapy. Patients with greater than 50% involvement of the liver parenchyma by a tumor, invasion of the main portal vein or vena cava, or extrahepatic disease were excluded. Serious bleeding complications were reported in 11% of patients, with one fatal variceal bleed. This resulted in modification of the protocol with early upper GI endoscopy and banding of any obvious esophageal varices to avoid such complication [86].

Similar to other bevacizumab-treatment protocols in other malignancies, bevacizumab, as a targeted agent, was used in combination with standard chemotherapeutic agents in patients with HCC. In a phase 2 trial of 33 patients with advanced HCC, 49% with extrahepatic disease, treatment with bevacizumab, gemcitabine, and oxaliplatin (GEMOX) resulted in an objective response rate of 20%, and stable disease in 27% of patients. The overall median survival time was 9.6 months [87]. A similar partial response rate of 20%, with overall disease control rate of 77.5%, was achieved in a recently published phase 2 trial of bevacizumab in combination with capecitabine and oxaliplatin in patients with advanced unresectable and untransplantable HCC [88]. Similar to bevacizumab, ramucirumab and IMC-1121B are new mAbs against VEGFR2 and they are currently being investigated in phase 2 trials for advanced HCC [56].

Cetuximab is a chimeric monoclonal antibody targeting the EGFR. EGFR is overexpressed in 40–70% of HCCs [89]. Cetuximab and other EGFR inhibitors have been shown to prevent the development of HCC in animal models [90]. In a phase 2 study that evaluated 30 patients with unresectable HCC treated with cetuximab alone, there was no objective response seen. The median overall survival was 9.6 months and progression-free survival was 1.4 months. Treatment was well tolerated, but no significant

antitumor activity was seen [91]. Cetuximab in combination with gemcitabine and oxaliplatin (GEMOX) was evaluated in 45 patients with advanced HCC. There were nine partial responses that lasted 2–14 months. Stable disease was observed in 40%, with an overall disease control rate of 60%. Progression-free survival and overall survival were 4.7 and 9.5 months, respectively [92]. It is unclear at this time whether the addition of cetuximab offers additional benefit over the GEMOX combination alone.

IGF1R (Insulin-like growth factor 1 receptor) is over expressed in 30–40% of patients with HCC. IGF1R plays an essential role in stimulating cellular growth and proliferation [93]. Currently, there are 4 mAbs against IGF1R (cixutumumab, AVE1642, BIIB022 and IMC-A12) under active investigation in patients with HCC in phase 1 and 2 trials (Table 3) [56].

mABs also target apoptosis pathways by activating the tumor necrosis factor–related apoptosis-inducing ligand (TRAIL). TRAIL induces apoptosis by binding to two death receptors (death receptor 4 and death receptor 5). Mapatumumab (TRM-1, HGS-ETR1) is a fully humanized agonistic mAB that targets and activates TRAIL receptor 1 (death receptor 4). Promising preclinical activity with mapatumumab has been observed. Currently, it is being investigated in phase 1 and 2 trials [56]. CS-1,008 (tigatuzumab), is a mAb against TRAIL receptor 2 (death receptor 5) being investigated in phase 2 trial for patients with HCC [56].

Enzyme Inhibitors

Enzymes play essential roles in the carcinogenesis process, as they regulate and catalyze multiple key steps. Recently, there has been an increasing interest to target these enzymes by specific inhibitors to add another level of blockade in the pathway of carcinogenesis (e.g. angiogenesis, cell proliferation).

Angiogenesis is a key process not only in hepatocarcinogenesis, but it is also linked to a higher incidence of metastases. Tumor extracellular matrix is composed of heparan sulfate proteoglycans (HSPGs), collagen, laminin and fibronectin. These extracellular elements help bind growth factors and aid in cell signaling that are necessary for tumor proliferation and angiogenesis. Heparanase is an endoglycosidase that contributes to angiogenesis by cleaving HSPG resulting in the loss of basement membrane integrity, and release of heparan sulfate bound angiogenic and growth-promoting factors that enhance cell invasion, migration, intravasation and extravasation. [94, 95]. HCCs are highly vascularized and depend on angiogenic factors for development. Targeting the heparanase enzyme provides another method by which the angiogenesis pathway may be inhibited. There are a few molecules that have been found to block such enzymes and are being developed as potential therapies [95–97] (Table 3). PI-88 is a reproducible mixture of highly sulfonated oligosaccharides. It is derived from the yeast Pichia (Hansenula) holstii NRRL Y-2,488 and is a powerful inhibitor of heparanase. PI-88 seems to have antitumour activity by inhibiting heparanase and also competing with heparan sulfate to bind VEGF and FGF [94, 96]. In a phase 2 trial it was well tolerated, and the most common adverse effects reported were neutropenia, thrombocytopenia, injection site

hemorrhage, PT prolongation, muscle spasm, and alopecia [95, 97]. Currently phase 2/3 trials are underway to further investigate PI-88 in HCC [56].

Nuclear factor-κB (NF-κB) controls multiple transcription process essential for hepatocarcinogenesis. Nuclear factor-κB (NF-κB) has been proposed as a major target of proteosome inhibitors. Bortezomib, a proteosome inhibitor, is a peptide that is approved for relapsed multiple myeloma and mantle cell lymphoma. Bortezomib binds and sequesters NF-κB preventing its transcriptional activity, which is essential for hepoatocarcinogenesis. Currently, a phase 2 trial has been completed and a phase 3 trial is underway [98]. Bcl-2 and p53 are also proposed targets of proteasome inhibitors (Table 3).

Histone deacetylase plays an important role in gene function. It can modulate expression of genes and thus influence production of proteins important in cell proliferation, signaling and angiogenesis. Deregulated histone deacetylases have been commonly associated with many kinds of cancers. Several drugs that act as inhibitors of histone deacetylase are currently under investigation; these include belinostat, LBH 589, panobinostat, resminostat and vorinostat [99].

Miscellaneous Agents

Retinoids and vitamin A have been shown to be necessary for development, maintenance, and overall survival of normal tissues. Patients with diets deficient in vitamin A have been seen to develop metaplasia of the eyes, intestines, respiratory and genitourinary tract. Retinoic Acid Receptor (RAR) is a ligand-induced transcriptional regulator. RARs are located in the nucleus and are of two types: RARα and RXR (retinoid X receptors) [100]. TAC101 (4-[3,5-*bis* (trimethylsilyl) benzamide] benzoic acid) is a synthetic retinoid and is able to bind RARα. TAC101 binds RAR, inhibits transcriptional activity related to carcinogenesis, and retards the growth of liver tumors after direct splenic or portal vein injection of tumor cell lines [101, 102]. Another drug that is being tested that works via inhibition of RAR is 56 Z-208. These RAR inhibitors are currently under investigation in phase 1 and 2 trials (Table 4).

AEG35156 is a 9-mer phosphorothioate antisense oligonucleotide caspase inhibitor that acts by targeting the X-linked inhibitor of apoptosis protein (XIAP) messenger RNA. XIAP prevents apoptosis induced by viral infection or production of caspases. By blocking XIAP function, the cells are more susceptible to cell death. [103, 104] AMG386 is an angiopoietin antagonist peptide-Fc fusion protein (peptibody) that selectively inhibits angiopoietin-1 and angiopoietin-2 binding to the Tie2 tyrosine kinase receptor (Table 4) [105].

Combination Therapy and Future Directions

Standard chemotherapeutic regimens currently in use for the treatment of malignant diseases typically use combinations of agents with different mechanisms of action. Combination therapies may result in synergistic action against cancer and

decrease the development of multidrug resistance. This may also allow for reduced doses that lessen toxicity. This same concept can be utilized in the new era of targeted therapy; targeted agents can be utilized in combination with each other, standard chemotherapy, or other therapeutic modalities (radiation therapy, and TACE) in the treatment of HCC. In a phase 2 study of sorafenib with doxorubicin versus doxorubicin alone in patients with advanced HCC with no prior systemic therapy, the median time to progression and median overall survival was significantly longer in the combination group (6.4 months vs 2.8 months, and 13.7 months vs 6.5 months, respectively). The degree to which this improvement may represent synergism between sorafenib and doxorubicin remains to be defined [106]. In a phase II study of 33 patients, treatment with bevacizumab and gemcitabine/oxaliplatin (GEMOX) resulted in an objective response rate of 20%, and stable disease in 27% of patients. The overall median survival time was 9.6 months [87]. A similar partial response rate of 20%, with overall disease control rate of 77.5%, was achieved in a recently published phase 2 trial of bevacizumab in combination with capecitabine and oxaliplatin in patients with advanced unresectable and untransplantable HCC [88].

The Sorafenib or Placebo in Combination with TACE in Hepatocellular carcinoma (SPACE) trial assesses the combination of sorafenib and TACE in patients with intermediate HCC. The aim of the study is to reduce risk of local relapse after this procedure [56]. Multiple other studies combining sorafenib with radiation therapy, including the use of yttrium-90 spheres, are ongoing [56].

Targeted therapies can be combined to block the same pathway at different levels to create a vertical blockade. This can lead to complete blockade of the molecular pathway, decrease resistance patterns to the targeted therapy, and block feedback loops within the pathway. Although such combinations may be more effective, greater overlapping toxicities may occur [107]. In a phase 1 study using sorafenib and bevacizumab in patients with solid tumors (ovarian cancer and renal cell carcinoma), Azad et al. showed a partial response rate of 59%; unfortunately, toxicities included hand-foot syndrome (76%) and hypertension (67%). Both toxicities resulted in dose modifications of both drugs [108]. For HCC this combination is currently under investigation in a NCI sponsored phase 1/2 trial as a first line therapy for patients with locally advanced or metastatic HCC [56].

A more interesting concept is to combine two agents that block two different pathways. These horizontal blockades may be more effective with a decrease in overlapping toxicities [109]. In a phase 2 study of erlotinib (a TKI against EGFR) and bevacizumab (an inhibitory mAb of VEGFR) used in combination in the treatment of advanced HCC, Thomas et al. showed a progression free survival at 16 weeks of 62.5%. Median overall survival was 15.65 months, with 10–20% grade 3 to 4 drug toxicities [110]. Because of the significant toxicities associated with the vertical blockade, most ongoing studies utilize the concept of horizontal blockade combining sorafenib with other available targeted therapies in a simultaneous or sequential fashion [56].

Cancer Stem Cells and New Targets in HCC

Over the last decade Cancer Stem Cells (CSCs) have gained increasing attention in its role in the evolution of carcinogenesis. In hepatocarcinogenesis, CSCs are thought to be at least partially responsible for the high incidence of recurrence after potentially curative local liver-directed therapy (resection, ablation, etc.). This is thought to be due to their resistance to standard chemotherapy and ability for self-renewal, proliferation, and production of heterogeneous cell lines [111]. CSCs in HCC can be identified by several cell surface antigens including CD133, CD90, CD44, OV6, and CD326 (EpCAM) [112]. Expression of CSCs specific surface antigens in HCC has been linked to overall worse outcomes, for example increased CD133 expression in HCC were correlated with advanced disease stage, shorter overall survival, and higher recurrence rates compared with patients with low CD133 expression [113]. CSCs in HCC have multiple unique and well preserved signaling pathways (i.e. Wnt/β-catenin signaling pathway, TGF-β family, Notch pathway, Hedgehog signaling, BMI1 signaling pathway, Stem cell signaling network, miRNA and Lin28 and let-7 signaling) that are different from common signaling pathways known in carcinogenesis [113]. Careful analysis and understanding of CSCs markers and signaling pathways has opened the door for a new era in targeted therapy for HCC (i.e. Anti-Self-renewal, Anti-CD133, Anti-CD44, Anti-EpCAM, and Anti-CD13). Several of these potential therapies are currently under active investigation in phase 1 and 2 clinical trials as a monotherapy or in combination with other targeted therapies [113].

Conclusion

Unresectable HCC presently has one proven systemic therapy and many other promising agents are under investigation. These agents are the result of an improved understanding of HCC pathogenesis at the molecular level. Multiple gene signatures have been identified and can be used to better classify HCC and predict clinical outcome and response to therapy. Additional work is required to determine the key genetic events involved in hepatocarcinogenesis. Due to the complex nature of the disease and the multiple molecular pathways involved, combination of targeted therapies appears to be the most logical approach delivered either simultaneously or sequentially. Patients who are candidates for systemic therapy should be evaluated by a multidisciplinary team with up to date knowledge of all available clinical trials.

References

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2009. CA Cancer J Clin. 2009;59:225–49.
2. El-Serag HB. Hepatocellular carcinoma: recent trends in the United States. Gastroenterology. 2004;127:S27–34.

3. Fattovich G, Stroffolini T, Zagni I, et al. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology. 2004;127:S35–50.
4. Sangiovanni A, Del Ninno E, Fasani P, et al. Increased survival of cirrhotic patients with a hepatocellular carcinoma detected during surveillance. Gastroenterology. 2004;126:1005–14.
5. Bugianesi E. Non-alcoholic steatohepatitis and cancer. Clin Liver Dis. 2007;11(1):191–207.
6. Liaw YF, Sung JJ, Chow WC, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med. 2004;351:1521–31.
7. Chang MH, Chen CJ, Lai MS, et al. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. N Engl J Med. 1997;336:1855–9.
8. Sherman M, Bruix J. Management of hepatocellular carcinoma: an update. Hepatology. 2011;53:1020–2.
9. Davila JA, Morgan RO, Richardson PA, et al. Use of surveillance for hepatocellular carcinoma among patients with cirrhosis in the United States. Hepatology. 2010;52:132–41.
10. El Serag HB, Siegel AB, Davila JA, Shaib YH, Cayton-Woody M, McBride R, et al. Treatment and outcomes of treating of hepatocellular carcinoma among Medicare recipients in the United States: a population- based study. J Hepatol. 2006;44:158–66.
11. Thomas MB, Zhu AX. Hepatocellular carcinoma: the need for progress. J Clin Oncol. 2005;23(13):2892–9.
12. Cabibbo G, Enea M, Attanasio M, Bruix J, Craxì A, Cammà C. A meta-analysis of survival rates of untreated patients in randomized clinical trials of hepatocellular carcinoma. Hepatology. 2010;51(4):1274–83.
13. Parkin DM, Bray FI, Devesa SS. Cancer burden in the year 2000. The global picture. Eur J Cancer. 2001;37(Suppl 8):S4–66.
14. International Working Party. Terminology of nodular hepatocellular lesions. Hepatology. 1995;22:983–93.
15. International Consensus Group for Hepatocellular Neoplasia. The International Consensus Group for Hepatocellular Neoplasia. Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. Hepatology. 2009;49:658–64.
16. Fernandez M, Semela D, Bruix J, et al. Angiogenesis in liver disease. J Hepatol. 2009;50:604–20.
17. Kaposi-Novak P, Libbrecht L, Woo HG, et al. Central role of c- Myc during malignant conversion in human hepatocarcinogenesis. Cancer Res. 2009;69:2775–82.
18. Zucman-Rossi J. Molecular classification of hepatocellular carcinoma. Dig Liver Dis. 2010;42(Suppl 3):S235–41.
19. Woo HG, Park ES, Thorgeirsson S, Kim YJ. Exploring genomic profiles of hepatocellular carcinoma. Mol Carcinog. 2011;50(4):235–43. doi:doi: 10.1002/mc.20691.
20. Iizuka N, Oka M, Yamada-Okabe H, et al. Oligonucleotide microarray for prediction of early intrahepatic recurrence of hepatocellular carcinoma after curative resection. Lancet. 2003;361:923–9.
21. Lee JS, Chu IS, Heo J, et al. Classification and prediction of survival in hepatocellular carcinoma by gene expression profiling. Hepatology. 2004;40:667–76.
22. Wang SM, Ooi LL, Hui KM. Identification and validation of a novel gene signature associated with the recurrence of human hepatocellular carcinoma. Clin Cancer Res. 2007;13:6275–83.
23. Woo HG, Park ES, Cheon JH, et al. Gene expression-based recurrence prediction of hepatitis B virus-related human hepatocellular carcinoma. Clin Cancer Res. 2008;14:2056–64.
24. Hoshida Y, Villanueva A, Kobayashi M, et al. Gene expression in fixed tissues and outcome in hepatocellular carcinoma. N Engl J Med. 2008;359:1995–2004.
25. Hernandez-Vargas H, Lambert MP, Le Calvez-Kelm F, et al. Hepatocellular carcinoma displays distinct DNA methylation signatures with potential as clinical predictors. PLoS One. 2010;5:e9749.
26. Budhu A, Jia HL, Forgues M, et al. Identification of metastasis-related microRNAs in hepatocellular carcinoma. Hepatology. 2008;47:897–907.

27. Gehrau R, Mas V, Archer KJ, Maluf D. Molecular classification and clonal differentiation of hepatocellular carcinoma: the step forward for patient selection for liver transplantation. Exp Rev Gastroenterol Hepatol. 2011;5(4):539–52.
28. Budhu A, Forgues M, Ye QH, et al. Prediction of venous metastases, recurrence, and prognosis in hepatocellular carcinoma based on a unique immune response signature of the liver microenvironment. Cancer Cell. 2006;10:99–111.
29. Okamoto M, Utsunomiya T, Wakiyama S, et al. Specific geneexpression profiles of noncancerous liver tissue predict the risk for multicentric occurrence of hepatocellular carcinoma in hepatitis C virus-positive patients. Ann Surg Oncol. 2006;13:947–54.
30. Villanueva A, Minguez B, Forner A, et al. Hepatocellular carcinoma: novel molecular approaches for diagnosis, prognosis, and therapy. Annu Rev Med. 2010;61:317–28.
31. Villanueva A, Hoshida Y, Battiston C, et al. Combining clinical, pathology, and gene expression data to predict recurrence in hepatocellular carcinoma. Gastroenterology. 2011;140(5):1501–12.
32. Ura S, Honda M, Yamashita T, et al. Differential microRNA expression between hepatitis B and hepatitis C leading disease progression to hepatocellular carcinoma. Hepatology. 2009;49:1098–112.
33. Wurmbach E, Chen YB, Khitrov G, et al. Genome-wide molecular profiles of HCV-induced dysplasia and hepatocellular carcinoma. Hepatology. 2007;45:938–47.
34. Nam SW, Park JY, Ramasamy A, et al. Molecular changes from dysplastic nodule to hepatocellular carcinoma through gene expression profiling. Hepatology. 2005;42:809–18.
35. Katoh H, Ojima H, Kokubu A, et al. Genetically distinct and clinically relevant classification of hepatocellular carcinoma: putative therapeutic targets. Gastroenterology. 2007;133:1475–86.
36. Van't Veer LJ, Bernards R. Enabling personalized cancer medicine through analysis of geneexpression patterns. Nature. 2008;452:564–70.
37. Hoshida Y, Toffanin S, Lachenmayer A, et al. Molecular classification and novel targets in hepatocellular carcinoma: recent advancements. Semin Liver Dis. 2010;30:35–51.
38. Tovar V, Alsinet C, Villanueva A, et al. IGF activation in a molecular subclass of hepatocellular carcinoma and pre-clinical efficacy of IGF-1R blockage. J Hepatol. 2010;52:550–9.
39. Villanueva A, Chiang DY, Newell P. Pivotal role of mTOR signaling in hepatocellular carcinoma. Gastroenterology. 2008;135:1972–83. 1983 e1-11.
40. Villanueva A, Llovet JM. Targeted therapies for hepatocellular carcinoma. Gastroenterology. 2011;140(5):1410–26. Epub 2011 Mar 13.
41. Lee JS, Chu IS, Mikaelyan A, et al. Application of comparative functional genomics to identify best-fit mouse models to study human cancer. Nat Genet. 2004;36:1306–11.
42. Minguez B, Tovar V, Chiang D, Villanueva A, Llovet JM. Pathogenesis of hepatocellular carcinoma and molecular therapies. Curr Opin Gastroenterol. 2009;25:186–94.
43. Hayashi PH, Di Bisceglie AM. The progression of hepatitis B- and C-infections to chronic liver disease and hepatocellular carcinoma: epidemiology and pathogenesis. Med Clin North Am. 2005;89(2):371–8.
44. Seeger C, Mason WS. Hepatitis B virus biology. Microbiol Mol Biol Rev. 2000;64(1):51–68.
45. Yuen MF, Wu PC, Lai VC, Lau JY, Lai CL. Expression of c-Myc, c-Fos, and c-jun in hepatocellular carcinoma. Cancer. 2001;911:106–12.
46. Chirillo P, Pagano S, Natoli G, Puri PL, Burgio VL, Balsano C, Levrero M. The hepatitis B virus X gene induces p53-mediated programmed cell death. Proc Natl Acad Sci USA. 1997;94:8162–7.
47. Tellinghuisen R. Interaction between hepatitis C virus proteins and host cell factors. Curr Opin Microbiol. 2002;5(4):419–27.
48. Kiyosawa K, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y, Purcell RH, Alter HJ. Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. Hepatology. 1990;12:671–5.
49. Giambartolomei S, Covone F, Levrero M, Balsano C. Sustained activation of the Raf/MEK/Erk pathway in response to EGF in s cell lines expressing the Hepatitis C Virus HCV core protein. Oncogene. 2001;20:2606–10.
50. Marra M, Sordelli IM, Lombardi A, Lamberti M. Molecular targets and oxidative stress biomarkers in hepatocellular carcinoma: an overview. J Transl Med. 2011;9:171.

51. Druker BJ, Guilhot F, O'Brien SG, Gathmann I, Kantarjian H. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Engl J Med. 2006;355(23):2408–17.
52. Demetri GD, von Mehren M, Blanke CD, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med. 2002;347:472–80.
53. Rutkowski P, Van Glabbeke M, Rankin CJ, et al. European Organisation for Research and Treatment of Cancer Soft Tissue/Bone Sarcoma Group, Southwest Oncology Group. Imatinib mesylate in advanced dermatofibrosarcoma protuberans: pooled analysis of two phase II clinical trials. J Clin Oncol. 2010;28(10):1772.
54. Llovet JM, Ricci S, Mazzaferro V, et al. SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008;359(4):378–90.
55. Cheng AL, Kang YK, Chen Z, 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.
56. www.clinicaltrials.gov.
57. Llovet JM, Di Bisceglie AM, Bruix J, et al. Design and endpoints of clinical trials in hepatocellular carcinoma. J Natl Cancer Inst. 2008;100:698–711.
58. Piscaglia F, Bolondi L. The intermediate hepatocellular carcinoma stage: should treatment be expanded? Dig Liver Dis. 2010;42(Suppl 3):S258–63.
59. Peck-Radosavljevic M, Greten TF, Lammer J, et al. Consensus on the current use of sorafenib for the treatment of hepatocellular carcinoma. Eur J Gastroenterol Hepatol. 2010;22(4):391–8.
60. Abou-Alfa GK, Schwartz L, Ricci S, et al. Phase II study of sorafenib in patients with advanced hepatocellular carcinoma. J Clin Oncol. 2006;24:4293–300.
61. Philip PA, Mahoney MR, Allmer C, et al. Phase II study of Erlotinib (OSI-774) in patients with advanced Hepatocellular cancer. J Clin Oncol. 2005;23:6657–63.
62. Thomas MB, Chadha R, Glover K, et al. Phase 2 study of erlotinib in patients with unresectable hepatocellular carcinoma. Cancer. 2007;110:1059–67.
63. Faivre S, Raymond E, Boucher E, et al. Safety and efficacy of sunitinib in patients with advanced hepatocellular carcinoma: an open-label, multicentre, phase II study. Lancet Oncol. 2009;10(8):794–800.
64. Zhu AX, Sahani DV, Duda DG, et al. Efficacy, safety, and potential biomarkers of sunitinib monotherapy in advanced hepatocellular carcinoma: a phase II study. J Clin Oncol. 2009;27(18):3027–35.
65. Toh H, Chen P, Carr BI, et al. A phase II study of ABT-869 in Hepatocellular carcinoma (HCC): interim analysis. J Clin Oncol. 2009;27:222s. abstr 4581.
66. Gupta N, Chiu Y, Toh HC, et al. Preliminary pharmacokinetics and safety comparison of Child Pugh A vs. Child Pugh B patients enrolled in a phase 2 study of Linifanib (ABT-869) in hepatocellular carcinoma [abstract]. 2010 ESMO Congress, Milan, Italy, Abstract number 3105.
67. Park JW, Finn RS, Kim JS, et al. Phase II, open-label study of brivanib as first-line therapy in patients with advanced Hepatocellular carcinoma. Clin Cancer Res. 2011;17:1973–83.
68. Ramanathan RK, Belani CP, Singh DA, et al. A phase II study of lapatinib in patients with advanced biliary tree and hepatocellular cancer. Cancer Chemother Pharmacol. 2009;64:777–83.
69. Trujillo JI. MEK inhibitors: a patent review 2008–2010. Expert Opin Ther Pat. 2011; 21(7):1045–69. Epub 2011 May 9.
70. O'Neil BH, Goff LW, Kauh JS, Strosberg JR, et al. Phase II study of the mitogen-activated protein kinase 1/2 inhibitor selumetinib in patients with advanced hepatocellular carcinoma. J Clin Oncol. 2011;29(17):2350–6. Epub 2011 Apr 25.
71. You H, Ding W, Dang H, Jiang Y, Rountree CB. c- Met represents a potential therapeutic target for personalized treatment in hepatocellular carcinoma. Hepatology. 2011;54(3):879–89. doi:doi: 10.1002/hep.24450 [Epub ahead of print].
72. A Santoro, M. Simonelli, P. Zucali, L. Rimassa, N. N. Senzer, L. H. Camacho, L. Bolondi, M. Lamar, G. Abbadessa, J. A phase Ib safety trial of ARQ 197 in cirrhotic patients with hepatocellular carcinoma (HCC). 2010 Gastrointestinal Cancers Symposium, Orlando, FL, Abstract 187.
73. T. Yau, C.-J. Yen, P.-J. Chen, Y. Chau, R. Lencioni, H. Kallender, L.H. Ottesen, R.T. A phase I/II study of Foretinib, a oral Multikinase Inhibitor targeting MET in advanced

HCC The International Liver Congress, 2011 (European Association for the study of the Liver Disease 2011) Berlin, Germany, 30 Mar – 3 Apr 2011.
74. Treiber G. mTOR inhibitors for hepatocellular cancer: a forward-moving target. Expert Rev Anticancer Ther. 2009;9(2):247–61.
75. Villanueva A, Chiang DY, Newell P, et al. Pivotal role of mTOR signaling in hepatocellular carcinoma. Gastroenterology. 2008;135(6):1972–83. 1983.e1-11.
76. Heuer M, Benko T, Cicinnati VR, Kaiser GM, Sotiropoulos GC, Baba HA, et al. Effect of low-dose rapamycin on tumor growth in two human hepatocellular cancer cell lines. Transplant Proc. 2009;41:359–65.
77. Toso C, Meeberg GA, Bigam DL, Oberholzer J, Shapiro AM, Gutfreund K, et al. De novo sirolimus-based immunosuppression after liver transplantation for hepatocellular carcinoma: long-term outcomes and side effects. Transplantation. 2007;83:1162–8.
78. Zhou J, Wang Z, Wu ZQ, Qiu SJ, Yu Y, Huang XW, et al. Sirolimusbased immunosuppression therapy in liver transplantation for patients with hepatocellular carcinoma exceeding the Milan criteria. Transplant Proc. 2008;40:3548–53.
79. Rizell M, Andersson M, Cahlin C, Hafstrom L, Olausson M, Lindner P. Effects of the mTOR inhibitor sirolimus in patients with hepatocellular and cholangiocellular cancer. Int J Clin Oncol. 2008;13:66–70.
80. Huynh H, Chow KP, Soo KC, Toh HC, Choo SP, Foo KF, et al. RAD001 (everolimus) inhibits tumor growth in xenograft models of human hepatocellular carcinoma. J Cell Mol Med. 2009;13:1371–80.
81. Huynh H, Ngo VC, Koong HN, Poon D, Choo SP, Thng CH, et al. Sorafenib and rapamycin induce growth suppression in mouse models of hepatocellular carcinoma. J Cell Mol Med. 2009;13:2673–83.
82. Zhu AX, Abrams TA, Miksad R, Blaszkowsky LS, et al. Phase 1/2 study of everolimus in advanced hepatocellular carcinoma. Cancer. 2011 Apr 27. doi: 10.1002/cncr.26165. [Epub ahead of print].
83. Reichert JM, Rosensweig CJ, Faden LB, et al. Monoclonal antibody successes in the clinic. Nat Biotechnol. 2005;23:1073–8.
84. Dancey J, Sausville EA. Issues and progress with protein kinase inhibitors for cancer treatment. Nat Rev Drug Discov. 2003;2:296–313.
85. Willett CG, Boucher Y, di Tomaso E, Duda DG, Munn LL, Tong RT, et al. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. Nat Med. 2004;10:145–7.
86. Siegel AB, Cohen EI, Ocean A, et al. Phase II trial evaluating the clinical and biologic effects of bevacizumab in unresectable hepatocellular carcinoma. J Clin Oncol. 2008;26(18):2992–8.
87. Zhu AX, Blaszkowsky LS, Ryan DP, Clark JW, Muzikansky A, Horgan K, et al. Phase II study of gemcitabine and oxaliplatin in combination with bevacizumab in patients with advanced hepatocellular carcinoma. J Clin Oncol. 2006;24:1898–903.
88. Sun W, Sohal D, Haller DG, Mykulowycz K, Rosen M, et al. Phase 2 trial of bevacizumab, capecitabine, and oxaliplatin in treatment of advanced hepatocellular carcinoma. Cancer. 2011;117(14):3187–92. doi:doi: 10.1002/cncr.25889. Epub 2011 Jan 24.
89. Buckley AF, Burgart LJ, Sahai V, Kakar S. Epidermal growth factor receptor expression and gene copy number in conventional hepatocellular carcinoma. Am J Clin Pathol. 2008;129:245–51.
90. Schiffer E, Housset C, Cacheux W, Wendum D, Desbois-Mouthon C, Rey C, et al. Gefitinib, an EGFR inhibitor, prevents hepatocellular carcinoma development in the rat liver with cirrhosis. Hepatology. 2005;41:307–14.
91. Zhu AX, Stuart K, Blaszkowsky LS, et al. Phase 2 study of Cetuximab in patients with advanced hepatocellular carcinoma. Cancer. 2007;110(3):581–9.
92. Asnacios A, Fartoux L, Romano O, et al. Gemcitabine plus oxaliplatin (GEMOX) combined with cetuximab in patients with progressive advanced stage hepatocellular carcinoma: results of a multicenter phase 2 study. Cancer. 2008;112(12):2733–9.
93. Hopfner M, Huether A, Sutter AP, Baradari V, Schuppan D, Scherubl H. Blockade of IGF-1 receptor tyrosine kinase has antineoplastic effects in hepatocellular carcinoma cells. Biochem Pharmacol. 2006;71:1435–48.

94. Turnbull J, Powell A, Guimond S. Heparan sulfate: decoding a dynamic multifunctional cell regulator. Trends Cell Biol. 2001;11:75–82.
95. Miao HQ, Liu H, Navarro E, Kussie P, Zhu Z. Development of heparanase inhibitors for anticancer therapy. Curr Med Chem. 2006;13:2101–11.
96. McKenzie EA. Heparanase: a target for drug discovery in cancer and inflammation. Br J Pharmacol. 2007;151:1–14.
97. Liu CJ, Lee PH, Lin DY, et al. Heparanase inhibitor PI-88 as adjuvant therapy for hepatocellular carcinoma after curative resection: a randomized phase II trial for safety and optimal dosage. J Hepatol. 2009;50(5):958–68. Epub 2009 Feb 15.
98. Spratlin JL, Pitts TM, Kulikowski GN, et al. Synergistic activity of histone deacetylase and proteasome inhibition against pancreatic and hepatocellular cancer cell lines. Anticancer Res. 2011;31(4):1093–103.
99. Lemoine M, Younes A. Histone deacetylase inhibitors in the treatment of lymphoma. Discov Med. 2010;10(54):462–70.
100. Chambon P. The retinoid signaling pathway: molecular and genetic analyses. Semin Cell Biol. 1994;5:115–25.
101. Murakami K, Wierzba K, Sano M, et al. TAC-101, a benzoic acid derivative, inhibits liver metastasis of human gastrointestinal cancer and prolongs the life-span. Clin Exp Metastasis. 1998;16:323–31.
102. Shibata J, Murakami K, Wierzba K, et al. Anticancer effect of 4-[3,5-bis (trimethylsilyl) benzamido] benzoic acid (TAC-101) against A549 non–small cell lung cancer cell line is related to its anti-invasive activity. Anticancer Res. 2000;20:3169–76.
103. Duckett CS, Li F, Wang Y, Tomaselli KJ, Thompson CB, Armstrong RC. Human IAP-like protein regulates programmed cell death downstream of Bcl-xL and cytochrome c. Mol Cell Biol. 1998;18(1):608–15.
104. Deveraux QL, Reed JC. IAP family proteins—suppressors of apoptosis. Genes Dev. 1999;13(3):239–52.
105. Herbst RS, Hong D, Chap L, Kurzrock R, Jackson E, et al. Safety, pharmacokinetics, andantitumor activity of AMG 386, a selective angiopoietin inhibitor, in adult patients with advanced solid tumors. J Clin Oncol. 2009;27(21):3557–65.
106. Abou-Alfa GK, Johnson P, Knox JJ, et al. Doxorubicin plus sorafenib vs doxorubicin alone in patients with advanced hepatocellular carcinoma: a randomized trial. JAMA. 2010;304(19):2154–60.
107. Azad NS, Aragon-Ching JB, Dahut WL, et al. Hand-foot skin reaction increases with cumulative sorafenib dose and with combination anti-vascular endothelial growth factor therapy. Clin Cancer Res. 2009;15(4):1411–6.
108. Azad NS, Posadas EM, Kwitkowski VE, Steinberg SM, Jain L, Annunziata CM, et al. Combination targeted therapy with sorafenib and bevacizumab results in enhanced toxicity and antitumor activity. J Clin Oncol. 2008;26:3709–14.
109. Camp ER, Summy J, Bauer TW, Liu W, Gallick GE, Ellis LM. Molecular mechanisms of resistance to therapies targeting the epidermal growth factor receptor. Clin Cancer Res. 2005;11:397–405.
110. Thomas MB, Morris JS, Chadha R, et al. Phase II trial of the combination of bevacizumab and erlotinib in patients who have advanced hepatocellular carcinoma. J Clin Oncol. 2009;27(6):843–50. Epub 2009 Jan 12.
111. Russo FP, Parola M. Stem and progenitor cells in liver regeneration and repair. Cytotherapy. 2011;13:135–44.
112. Oishi N, Wang XW. Novel therapeutic strategies for targeting liver cancer stem cells. Int J Biol Sci. 2011;7(5):517–35.
113. Song W, Li H, Tao K, et al. Expression and clinical significance of the stem cell marker CD133 in hepatocellular carcinoma. Int J Clin Pract. 2008;62:1212–8.

Toward the Goal of Personalized Therapy in Pancreatic Cancer by Targeting the Molecular Phenotype

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Abstract The purpose of this article is to provide a critical review of the molecular alterations in pancreatic cancer that are clinically investigated as therapeutic targets and their potential impact on clinical outcomes. Adenocarcinoma of exocrine pancreas is generally associated with poor prognosis and the conventional therapies are marginally effective. Advances in understanding the genetic regulation of normal and neoplastic development of pancreas have led to development and clinical evaluation of new therapeutic strategies that target the signaling pathways and molecular alterations in pancreatic cancer. Applications have begun to utilize the genetic targets as biomarkers for prediction of therapeutic responses and selection of treatment options. The goal of accomplishing personalized tumor-specific therapy with tolerable side effects for patients with pancreatic cancer is hopefully within reach in the foreseeable future.

Keywords Pancreatic cancer • Genetic targets • Biomarkers • Personalized therapy

Introduction

Pancreatic adenocarcinoma, the most common form of cancer in the exocrine pancreas, has posed a serious health threat and remained a great challenge in medicine. In the United States, the incidence and mortality rate of pancreatic cancer have been rising. This disease is the fourth most common cause of cancer-related death in

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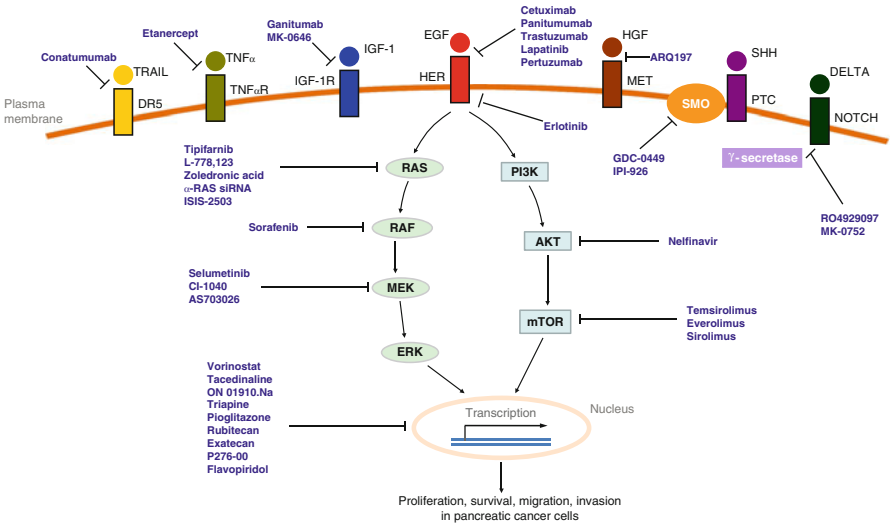

Fig. 1 A schematic diagram to illustrate the genetic targets for therapeutics in pancreatic adenocarcinoma. *DR5*, death receptor 5; *EGF*, epidermal growth factor; *HER*, human epidermal growth factor receptor; *HGF*, hepatocyte growth factor; *HH*, hedgehog; *IGF-1R*, insulin growth factor-1 receptor; *PTC*, patched; *SMO*, smoothened; *TNFαR*, tumor necrosis factor α receptor; *TRAIL*, tumor necrosis factor apoptosis-inducing ligand

both genders, and it is associated with the lowest 5-year relative survival rate (6%) [1]. In the minority of patients whose pancreatic adenocarcinoma is incidentally discovered at an early-localized stage, they can be potentially cured by surgical resection of the tumor. Patients with pancreatic adenocarcinoma often develop non-specific symptoms such as nausea, abdominal pain, and weight loss. In patients whose tumor is located in the head of pancreas, they typically present with painless jaundice, acholic stool, and dark urine. These signs are related to biliary obstruction by tumor, which is often locally advanced at the time of diagnosis. When the primary tumor is located in the body or tail of pancreas, the patients may remain asymptomatic or complain of pain, and the tumor has typically progressed and metastasized when diagnosed. Current therapy for locally advanced pancreatic adenocarcinoma generally involves chemotherapy including gemcitabine, 5-fluorouracil, or capecitabine administered concurrently with radiation therapy. For advanced or metastatic pancreatic adenocarcinoma, palliative systemic chemotherapy includes gemcitabine-based combination with capecitabine, erlotinib, docetaxel, or *nab*-paclitaxel, and also FOLFIRINOX (5-fluorouracil, leucovorin, oxaliplatin, and irinotecan). However, these treatment modalities are generally palliative and marginally beneficial for prolonging survival. Even if the pancreatic tumors initially respond to treatment, they will eventually become resistant to the conventionally used agents [2].

Accumulating evidence indicates that therapeutic targeting of the genetic abnormalities in malignant neoplasia produces improvement of treatment response in a

variety of human cancers. Many of the genetic alterations during malignant transformation of pancreatic epithelia and multi-step progression of the pre-malignant precursors into invasive adenocarcinoma have been identified [3]. Animal studies have progressively revealed the molecular mechanisms underlying the interactions among various oncogenes and tumor suppressor genes in pancreatic tumorigenesis [4]. Isolation of the pancreatic cancer stem cells and their treatment with agents that target the developmental regulators suggests the potential of this approach to eradicate these cells that are typically chemoresistant [5]. The importance of targeting the pancreatic tumor stroma has been demonstrated as a critical component in improving therapy [6]. In this article, the genetic alterations involved in the pathogenesis of pancreatic adenocarcinoma are reviewed. The clinical investigations of therapeutic agents directed at the genetic targets as well as the emerging utility of genetic biomarkers as therapeutic indicators in pancreatic adenocarcinoma are discussed. Special emphasis is placed on the impact of the molecularly targeted agents on treatment response and how future studies can be improved in hopes for attaining the goal of personalized therapy in pancreatic cancer.

Genetic Alterations Involved in Pathogenesis of Pancreatic Adenocarcinoma

In the multistage pancreatic carcinogenesis, a number of genetic abnormalities that accompany the histological changes in pancreatic adenocarcinoma and its precursor lesions (pancreatic intra-epithelial neoplasia or PanINs) have been identified and characterized [7–10]. During the early steps of malignant transformation of pancreatic epithelia, activating mutations in *K-RAS* and shortening of telomeres occur, and expression of epidermal growth factor and its receptor are up-regulated. Loss-of-function mutations in the tumor suppressor genes including $p16^{CDKN2A}$, *p53*, *DPC4*, *BRCA2*, and *STK11*/*LKB1* are also present. Aberrantly expressed developmental regulators including *Hedgehog*, *Notch*, and *WNT* are involved. Multiple genetic studies in animal models of pancreatic organogenesis and cancer have generated insights into the molecular basis of the pathogenic mechanisms underlying the initiation, development, and progression of pancreatic neoplasia.

A number of those genetic alterations have been clinically investigated as therapeutic targets in patients with pancreatic adenocarcinoma and they can be classified in the major groups as listed in Table 1. These molecules have been clinically investigated as therapeutic targets in patients with pancreatic adenocarcinoma by using chemical inhibitors and antibodies. Some of these clinical trials have been completed and some are ongoing. In the following sections, each group of genetic targets will be described, the clinical trials that investigate the therapeutic efficacy of targeting the genetic abnormalities mentioned, and the results of the completed clinical studies discussed.

Table 1 Selected genetic targets for therapeutics in human pancreatic adenocarcinoma

Groups of genetic targets	Genetic targets
Receptor tyrosine kinases and ligands	Epidermal growth factor (EGF)/EGF receptor (EGFR)
	Human epidermal growth factor receptor 2 (HER2)
	Insulin-like growth factor-1 (IGF-1)/ IGF-1receptor (IGF-1R)
	Hepatocyte growth factor (HGF)/HGFR (MET)
Signal transducers	RAS
	MEK
	AKT
	Mammalian target of rapamycin (mTOR)
	Hsp90
Nuclear targets	Histone deacetylases (HDACs)
	Polo-like kinase 1 (Plk1)
	Ribonucleotide reductase (RR)
	Peroxisome proliferator-activator receptor γ (PPARγ)
	DNA topoisomerase I
	Cyclin-dependent kinases (CDKs)
Death signals	Tumor necrosis factor α (TNFα)
	Tumor necrosis factor-related apoptosis inducing ligand (TRAIL) receptor 2 (TR-2)
Developmental pathways	Hedgehog
	Notch
Stromal microenvironment	Secreted protein acidic and rich in cysteine (SPARC)
	Matrix metalloproteinases (MMPs)
	Cyclooxygenase-2 (COX-2)
	Vascular endothelial growth factor (VEGF)/ VEGF receptor (VEGFR)
	Chemokine receptor 2 (CCR2)
	Integrin α5β1
Antigens and Immunodulatory signals	Mesothelin
	5T4
	ASG-5
	Carcinoembryonic antigen (CEA)
	Prostate stem cell antigen (PSCA)
	Telomerase
	CD40
	Cytotoxic T-lymphocyte associated antigen-4 (CTLA-4)
	Programmed death-1 receptor (PD-1)
	Dipeptidyl peptidase IV (DPP-IV)

Genetic Alterations as Therapeutic Targets

Receptor Tyrosine Kinases and Ligands

Trans-plasma membrane receptors with tyrosine kinase activities and their ligands play crucial roles in the proliferation, differentiation, migration of pancreatic epithelia during development. These include epidermal growth factor, fibroblast growth

factor, insulin-like growth factor-1, hepatocyte growth factor, and their receptors. Serine/threonine kinases such as transforming growth factor β and receptor are also involved. Over-expression and/or abnormal activation of the receptor tyrosine kinases and/or their ligands can contribute to pathogenesis of pancreatic neoplasia. Preclinical studies indicate that targeted inhibition of these receptors and ligands can impede pancreatic tumor growth, and many clinical trials have been developed to validate these findings for therapies in patients with pancreatic adenocarcinoma.

Epidermal Growth Factor (EGF)

The EGF receptor (EGFR) family of protein tyrosine kinases include HER (human EGFR or ERBB-1), HER2 (ERBB-2), HER-3 (ERBB-3), and HER-4 (ERBB-4) [11]. The ligands of the EGFR family including EGF itself are mitogens that control proliferation and differentiation of epidermal and mesenchymal cells [12, 13]. Immunohistochemical analysis indicates that EGFR and EGF are over-expressed in most of the pancreatic cancer specimens [14–16]. Results of these studies suggest an opportunity to impede pancreatic tumor growth by disrupting the autocrine loop of mitogenic signaling. Therapeutic agents that target the HERs have been developed including antibodies and small molecules as follows:

Cetuximab and panitumumab are monoclonal antibodies that prevent EGF-induced receptor activation and dimerization by binding to the extracellular domain and trans-membrane domain of EGFR, respectively. Cetuximab and panitumumab have been shown to inhibit EGF-induced proliferation of tumor cells. Clinical studies suggest that cetuximab in combination with cytotoxic drugs does not provide additional survival benefits in advanced pancreatic adenocarcinoma [17–19] (Table 2). However, a number of clinical trials have been launched to evaluate the efficacy of cetuximab and panitumumab either alone or as combination therapies in various stages of pancreatic adenocarcinoma (Table 2).

Erlotinib is an oral small molecule that inhibits the tyrosine kinase activity of EGFR by competing with adenosine triphosphate for the binding site of the intracellular catalytic domain of EGFR. This results in reversible inhibition of EGFR-mediated signal transduction and the associated cancer-promoting effects. In a phase II trial, the combination of erlotinib with gemcitabine provides a small but statistically significant survival benefit over gemcitabine alone [20] (Table 2). Since then, a large number of clinical trials have been conducted to evaluate the combination of erlotinib with cytotoxic drugs, other targeted agents, or radiation in localized and advanced pancreatic adenocarcinoma (Table 2).

Trastuzumab and pertuzumab are monoclonal antibodies that bind to HER2 and induce antibody-dependent cell-mediated cytotoxicity against the HER2-overexpressing cancer cells. Lapatinib is a small molecule inhibitor that reversibly inhibits the kinase activity of HER1 and HER2 and thus their phosphorylation, and also phosphorylation of AKT, ERK-1, and ERK-2. Clinical trials are ongoing to investigate if these targeted agents show any therapeutic efficacy in pancreatic adenocarcinoma at various stages (Table 2).

Table 2 Targeting receptor tyrosine kinases in pancreatic adenocarcinoma

Genetic targets	Targeted agents	Pancreatic adenocarcinoma	Clinical trial	Comments	Clinical Trials.gov Identifier	Ref.
EGFR	Cetuximab	Locally advanced or metastatic	Phase II	In combination with gemcitabine as first-line therapy. Median overall survival 7.1 months. Median progression-free survival 3.8 months.	–	[17]
EGFR	Cetuximab	Locally advanced or metastatic	Phase II	Gemcitabine and cisplatin +/− cetuximab as first-line therapy. Median overall survival 3.4 months (cetuximab-treated) versus 4.2 months (non-cetuximab treated), P=0.739. Median progression-free survival 7.5 months (cetuximab-treated) versus 7.8 months (Non-cetuximab treated), $P=0.847$	NCT00536614	[18]
EGFR	Cetuximab	Locally advanced unresectable or metastatic	Phase III	In combination with gemcitabine versus gemcitabine alone as first-line therapy. Median survival 6.3 months (cetuximab + gemcitabine) versus 5.9 months (gemcitabine), $P=0.23$	NCT00075686	[19]
EGFR	Cetuximab	Metastatic	Phase II	In combination with bevacizumab +/− gemcitabine as first-line therapy. Terminated prematurely due to lack of efficacy in both arms.	NCT00326911	–
EGFR	Cetuximab	Stage I, II, III	Phase II	In combination with gemcitabine and radiotherapy as first-line therapy. Ongoing but not recruiting.	NCT00225784	–
EGFR	Cetuximab	Locally advanced, unresectable, non-metastatic	Phase II	Cetuximab + gemcitabine + oxaliplatin as neoadjuvant therapy, followed by either surgery or capecitabine + EBRT. Recruitment status unknown.	NCT00408564	–
EGFR	Cetuximab	Locally advanced, inoperable, non-metastatic	Phase II	Cetuximab and 3-dimensional conformal IGRT. Completed.	NCT00599833	–
EGFR	Cetuximab	Locally advanced, metastatic, or recurrent	Phase II	In combination with gemcitabine as first-line therapy. Completed.	NCT00005591	–

EGFR	Cetuximab	Locally advanced, unresectable	Phase I	In combination with gemcitabine and radiation therapy. Completed.	NCT00467116	–
EGFR	Cetuximab	Resected (R0 or R1)	Phase II	In combination with gemcitabine as adjuvant therapy. Ongoing but not recruiting.	NCT00395252	–
EGFR	Cetuximab	Resected	Phase II	As adjuvant therapy, either cetuximab or bevacizumab, in combination with gemcitabine, capecitabine and radiotherapy. Recruitment status unknown.	NCT00305877	–
EGFR, HER2	Cetuximab, Trastuzumab	Metastatic	Phase II	As first-line therapy. In combination with Gemcitabine. Currently recruiting.	NCT01204732	–
EGFR	Panitumumab	Locally advanced unresectable or metastatic	Phase II	In combination with gemcitabine as first-line therapy. Terminated because overall survival not improved.	NCT00613730	–
EGFR	Panitumumab	Locally advanced unresectable	Phase II	Panitumumab fluorouracil infusion + EBRT followed by panitumumab gemcitabine, then panitumumab. Ongoing but not recruiting.	NCT00601627	–
EGFR	Erlotinib	Metastatic	Phase II	In combination with gemcitabine v s gemcitabine alone as first-line therapy. Median overall survival 6.24 months (erlotinib + gemcitabine versus 5.91 months (gemcitabine), $P=0.038$	–	[20]
EGFR	Erlotinib	Metastatic	Phase II	In combination with gemcitabine with or without panitumumab as first-line therapy. Completed.	NCT00550836	–
EGFR	Erlotinib	Resectable	Phase II	In combination with gemcitabine as neoadjuvant therapy. Currently recruiting.	NCT01389440	–
EGFR	Erlotinib	Resectable	Phase II	In combination with gemcitabine +/− EBRT as pre-operative therapy. Ongoing but not recruiting.	NCT00766636	–
EGFR	Erlotinib	Resected Stage I/II	Phase II	In combination with capecitabine and EBRT; then in combination with gemcitabine as adjuvant therapy. Currently recruiting.	NCT00962520	–

(continued)

Table 2 (continued)

Genetic targets	Targeted agents	Pancreatic adenocarcinoma	Clinical trial	Comments	Clinical Trials.gov Identifier	Ref.
EGFR	Erlotinib	Metastatic	Phase II	In combination with capecitabine.as first-line therapy. Completed.	NCT00873353	–
EGFR	Erlotinib	Metastatic	Phase II	In combination with gemcitabine and sorafenib as first-line therapy. Ongoing but not recruiting.	NCT00696696	–
EGFR	Erlotinib	Operable	Phase II	In combination with gemcitabine as neoadjuvant therapy and adjuvant therapy. Currently recruiting.	NCT00733746	–
EGFR	Erlotinib	Locally advanced unresectable or metastatic	Phase I	In combination with gemcitabine and capecitabine as first-line therapy. Ongoing but not recruiting.	NCT00480584	–
EGFR	Erlotinib	Locally advanced or metastatic	Phase II	In combination with selumetinib as second-line therapy in tumor refractory to gemcitabine. Currently recruiting.	NCT01222689	–
EGFR	Erlotinib	Resectable or locally advanced	Phase I/II	In combination with bevacizumab and radiation as first-line therapy. Ongoing but not recruiting.	NCT00735306	–
EGFR	Erlotinib	Locally advanced of metastatic	Phase II	In combination with gemcitabine and bevacizumab as first-line therapy. Completed	NCT00366457	–
EGFR	Erlotinib	Unresectable or metastatic	Phase I	In combination with gemcitabine, oxaliplatin, and EBRT as first-line therapy. Ongoing but not recruiting.	NCT00266097	–
EGFR	Erlotinib	Locally advanced or metastatic	Phase I/II	In combination with gemcitabine, bevacizumab, and capecitabine as first-line therapy. Ongoing but not recruiting.	NCT00260364	–
EGFR	Erlotinib	Unresectable	Phase II	In combination with sorafenib as first-line therapy. Ongoing but not recruiting.	NCT00837876	–

EGFR	Erlotinib	Advanced	Phase I	In combination with gemcitabine and capecitabine as second-line therapy following first-line chemotherapy. Recruitment status unknown.	NCT00885066	–
EGFR	Erlotinib	Locally advanced or metastatic	Phase II	In combination with genistein and gemcitabine as first-line therapy. Completed.	NCT00376948	–
EGFR	Erlotinib	Resectable	Phase I	In combination with gemcitabine and hypofractionated radiation as neoadjuvant therapy. Recruitment status unknown.	NCT00243854	–
EGFR	Erlotinib	Locally advanced, unresectable	Phase I	In combination with gemcitabine and radiation therapy, followed by maintenance therapy including erlotinib and gemcitabine. Completed.	NCT00063947	–
EGFR	Erlotinib	Stage I, II	Phase II	Erlotinib as neoadjuvant therapy. Following surgical resection, erlotinib + capecitabine + gemcitabine + radiation as adjuvant therapy. Completed.	NCT00313560	–
EGFR	Erlotinib	Metastatic	Phase I	In combination with GDC-0449 with or without gemcitabine. Recruitment status unknown.	NCT00878163	–
EGFR	Erlotinib	Advanced	Phase I	In combination with gemcitabine and oxaliplatin as first-line therapy. Currently recruiting.	NCT00987766	–
EGFR	Erlotinib	Locally advanced, unresectable, or metastatic	Phase I	In combination with gemcitabine and Nab-paclitaxel as first-line therapy. Currently recruiting.	NCT01010945	–
HER2	Trastuzumab	Regionally confined resectable or unresectable	Phase II	In combination with gemcitabine and EBRT as first-line therapy. Completed.	NCT00005926	–
HER1/ HER2	Lapatinib	Metastatic	Phase II	In combination with capecitabine as first-line therapy. Recruitment status unknown.	NCT00962312	–
HER2 **EGFR**	Trastuzumab Cetuximab	Metastatic	Phase I/II	Combination of cetuximab and trastuzumab as second-line therapy in patients with disease progression after previous gemcitabine-based treatment. Recruitment status unknown.	NCT00923299	–

(continued)

Table 2 (continued)

Genetic targets	Targeted agents	Pancreatic adenocarcinoma	Clinical trial	Comments	Clinical Trials.gov Identifier	Ref.
HER2, EGFR	Pertuzumab, Erlotinib	Locally advanced or metastatic	Phase II	As second-line therapy following tumor progression through gemcitabine. Terminated because of extreme toxicity.	NCT01108458	–
IGF-1R	Ganitumab (AMG-479)	Metastatic	Phase III	In combination with gemcitabine.as first-line therapy. Currently recruiting.	NCT01231347	–
IGF-1R	Ganitumab (AMG-479)	Metastatic	Phase Ib/II	In combination with gemcitabine as first-line therapy. Ongoing but not recruiting.	NCT00630552	–
IGF-1R	Ganitumab (AMG-479)	Locally advanced	Phase II	In combination with gemcitabine as first-line therapy. Not yet open for recruitment.	NCT01318642	–
IGF-1R	Ganitumab (AMG 479)	Locally advanced	Phase I	Ganitumumab + gemcitabine as induction therapy, followed by ganitumumab + capecitabine + 3-dimensional CRT, then ganitumumab gemcitabine as maintenance therapy. Not yet open for recruitment.	NCT01298401	–
IGF-1R	MK-0646	Metastatic	Phase I/II	In combination with gemcitabine and with or without erlotinib as first-line therapy. Currently recruiting.	NCT00769483	–
MET	ARQ 197	Locally advanced unresectable or metastatic	Phase II	Versus gemcitabine as first-line therapy. Completed.	NCT00558207	–

EGF epidermal growth factor, *IGF-1R* insulin-like growth factor 1 receptor, *MET* hepatocyte growth factor receptor, *EBRT* external beam radiation therapy, *IGRT* image guided radiotherapy, *CRT* conformal radiation therapy

Insulin-Like Growth Factor-1 (IGF-1)

IGF-1 receptor (IGF-1R) is a tyrosine kinase that belongs to the family of insulin receptor, and activation of IGF-1R stimulates cellular proliferation and suppresses apoptosis. Ganitumab (AMG-479) and MK-0646 are monoclonal antibodies that bind to IGF-1R, block its binding with IGF-1, and prevent the subsequent activation of PI3K/AKT signaling. Ganitumab inhibits pancreatic cancer cell growth and survival and produces additive growth inhibitory effects when combined with gemcitabine [21]. Clinical trials using ganitumab or MK-0646 as combination therapies are ongoing to evaluate if blocking IGF-1R provides any additional benefit in advanced pancreatic adenocarcinoma (Table 2).

Hepatocyte Growth Factor (HGF)

The proto-oncogene c-*MET*-encoded tyrosine kinase is the receptor of hepatocyte growth factor (HGF, also called scatter factor) and over-expressed or mutated in various types of tumor cells including pancreatic adenocarcinoma [22, 23] and pancreatic cancer stem cells [24]. MET plays important roles in tumor cell proliferation, survival, invasion, and metastasis, and tumor angiogenesis. ARQ 197 is a small molecule that binds to MET and disrupts MET-mediated signal transduction pathways, inducing tumor cell death. A clinical trial to test the efficacy of ARQ 197 has been completed (Table 2).

A practical concern about the therapeutic use of EGFR inhibitor in pancreatic adenocarcinoma stems from the fact that activating mutations in *K-RAS*, a downstream effector of EGFR, are present in most of the pancreatic adenocarcinoma specimens examined. It has been shown that a prerequisite for cetuximab and panitumumab to be effective as anti-tumor agents for therapies in colon cancer is the presence of wild-type *K-RAS*. Another factor for erlotinib to produce anti-tumor effect is activating mutation in the tyrosine kinase of EGFR, as demonstrated in lung adenocarcinoma. In pancreatic adenocarcinoma, activating mutation in *K-RAS* is highly frequent whereas activating mutation in EGFR tyrosine kinase is not common. Thus, it is questionable if these antibodies or small molecules that target EGFR can be expected to produce any meaningful clinical benefits in the majority of patients with pancreatic adenocarcinoma. However, recent evidence suggests that a sub-population of pancreatic adenocarcinoma with wild-type *K-RAS* may benefit from the therapeutic use of erlotinib [25].

Signal Transducers

The growth factors including EGF-, IGF-1-, and HGF-induced signals are transduced by multiple cytoplasmic pathways that mediate the mitogenic effects on pancreatic epithelia during normal and cancerous development of exocrine pancreas.

In general, binding of these growth factor to their receptors leads to activation of RAS that plays a key role in mediating mitogen-induced signaling and functions in cellular proliferation, survival, differentiation, and migration. The RAS-mediated pathways include the RAF/MEK/ERK, PI3K/AKT/mTOR, RalGDS/RAL/PLD1, and others [26].

RAS

In most of pancreatic tumors, activating mutations in codon 12 of the transforming gene Kirsten Rous sarcoma virus (*K-RAS*) are present [27, 28]. K-RAS is an intracellular, membrane-bound protein belonging to the superfamily of small guanosine triphosphate-binding proteins (GTPases) that are capable of hydrolyzing GTP to guanosine diphosphate (GDP). For K-RAS to mediate its cellular functions, post-translational prenylation of K-RAS is necessary for association of K-RAS with plasma membrane. This process of prenylation involves addition of a farnesyl isoprenoid moiety by the enzyme farnesyltransferase (FTase). Studies in animal models indicate that K-*Ras* mutations contribute to the initiation and progression of pancreatic tumors [4]. Therapeutic strategies have been developed by interfering with farnesylation of K-RAS using chemical inhibitors or expression of RAS using anti-sense oligonucleotides [29].

Tipifarnib (also known as R115777) is a small molecule that binds to and inhibits farnesyltransferase (FTase), thereby preventing activation of RAS and the subsequent signaling and tumor growth. Tipifarnib alone is ineffective for treatment of advanced pancreatic cancer [30, 31]. The combination of Tipifarnib with gemcitabine does not provide additional survival benefit [32]. An explanation proposed for the lack of clinical efficacy observed in these studies is that the FTase inhibitor-inhibited RAS isoforms can undergo alternative geranylgeranylation catalyzed by geranylgeranyl transferase (GGTase) I. However, the dual inhibitor of FTase and GGTase, L-778,123, shows minimal efficacy when used in combination with radiation therapy for patients with locally advanced pancreatic adenocarcinoma [33]. Indeed, there is no inhibition of K-RAS prenylation detected in the peripheral blood mononuclear cells from L-778,123-treated patients [34]. Accumulating evidence indicates that zoledronic acid, a bisphosphonate with anti-resorptive property, exerts direct anti-tumor activities by blocking the enzymes involved with the synthesis of mevalonic acid including FTase and GGTase [35]. Preclinical studies indicate that zoledronic acid produces anti-tumor effects on pancreatic cancer cells [36, 37]. Currently, zoledronic acid is being tested clinically as part of a neoadjuvant therapy (Table 3).

Mutant K-RAS peptides have been used as the tumor-specific antigen to stimulate the immune response directed at pancreatic adenocarcinoma (Table 3). In the adjuvant setting, patients with resected pancreatic tumor were vaccinated intradermally with mutant K-RAS peptides in combination with granulocyte-macrophage colony-stimulating factor [38]. Peptide-specific immunity was induced in 58% patients, and the median survival is significantly prolonged in the patients who demonstrated an immune response than those who did not. A long-term follow up of those patients with resected pancreatic tumor following vaccination against mutant K-RAS suggests

Table 3 Targeting cytoplasmic signal transducers in pancreatic adenocarcinoma

Genetic targets	Targeted agents	Pancreatic adenocarcinoma	Clinical trial	Comments	ClinicalTrials.gov Identifier	Ref.
FTase	Tipifarnib	Metastatic	Phase II	As first-line therapy. No objective response. Median time to progression 4.9 weeks. Median survival 19.7 weeks.	NCT00005843	[30]
FTase	Tipifarnib	Unresectable locally advanced or metastatic	Phase II	As first-line therapy. Median survival 2.6 months, 6-month survival 19%, median time to treatment failure 1.4 month. Ongoing but not recruiting.	NCT00005832	[31]
FTase	Tipifarnib	Locally advanced unresectable or metastatic	Phase III	Gemcitabine in combination with either tipifarnib or placebo as first-line therapy. Median overall survival 193 days (Tipifarnib + gemcitabine) versus 182 days (gemcitabine), $P=0.75$	–	[32]
FTase and GGTase	L-778,123	Locally advanced	Phase I	In combination with radiotherapy. Partial response by radiological measurement in 1 of 8 patients who completed therapy.	–	[33]
FTase	Tipifarnib	Locally advanced	Phase II	In combination with paclitaxel, gemcitabine, and radiotherapy versus paclitaxel + gemcitabine + radio-therapy. Completed.	NCT00026104	–
K-RAS	Zoledronic acid	Resectable	Phase I	As neoadjuvant therapy. Currently recruiting.	NCT00892242	–
K-RAS	Mutant K-RAS peptide	Advanced	Phase I/II	20 of 34 patients showed immune response. Median survival 148 days (immune responders) versus 61 days (immune non-responders), $P=0.0002$.	–	[38]

(continued)

Table 3 (continued)

Genetic targets	Targeted agents	Pancreatic adenocarcinoma	Clinical trial	Comments	ClinicalTrials.gov Identifier	Ref.
K-RAS	Mutant K-RAS peptide	Resected	Phase I/II (10 year-follow up)	17 of 20 patients showed immune response to the vaccine. Median survival 28 months (immune responders) versus 27.5 months (all patients). 5-year survival 29% (immune responders) versus 22% (all patients). 10-year survival 20% (evaluable patients) versus 0% (non-vaccinated patients).	–	[39]
K-RAS	Mutant K-RAS peptide-DETOX™ vaccine	Resected	Phase II	As adjuvant therapy. Median disease-free survival 35.2 months. Median overall survival 44.4 months.	–	[40]
K-RAS	Mutant K-RAS peptide	Metastatic	Phase I	One of eight patients mounted a positive cellular immune response (cytotoxic T lymphocyte and interferon-γ.	–	[41]
K-RAS	Anti-K-RAS^{G12D} siRNA (siG12D LODER)	Operable	Phase 0	Using EUS biopsy needle. Currently recruiting.	NCT01188785	–
K-RAS	Anti-K-RAS^{G12D} siRNA (siG12D LODER)	Locally non-operable advanced	Phase I	Using EUS biopsy needle. Currently recruiting.	NCT01188785	–
H-RAS	ISIS-2503	Locally advanced or metastatic	Phase II	In combination with gemcitabine as first-line therapy. Unclear benefit. Median survival 6.6 months. Response rate 10.4%.	NCT00006467	[44]
H-RAS	ISIS 2503	Advanced, inoperable	Phase II	As first-line therapy. Completed.	NCT00005594	–

RAS	REOLYSIN®	Advanced or metastatic	Phase II	In combination with Gemcitabine. Currently recruiting.	NCT00998322	–
MEK	Selumetinib (AZD6244)	Advanced or metastatic	Phase II	As second-line therapy versus capecitabine in advanced or metastatic tumor pretreated with a gemcitabine-based regimen. Median survival selumetinib (5.4 months) versus capecitabine (5.0 months), $P=0.92$.	NCT00372944	[49]
MEK	Selumetinib (AZD6244)	Locally advanced or metastatic	Phase II	In combination with erlotinib as second-line therapy in tumor refractory to gemcitabine. Currently recruiting.	NCT01222689	–
MEK1 and MEK2	CI-1040	Advanced (inoperable or metastatic)	Phase II	As first-line therapy. Completed.	NCT00033384	–
MEK	AS703026	Metastatic	Phase II	In combination with gemcitabine (vs. gemcitabine alone) as first-line therapy. Currently recruiting.	NCT01016484	–
AKT	Nelfinavir	Locally advanced	Phase I	In combination with gemcitabine, cisplatin, and radiotherapy as first-line therapy. Acceptable toxicity and therapeutic efficacy.	–	[55]
AKT	Nelfinavir	Locally advanced	Phase 1	In combination with SRT, gemcitabine, fluorouracil, and leucovorin as neoadjuvant therapy. Currently recruiting.	NCT01068327	–
mTOR	Temsirolimus	Metastatic	Phase II	As first-line therapy. Completed. No objective response or disease stability.	NCT00075647	[58]

(continued)

Table 3 (continued)

Genetic targets	Targeted agents	Pancreatic adenocarcinoma	Clinical trial	Comments	ClinicalTrials.gov Identifier	Ref.
mTOR	Everolimus	Metastatic	Phase II	In combination with erlotinib as second-line therapy in patients who had prior gemcitabine-based regimen . Completed. No objective response or disease stability.	NCT00640978	[58]
mTOR	Temsirolimus	Metastatic	Phase 1	In combination with gemcitabine as first-line therapy. Currently recruiting.	NCT00593008	–
mTOR	Everolimus	Locally advanced and/or metastatic	Phase I/II	In combination with gemcitabine as first-line therapy. Ongoing but not recruiting.	NCT00560963	–
mTORC1	Sirolimus	Locally advanced or metastatic	Phase II	As second-line therapy in patients with disease progression after one prior gemcitabine-based regimen. Completed.	NCT00499486	–
HSP90	STA-9090	Metastatic	Phase II	As second-line therapy in patients that have progressed or did not tolerate one line of prior therapy. Currently recruiting.	NCT01227018	–

FTase farnesyltransferase, *GGTase* geranylgeranyltransferase, *siG12D LODER* (Local Drug EluteR) is a miniature biodegradable polymeric matrix designed to release anti K-RAS^{G12D} siRNA regionally within a pancreatic tumor (Silenseed Ltd), *mTORC1* mammalian target of rapamycin complex 1, *EUS* endoscopic ultrasound, *SRT* stereotactic radiation therapy, *HSP90* heat shock protein 90

that this adjuvant treatment deserves further evaluation [39]. In another clinical trial, subcutaneous vaccination with mutant K-RAS in patients with resected pancreatic adenocarcinoma, three of five patients produced immune response and they remain without evidence of disease; the other two patients who did not show immune response had progression of disease [40]. In patients with metastatic pancreatic adenocarcinoma, one of eight patients developed specific immune response following vaccination with mutant K-RAS peptides [41]. Taken together, vaccination with mutant K-RAS peptides supports further evaluation of its potential value as an adjuvant therapy following resection of pancreatic adenocarcinoma.

Anti-sense oligonucleotides directed against *K-RAS*^{G12D} have been developed to inhibit expression of K-RAS^{G12D} protein. Currently, there are clinical trials ongoing to evaluate the efficacy of this approach in localized tumor or in combination with gemcitabine for patients with metastatic disease (Table 3). Besides K-RAS, the isoform H-RAS has been implicated in pancreatic tumor growth [42]. ISIS-2503 is an anti-sense oligonucleotide that inhibits translation of *H-RAS* mRNA [43]. The combination of ISIS-2503 with gemcitabine in locally advanced or metastatic pancreatic adenocarcinoma shows low response rate and unclear benefits [44] (Table 3).

Respiratory Enteric Orphan virus (reovirus) is an oncolytic virus, and it is non-pathogenic in normal cells [45]. This is because viral synthesis of double-stranded RNA activates protein kinase R (PKR) that helps protect the host cells from viral infection. Tumor cells with activated RAS signaling are deficient in PKR and thus unable to elicit an anti-viral response. In reovirus-infected pancreatic cancer cells, most of which contain activated RAS, the virus will multiply and cause lysis of tumor cells [46]. This approach of using reoviral infection of pancreatic adenocarcinoma exploits the presence of oncogenic K-RAS in the tumor cells, and its potential efficacy remains to be demonstrated (Table 3).

MEK

RAF, MEK, and ERK are intracellular protein serine/threonine kinases and they act as downstream mediators of various mitogenic signals including EGF-induced signaling. Growth factor-induced or oncogenic activation of RAS leads to phosphorylation of RAF, which in turn phosphorylates MEK and subsequently ERK. ERK will then activate transcription factors and expression of target genes for cellular proliferation. Small molecule MEK inhibitors bind non-competitively to a specific binding pocket adjacent to the ATP binding site of MEK [47]. CI-1040 is an oral small molecule inhibitor of MEK and it produces partial response in a patient with pancreatic cancer [48]. The MEK inhibitor selumetinib shows similar efficacy as capecitabine in gemcitabine-pretreated pancreatic cancer [49]. Several clinical studies are ongoing to assess the anti-pancreatic tumor activity of MEK inhibitors (Table 3).

AKT

AKT, also known as protein kinase B, is a serine-threonine protein kinase and an important mediator of the phosphatidylinositol-3-kinase (PI3K) pathway. PI3Ks are

lipid kinases that transduce signaling cascades and regulate a variety of cellular processes including survival and growth [50]. Class IA PI3K can be activated through interaction with receptor tyrosine kinases such as EGFR, or through binding to active K-RAS or G-protein coupled receptors. Activated PI3K phosphorylates phosphatidylinositols in the plasma membrane, leading to recruitment and subsequent activation of AKT. In 40-70% of pancreatic adenocarcinoma specimens, expression of AKT2 protein and the phosphorylated or active form of AKT is elevated [51, 52]. In a mouse model of pancreatic adenocarcinoma, deletion of PTEN that acts as a negative regulator of AKT promotes neoplastic transformation of pancreatic epithelia [53].

Nelfinavir is an anti-human immunodeficiency virus (HIV) protease inhibitor that is also found to inhibit AKT [54]. In a phase I trial, nelfinavir was used in combination with gemcitabine, cisplatin, and radiotherapy in locally advanced pancreatic adenocarcinoma (Table 3). In six of ten patients who completed chemoradiotherapy, complete resection was possible, including one tumor with complete pathologic response. In five of ten patients who completed chemoradiotherapy, partial CT responses were observed [55]. A dose escalation study of nelfinavir mesylate in a neoadjuvant chemoradiation regimen is designed for locally advanced pancreatic adenocarcinoma (Table 3).

Mammalian Target of Rapamycin (mTOR)

The signaling molecule mTOR is a protein serine-threonine kinase and it functions as a key effector downstream of AKT [56]. The active phosphorylated form of mTOR is expressed in 55% of pancreatic adenocarcinoma specimens [57]. The clinically used inhibitors of mTOR such as temsirolimus and everolimus have been investigated in clinical trials.

Temsirolimus (CCI-779) is an analog of rapamycin, and it binds to and inhibits mTOR, thereby preventing its subsequent signaling and tumor cell growth. Temsirolimus shows anti-tumor activity in the mouse xenograft model of pancreatic cancer [57]. In a phase II trial, temsirolimus did not produce any objective response or disease stability in patients with metastatic pancreatic adenocarcinoma [58], presumably related to a negative feedback loop from inhibition of mTOR causing activation of AKT. An ongoing study is designed to determine MTD of temsirolimus in combination with gemcitabine for treatment of metastatic pancreatic adenocarcinoma (Table 3).

Everolimus is a synthetic derivative of the naturally occurring sirolimus that possesses immunosuppressive and anti-angiogenic activities. Everolimus binds to FK binding protein-12 (FKBP-12), and the resulting complex binds to and inhibits mTOR. In a phase II trial using a combination of everolimus and erlotinib, no objective response or disease stability was demonstrated in patients with metastatic pancreatic adenocarcinoma, suggesting the need for a broader targeting of the PI3K pathway [58]. A dose finding study of everolimus in combination with gemcitabine in locally advanced and/or metastatic pancreatic cancer is ongoing (Table 3).

Heat Shock Protein (Hsp90)

Hsp90 is a molecular chaperone that is up-regulated in various types of tumor cells. It plays a key role in the stability and function of proteins involved in signal transduction, cell cycle regulation, and apoptosis. STA-9090 is a small molecule that binds to and inhibits Hsp90, resulting in proteasomal degradation of kinases such as KIT, EGFR, and BCR-ABL and thus inhibition of cell proliferation. A clinical trial is ongoing to determine the therapeutic efficacy of STA-9090 in patients with metastatic pancreatic adenocarcinoma (Table 3).

Nuclear Targets

A number of molecules located in the nuclei have been utilized as genetic targets for therapies in pancreatic adenocarcinoma, and they have been investigated in clinical trials. These nuclear targets include histone deacetylases, polo-like kinase 1, ribonucleotide reductase, peroxisome proliferator-activated receptor γ, DNA topoisomerase I, and cyclin-dependent kinases. These molecules are known to play fundamental roles in various cellular functions such as nuclear division, cell cycle division, and transcription. The rationale of targeting these essential molecules lies on the abnormally high proliferative rate of cancer cells. Whether these nuclear targets are effective for improving treatment response in patients with pancreatic adenocarcinoma remains to be determined.

Histone Deacetylases (HDACs)

The acetylation status of histones is dynamically modulated by the opposing actions of histone acetyltransferases and HDACs. By catalyzing the removal of acetyl groups from lysine residues within the tails of nucleosomal histones, particularly H3 and H4, HDACs-induced hyperacetylation of nucleosomal histones results in a relaxed and accessible conformation of chromatin that modulates transcriptional activity and affects diverse cellular effects [59, 60]. Vorinostat (or suberoylanilide hydroxamic acid, SAHA) is a synthetic derivative of hydroxamic acid, and it inhibits the deacetylase activity of class I and class II HDACs by binding to the catalytic domain of HDACs and chelating the zinc ion in the catalytic pockets of HDACs. This results in hyperacetylation of histone proteins and leads to up-regulation as well as down-regulation of a variety of genes including the cyclin-dependent kinase inhibitor $p21^{CDKN1A}$. Besides, vorinostat induces hyperacetylation of non-histone proteins such as TP53 and heat-shock protein 90. These actions of vorinostat contribute to its anti-tumor effects by inducing cell cycle arrest and apoptosis in a variety of cancer cells including pancreatic adenocarcinoma [61–63]. The clinical efficacy of vorinostat has been tested by using it alone or in combination with 5-fluorouracil and radiation (Table 4). Tacedinaline is a relatively specific inhibitor of HDAC1 and HDAC2, its use in combination with gemcitabine is being evaluated (Table 4).

Table 4 Nuclear targeting in pancreatic adenocarcinoma

Genetic targets	Targeted agents	Tumor stage	Clinical trial	Comments	ClinicalTrials.gov Identifier	Reference
HDACs	Vorinostat	Locally advanced	Phase I/II	As a first-line therapy. In combination with infusional 5-fluorouracil and radiation therapy. Ongoing but not recruiting.	NCT00948688	–
HDACs	Vorinostat	Non-metastatic (resectable, borderline resectable, or un-resectable)	Phase I	Currently recruiting.	NCT00983268	–
HDAC1, HDAC2	Tacedinaline (CI-994)	Locally advanced, unresectable or metastatic	Phase II	Tacedinaline+gemcitabine versus placebo+gemcitabine as first-line therapy. Recruitment status unknown.	NCT00004861	–
Plk-1	ON 01910.Na	Metastatic	Phase II/III	In combination with gemcitabine versus gemcitabine as first-line therapy. Currently recruiting.	NCT00366457	–
RR	Triapine (3-AP)	Locally advanced or metastatic	Phase II	Early study closure due to toxicities and lack of meaningful clinical benefit.	–	[68]
RR	Triapine (3-AP)	Locally advanced, unresectable, and non-metastatic (stage III)	Phase I	In combination with radiation therapy. Recruitment status unknown.	NCT00288903	–

PPARγ	Pioglitazone	Metastatic	Phase I	As second-line therapy in patients with disease progression after gemcitabine-based treatment. Currently recruiting.	NCT00867126	–
DNA Topo-isomerase I	Rubitecan (9-nitro-camptothecin)	Locally advanced or metastatic	Phase III	Rubitecan versus most appropriate therapy in patients with recurrent or refractory tumor previously treated with chemotherapy (other than gemcitabine alone or 5-fluorouracil as a radiosensitizer). Recruitment status unknown.	NCT00005870	–
DNA Topo-isomerase I	Rubitecan (Orathecin)	Non-resectable (stage II-IV)	Phase II/III	Orathecin with or without gemcitabine as first-line therapy. Completed.	NCT00113256	–
DNA Topo-isomerase I	Exatecan (DX-8951f)	Metastatic	Phase II	Exatecan as first-line therapy. Recruitment status unknown.	NCT00003951	–
DNA Topo-isomerase I	Exatecan (DX-8951f)	Metastatic	Phase III	Exatecan with or without gemcitabine as first-line therapy. Median survival 6.7 months (Exatecan + gemcitabine) versus 6.2 months (gemcitabine), $P=0.52$	–	[73]
CDK4-cyclin D1	P276-00	Localy advanced or metastatic	Phase I/II	In combination with gemcitabine as first-line therapy. Ongoing but not recruiting.	NCT00898287	–
CDKs	Flavopiridol (Alvocidib)	Refractory metastatic	Phase II	In combination with docetaxel. Median survival 4.2 months. Minimal activity and significant toxicity.	NCT00331682	[76]
CDKs	Flavopiridol (Alvocidib)	Locally advanced, unresectable	Phase I	In combination with radiation therapy followed by gemcitabine-based regimen. Completed.	NCT00047307	–

HDACs histone deacetylases, *Plk-1* polo-like kinase 1, *RR* ribonucleotide reductase, *PPARγ* peroxisome proliferator-activated receptor γ, *CDKs* cyclin-dependent kinases

Polo-Like Kinase 1 (Plk1)

Plk1 is an important regulator of mitosis and it modulates transition through the G_2/M phases by influencing activation the CDC25C phosphatase and cyclin B1 [64]. Plk1 is over-expressed in a variety of cancers including pancreatic cancer [65, 66]. ON01910.Na is a small-molecule drug that induces mitotic arrest and apoptosis by disrupting Plk1-mediated G_2/M cell cycle transition [67]. A clinical study using ON01910.Na is ongoing to evaluate its potential efficacy in metastatic pancreatic adenocarcinoma (Table 4).

Ribonucleotide Reductase (RR)

RR catalyzes the synthesis of deoxyribonucleoside diphosphates, the nucleotide precursors for DNA synthesis, from ribonucleoside diphosphates, and it plays a central role in cell proliferation. Over-expression of RR has been related to tumorigenesis, metastasis, and drug resistance. Treatment of cancer attempts to aim at inhibition of RR by developing the agent called Triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone or 3-AP). A phase II trial using Triapine in patients with advanced pancreatic adenocarcinoma indicates severe toxicity and no meaningful clinical benefit [68]. A phase I trial using Triapine in combination with radiation therapy is open for patients with locally advanced and unresectable pancreatic tumor (Table 4).

Peroxisome Proliferator-Activated Receptor γ (PPARγ)

PPARγ is a transcription factor that belongs to the nuclear receptor superfamily of ligand-activated nuclear transcription factors [69]. PPARγ is strongly expressed in adipose tissue and it regulates adipocyte differentiation. Pioglitazone is a derivative of thiazolidinediones (TZDs), which are anti-diabetic drugs and ligands for PPARγ, and TZDs have been shown to inhibit the proliferation of various cancers including pancreatic cancer [70]. An ongoing clinical trial aims to evaluate the potential efficacy of Pioglitazone in metastatic pancreatic adenocarcinoma (Table 4).

DNA Toposisomerase I

DNA topoisomerase I functions in relaxing supercoiled DNA during replication and transcription of DNA. Camptothecin is a naturally occurring alkaloid extracted from the tree Camptotheca acuminate and it inhibits DNA topoisomerase I. It binds to topoisomerase I and stabilizes the topoisomerase I/DNA cleavable complex, inducing single-strand breaks in DNA and preventing their repair. By interfering with synthesis of DNA and RNA during cell division, camptothecin causes apoptotic death of rapidly proliferating cells and tumor cells.

Irinotecan, rubitecan (9-nitro-camptothecin), and exatecan are semi-synthetic analogues of the topoisomerase I inhibitor camptothecin. Irinotecan, when used in combination with 5-fluorouracil, leucovorin, and oxaliplatin, produces significant survival benefits and toxicity as compared to gemcitabine in patients with metastatic

pancreatic adenocarcinoma [71]. In pre-clinical studies, rubitecan shows a broad spectrum of anti-tumor activities, and in phase I/II trials, it exhibits appreciable activity against pancreatic cancer [72]. A phase II/III trial using rubitecan in non-resectable pancreatic adenocarcinoma is completed (Table 4). Results of the phase II trial using exatecan alone in metastatic pancreatic adenocarcinoma is unknown (Table 4), but in a phase III trial of patients with metastatic pancreatic adenocarcinoma, exatecan showed no significant survival benefit when used in combination with gemcitabine as compared to gemcitabine alone [73].

Cyclin-Dependent Kinases (CDKs)

CDKs form functional complexes, which promote progression of cell cycle from G_1 phase to S phase. Agents have been developed to inhibit CDK/cyclin complex and induce cell cycle arrest and apoptosis, thus inhibiting tumor cell proliferation. P276-00 is a flavone and it binds to and inhibits CDK4/cyclin D1, CDK1/cyclin D, and CDK9/cyclin T1, resulting in cell cycle arrest in G_1-S phases [74]. This in turn leads to induction of apoptosis and subsequent inhibition of tumor cell proliferation. A phase I/II trial is ongoing using P276-00 in combination with gemcitabine for locally advanced or metastatic pancreatic adenocarcinoma (Table 4).

Flavopiridol (alvocidib or HMR-1275) is a semi-synthetic alkaloid that prevents phosphorylation of CDKs and by repressing cyclin D1 and cyclin D3. These actions result in tumor cell proliferation by inducing cell cycle arrest and apoptotic cell death. Flavopiridol has been shown to potentiate the anti-tumor activities of chemotherapeutic agents [75]. In a phase II trial, the combination of flavopiridol and docetaxel produced minimal anti-pancreatic cancer activity but significant toxicity [76] (Table 4).

Death Signals

The signaling components that induce cell death have been exploited for anti-tumor effects in various tumors including pancreatic adenocarcinoma [77]. Tumor necrosis factor (TNF) is a cytokine that stimulates immune response and causes necrosis in certain types of tumor cells. The members 10A and 10B of the TNF receptor superfamily bind to another cytokine called tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and cause cancer cell death.

TNFα

Etanercept is a recombinant chimeric protein (TNFR:Fc) consisting of the extracellular ligand-binding region of TNF receptor 2 linked to the constant Fc portion of IgG1. Etanercept binds to and prevents TNFα from interacting with endogenous TNFR on the cell surface, resulting in reduced inflammation and tumor growth [78]. A clinical study to evaluate the potential efficacy of Etanercept in advanced pancreatic adenocarcinoma has been completed (Table 5).

Table 5 Targeting of death signals in pancreatic adenocarcinoma

Genetic targets	Targeted agents	Pancreatic adenocarcinoma	Clinical trial	Comments	Clinical Trials.gov Identifier	Reference
TNFα	Etanercept	Advanced	Phase I/II	In combination with gemcitabine as first-line therapy. Completed.	NCT00201838	–
TR-2 or DR5	Conatumumab (AMG 655)	Locally advanced, inoperable, non-metastatic	Phase I/II	In combination with gemcitabine as induction therapy, followed by Conatumumab + capecitabine + 3-dimensional CRT, and then Conatumumab + gemcitabine as maintenance therapy. Not yet open for recruitment.	NCT01017822	–
TR-2 or DR-5	Conatumumab (AMG 655)	Metastatic	Phase Ib/II	As first-line therapy. In combination with gemcitabine. Ongoing but not recruiting.	NCT00630552	–

TNFα tumor necrosis factorα, *TR-2* tumor necrosis factor-related apoptosis- inducing ligand (TRAIL) receptor 2, *DR5* death receptor 5, *CRT* conformal radiation therapy

TRAIL Receptor 2 (TR-2)

TR-2 (also called death receptor 5 or DR5) is a member of the TNF receptor superfamily and expressed in many types of tumor cells including pancreatic cancer [79]. Conatumumab (AMG 655) is a monoclonal agonist antibody directed against TR-2. By mimicking the activity of native TRAIL, conatumumab binds to and activates TR-2, thereby triggering caspase-dependent apoptosis in tumor cells. In pre-clinical studies, conatumumab inhibits the growth of human tumor, both alone or in combination with cytotoxic chemotherapeutic agents [77]. Ongoing clinical trials aim to evaluate the potential efficacy of conatumumab in locally advanced or metastatic pancreatic adenocarcinoma (Table 5).

Developmental Pathways

The major signaling pathways that play crucial roles in pancreas during embryonic development include EGF (see Sect. 3.1.), Hedgehog, Notch, fibroblast growth factor, transforming growth factor β (TGFβ), and retinoids. Aberrant expression and/or activity of the developmental pathways have been observed in the early steps of pancreatic carcinogenesis [9, 80]. Targeting the components of the developmental pathways of pancreas hypothetically enables intervention during the early stages of malignant transformation in pancreatic neoplasia and pancreatic cancer stem cells.

Hedgehog

Upon binding of the secreted ligand (Hedgehog) to the Patched receptor (PTC) on the target cell, the ligand-bound PTC releases Smoothened (SMO), which becomes uninhibited and activates the GLI family of transcription factors. As a result, target genes including *PTC* are expressed, leading to epithelial proliferation and survival in normal and cancerous pancreas [81–83]. GDC-0449 is an oral small molecule inhibitor of SMO, and it produces anti-tumor responses in patients with medulloblastoma [84] and basal cell carcinoma [85]. Inhibition of the hedgehog pathway offers the advantage of therapeutically targeting the primary pancreatic tumor and the associated stroma, and possibly the pancreatic cancer stem cells [86]. A clinical trial aims to test the hypothesis that GDC-0449 in combination with cytotoxic agents may increase progression-free survival in patients with pancreatic adenocarcinoma (Table 6). IPI-926 is another oral inhibitor of the hedgehog pathway by inactivating SMO. Two clinical studies are currently open to determine the safety and efficacy of IPI-926 in combination with conventional chemotherapy (Table 6).

Table 6 Targeting developmental pathways in pancreatic adenocarcinoma

Genetic targets	Targeted agents	Tumor stage	Clinical trial	Comments	Clinical Trials.gov Identifier	Ref.
SMO	GDC-0449	Metastatic	Phase II	In combination with Gemcitabine and Nab-Paclitaxel as first-line therapy. Currently recruiting.	NCT01088815	–
SMO	GDC-0449	Metastatic	Phase I	In combination with erlotinib with or without gemcitabine. Recruitment status unknown.	NCT00878163	–
SMO	GDC-0449	Resectable	Phase II	As neoadjuvant therapy. Currently recruiting.	NCT01096732	–
SMO	IPI-926	Metastatic	Phase Ib/II	Phase Ib study for dose escalation of IPI-926. Phase II study IPI-926 used at MTD. In combination with gemcitabine. Currently recruiting.	NCT01130142	–
SMO	IPI-926	Inoperable (locally advanced or metastatic)	Phase I	In combination with FOLFIRINOX as first-line therapy. Not yet open for recruitment.	NCT01383538	–
γ-secretase	RO4929097	Stage I, II	Phase I	As neoadjuvant therapy, followed by surgical resection. Currently recruiting.	NCT01192763	–
γ-secretase	RO4929097	Metastatic	Phase II	As second-line therapy in patients previously treated with chemotherapy, preferably gemcitabine-based regimen. Currently recruiting.	NCT01232829	–
γ-secretase	RO4929097	Locally advanced or metastatic	Phase I	In combination with gemcitabine as first-line therapy. Currently recruiting.	NCT01145456	–
γ-secretase	MK-0752	Metastatic	Phase I/IIa	In combination with gemcitabine as first-line therapy. Currently recruiting.	NCT01098344	–

SMO smoothened, *MTD* maximum tolerated dose

Notch

Upon binding of a Notch ligand expressed on a stromal cell to a Notch receptor expressed on an epithelial cell, the Notch ligand-receptor interaction triggers the cytoplasmic enzyme γ-secretase to mediate cleavage of the intracellular domain (ICD) of the ligand-bound Notch receptor. The Notch ICD translocates to the nucleus and then forms a complex that mediates transcription of the target genes that function in normal development and tumorigenesis [87]. Inhibition of Notch signaling has been shown to block the activity of γ-secretase and the subsequent signaling and cellular events in pancreatic cancer [88, 89]. RO4929097 and MK-0752 are inhibitors of γ-secretase, thus preventing the release of the Notch ICD and the resulting transcription of the target genes. Several clinical trials are ongoing to determine the potential efficacy of these γ-secretase inhibitors in all stages of pancreatic adenocarcinoma (Table 6).

Stromal Microenvironment

Pancreatic adenocarcinoma is characterized by the presence of strong desmoplastic reaction. The tumor-associated stroma is a complex microenvironment composed of an extracellular matrix, activated fibroblasts, inflammatory cells, and aberrantly formed vasculatures [6]. The vascular deficiency in the stromal matrix has been shown as a contributing factor to therapeutic resistance by impeding delivery of therapeutics such as gemcitabine [86]. A number of components in the tumor-associated stroma including secreted protein acidic and rich in cysteine (SPARC), matrix metalloproteinases (MMPs), cyclooxygenase 2 (COX-2), vascular endothelial growth factor (VEGF), CCR2, and integrin have been investigated as therapeutic targets for intervention (Table 7).

Secreted Protein Acidic and Rich in Cysteine (SPARC)

SPARC is a glycoprotein secreted in the extracellular matrix, and it is capable of binding has calcium and albumin. Genetic deletion of SPARC in mice results in enhanced growth of pancreatic adenocarcinoma xenograft, and this is associated with reduction of extracellular matrix and apoptotic tumor cells [90]. It has been demonstrated that SPARC is over-expressed in pancreatic adenocarcinoma and other solid tumors. On the basis of these observations, it was hypothesized that the presence of SPARC would facilitate intra-tumoral accumulation of albumin-bound cytotoxic drugs such as the albumin-stabilized nanoparticle formulation containing paclitaxel (*nab*-paclitaxel). To test this hypothesis, a phase I/II trial using a combination of *nab*-paclitaxel and gemcitabine showed remarkable response and survival rates [91]. Further support of the hypothesis is obtained by demonstrating that *nab*-paclitaxel either alone or in combination with gemcitabine produced depletion of

Table 7 Targeting tumor-associated stroma in pancreatic adenocarcinoma

Genetic targets	Targeted agents	Tumor stage	Clinical trial	Comments	Clinical trials.gov Identifier	Reference
SPARC	*nab*-paclitaxel	Metastatic	Phase I/II	Combination of *nab*-paclitaxel and gemcitabine showed a response rate of 48%, median overall survival 12.2 months, and 1-year survival 48%.	–	[91]
MMPs	Marimastat	Unresectable	Phase III	Marimastat versus gemcitabine as first-line therapy. Median survival 125 days (Marimastat) versus 167 days (gemcitabine), $P=0.19$	–	[96]
MMPs	Marimastat	Unresectable	Phase III	Marimastat with or without gemcitabine as first-line therapy. Median survival 165.5 days (Marimastat + gemcitabine) versus 164 days (gemcitabine), $P=0.95$	–	[97]
MMP-2, -3, -9, -13	BAY 12-9566	Unresectable, locally advanced, or metastatic	Phase III	BAY 12–9566 or gemcitabine as first-line therapy. Median survival 3.74 months (BAY 12–9566) versus 6.59 months (gemcitabine), $P<0.001$. Median progression-free survival 1.68 months (BAY 12–9566) versus 3.5 months (gemcitabine), $P<0.001$	–	[98]
COX-2	Celecoxib	Metastatic	Phase II	In combination with gemcitabine and cisplatin as first-line therapy. Median survival 5.8 months. 6-month survival 46%.	–	[104]
VEGF	Bevacizumab	Metastatic	Phase II	In combination with gemcitabine as first-line therapy. Completed. Median survival 8.8 months. Median progression-free survival 5.4 months.	NCT00028834	[105]

VEGF	Bevacizumab	Locally advanced or metastatic	Phase III	In combination with gemcitabine versus gemcitabine alone as first-line therapy. Median overall survival and median progression-free survival 5.8 months and 3.8 months (gemcitabine/bevacizumab) versus 5.9 months and 2.9 months (gemcitabine/placebo), both not statistically significant.	NCT00088894	[106]
VEGF	Bevacizumab	Metastatic or locally advanced unresectable	Phase II	In combination with gemcitabine and capecitabine as first-line therapy. Median overall survival 9.8 months. Median progression-free survival 5.8 months. Radiological response rate 22%.	NCT00100815	[107]
VEGF	Bevacizumab	Locally advanced, unresectable	Phase II	Bevacizumab + capecitabine EBRT, followed by bevacizumab gemcitabine. Completed. Median survival (11.9 months) and median progression-free survival (8.6 months).	NCT00114179	[108]
VEGF	Bevacizumab	Metastatic	Phase II	In combination with FDR gemcitabine and cisplatin as first-line therapy. Median survival 8.2 months. Median time to progression 6.6 months.	NCT00126633	[109]
VEGF	Bevacizumab	Metastatic	Phase II	As second-line therapy in patients with gemcitabine-refractory tumor. Median time to progression 40 days. Median survival 102 days.	NCT00365144	[110]
VEGF	Bevacizumab	Locally advanced or metastatic	Phase II	In combination with gemcitabine and erlotinib as first-line therapy. Completed.	NCT00366457	–

(continued)

Table 7 (continued)

Genetic targets	Targeted agents	Tumor stage	Clinical trial	Comments	Clinical trials.gov Identifier	Reference
VEGF	Bevacizumab	Potentially resectable	Phase II	As pre-operative therapy. In combination with FDR gemcitabine and RFRT. Ongoing but not recruiting.	NCT00557492	–
VEGF	Bevacizumab	Metastatic	Phase II	As second-line therapy in tumor pre-treated with gemcitabine-based regimen. Alone or in combination with docetaxel. Completed.	NCT00066677	–
VEGF	Bevacizumab	Metastatic	Phase II	As first-line therapy. In combination with gemcitabine and oxaliplatin. Completed.	NCT00112528	–
VEGF	Bevacizumab	Resectable or locally advanced	Phase I/II	In combination with erlotinib and radiation as first-line therapy. Ongoing but not recruiting.	NCT00735306	–
VEGF	Bevacizumab	Metastatic	Phase II	In combination with bevacizumab +/– gemcitabine as first-line therapy. Terminated prematurely due to lack of efficacy in both arms.	NCT00326911	–
VEGF	Bevacizumab	Locally advanced or metastatic	Phase I/II	In combination with erlotinib, gemcitabine, and capecitabine as first-line therapy. Ongoing but not recruiting.	NCT00260364	–
VEGF	Bevacizumab	Resected	Phase I/II	In combination with gemcitabine as adjuvant therapy. Recruitment status unknown.	NCT00410774	–
VEGF	Bevacizumab	Locally advanced or metastatic	Phase II	Bevacizumab and gemcitabine in combination with either cetuximab or erlotinib. Completed.	NCT00091026	-

VEGF	Bevacizumab	Resected	Phase II	As adjuvant therapy, either bevacizumab or cetuximab, in combination with gemcitabine, capecitabine and radiotherapy. Recruitment status unknown.	NCT00305877	–
VEGF	Bevacizumab	Locally advanced	Phase I/II	In combination with 5-fluorouracil, oxaliplatin, and EBRT, followed by bevacizumab gemcitabine. Terminated due to poor accrual.	NCT00307723	–
CCR2	PF-04136309	Borderline resectable and locally advanced	Phase I	In combination with FOLFIRINOX as first-line therapy. Not yet open for recruitment.	NCT01413022	–
Integrin α5β1	Volociximab (M200)	Metastatic	Phase II	In combination with gemcitabine as first-line therapy. Completed.	NCT00401570	–

CCR2 chemokine receptor 2, *COX-2* cyclooxygenase-2, *FDR* fixed-dose rate, *MMPs* matrix metalloproteinases, *RFRT* rapid-fractionation radiotherapy, *SPARC* secreted protein acidic and rich in cysteine, *VEGF* vascular endothelial growth factor

the desmoplastic stroma of pancreatic tumor in mouse xenografts. These data suggest the combination of *nab*-paclitaxel and gemcitabine as a promising therapeutic regimen that is expected to be further evaluated in a phase III trial. Moreover, future clinical investigations are anticipated to determine the efficacy and toxicity of *nab*-paclitaxel and gemcitabine as neoadjuvant or adjuvant therapy as well as in combination with other cytotoxic chemotherapeutics and targeted agents.

Matrix Metalloproteinases (MMPs)

The MMPs comprise a large family of zinc-dependent proteases capable of degrading the extracellular matrix [92]. The two isozymes MMP-2 and MMP-9 are over-expressed in pancreatic cancer, and this correlates with invasion and metastasis of the primary tumor [93]. Pre-clinical studies indicate that inhibitors of MMPs are effective in reducing the number and size of metastatic lesions of pancreatic tumors [94, 95]. Inhibitors of MMPs have been developed and tested clinically in patients with pancreatic adenocarcinoma including marimastat and BAY 12–9566. In the phase II trial using marimastat in patients with unresectable pancreatic tumor, no significant difference in overall survival between the marimastat-treated group and the gemcitabine-treated group [96]. In the phase III trial using marimastat with or without gemcitabine in patients with advanced pancreatic adenocarcinoma, addition of marimastat to gemcitabine does not improve the overall survival [97]. In the phase III trial using another MMP inhibitor BAY 12–9566 versus gemcitabine in patients with locally advanced or metastatic pancreatic adenocarcinoma, overall survival and progression-free survival in the BAY 12-9566-treated group are significantly inferior to that treated with gemcitabine [98].

Cyclooxygenase-2 (COX-2)

The enzyme COX-2 catalyzes the formation of prostaglandins that play an important role in inflammation and they are implicated in cancer progression and chemoresistance [99]. The isozyme COX-2 is over-expressed in pancreatic adenocarcinoma [100–102]. Pre-clinical studies show that the small molecule inhibitor of COX2, celecoxib, reduces pancreatic cancer cell growth and sensitizes the tumor cells to gemcitabine-induced cytotoxicity [103]. The phase II study using celecoxib, gemcitabine, and cisplatin in patients with metastatic pancreatic adenocarcinoma suggests that celecoxib does not provide additional improvement of survival [104].

Vascular Endothelial Growth Factor (VEGF) and VEGF Receptor (VEGFR)

VEGF is a cytokine that stimulates angiogenesis by inducing proliferation of endothelial cells. By interfering with neovascularization and blood supply of cancer cells, the use of anti-VEGFR inhibitors is expected to cause cessation of tumor growth. Since endothelial cells are normally non-proliferative, inhibitors of VEGF signaling are expected to produce relatively specific actions on tumor rather than normal tissues.

Bevacizumab is a monoclonal antibody that binds to VEGFα and prevents its interaction with VEGFR, thus inhibiting neovascularization and tumor growth. Several clinical trials that evaluated the efficacy of bevacizumab in combination with other therapeutic agents have been completed and a number of them are ongoing (Table 7). In a phase II trial with metastatic pancreatic adenocarcinoma, the combination of bevacizumab and gemcitabine produced partial responses in 21% of patients, whereas 46% patients had stable disease [105]. The subsequent phase III trial indicates no improvement of survival by addition of bevacizumab to gemcitabine for advanced pancreatic adenocarcinoma [106]. In a phase II trial with advanced pancreatic cancer, the combination of bevacizumab, gemcitabine, and capecitabine, the median progression-free survival and overall survival are 5.8 months and 9.8 months, respectively [107]. In a phase II study of locally advanced pancreatic cancer, addition of bevacizumab to concurrent capecitabine and radiation followed by maintenance bevacizumab and gemcitabine resulted in a median survival similar to prior studies by the Radiation Therapy Oncology Group [108]. In a phase II trial of metastatic pancreatic adenocarcinoma, a combination of bevacizumab, fixed-dose rate gemcitabine and low dose cisplatin shows modest efficacy [109]. Similarly, in another phase II study of gemcitabine-refractory metastatic pancreatic adenocarcinoma, a combination of bevacizumab and erlotinib produces minimal effect [110]. A number of clinical trials using bevacizumab either alone or in combination with other agents have been completed or ongoing (Table 7). However, it is important to note that the stromal microenvironment of pancreatic adenocarcinoma is deficient in vasculatures, thus impeding delivery of therapeutics such as gemcitabine [86]. This raises concern about the questionable use of anti-neoangiogenic agents in the treatment of patients with pancreatic adenocarcinoma.

Chemokine Receptor 2 (CCR2)

CCR2 is a G-protein coupled receptor expressed on the surface of monocytes and macrophages [111]. Upon binding of the endothelium-derived chemokine ligand CLL2 to CCR2, the resulting migration and infiltration of these cells play an important role in inflammation, angiogenesis, as well as tumor cell proliferation and migration [112]. PF-04136309 specifically binds to CCR2 and prevents it from binding with CLL2. This results in inhibition of CCR2 activation, thus reducing inflammation, angiogenesis, tumor cell migration, and tumor cell proliferation. A phase I study using PF-04136309 in combination with FOLFIRINOX in patients with borderline respectable and locally advanced pancreatic adenocarcinoma is planned (Table 7).

Integrin α5β1

Integrins are heterodimeric signaling and adhesion molecules, and they regulate proliferation, survival, and migration of endothelial cells [113]. Integrin α5β1 plays an important role in angiogenesis [114, 115]. Volociximab is a monoclonal antibody

directed against integrin α5β1, causing endothelial cell death and inhibiting angiogenesis and tumor growth [116]. A phase II study using a combination of volociximab and gemcitabine in metastatic pancreatic adenocarcinoma has been completed (Table 7).

Antigens and Immunomodulatory Signals

Genetic targets expressed on pancreatic tumor cells or on immune modulator cells have been exploited to enhance the anti-tumor immune response. The molecules expressed on pancreatic cancer cells including mesothelin, 5T4, AGS-5, CEA, PCSA, and telomerase have been used as ligands for tumor-specific delivery of cytotoxic agents or as antigens for attack of cancer cells by the immune system. The molecules expressed on immune modulator cells include CD40, cytotoxic T lymphocyte associated antigen-4 (CTLA-4), programmed death-1 (PD-1), dipeptidyl peptidase IV (DPP-IV) have been used for stimulating anti-cancer immune response or reversing its suppression.

Mesothelin

Mesothelin is a glycosylphosphatidylinositol-anchored membrane glycoprotein whose expression is normally restricted to mesothelial cells but aberrantly expressed in a variety of carcinomas including pancreatic adenocarcinoma [117, 118]. SS1(dsFv)-PE38, also known as SS1P, is a recombinant immunotoxin consisting of an anti-mesothelin disulfide-stabilized Fv fragment and the 38 kDa fragment of *Pseudomonas aeruginosa* exotoxin A [119]. In pre-clinical studies, SS1P has been shown to be cytotoxic in mesothelin-expressing cancer cells. A phase I trial of patients with chemoresistant mesothelin-expressing solid tumors including pancreatic adenocarcinoma indicates that SS1P is well tolerated with modest anti-tumor activity [120] (Table 8). Future phase II trials are planned to further evaluate the efficacy of a combination of SS1P and chemotherapy.

5T4

The tumor-associated antigen 5T4 is a trophoblast glycoprotein that is expressed in various tumor cells including pancreatic cancer [121, 122]. Naptumomab estafenatox (ABR-217620) is an immunotoxin consisting of a mutated variant of the superantigen *staphylococcal enterotoxin E* (SEA/E-120) linked to the antigen-binding fragment (Fab) moiety of a monoclonal antibody that recognizes the 5T4 antigen. The Fab binds to 5 T4, and the superantigen (SEA/E-12) induces a cytotoxic T-cell response. In two phase I studies of patients with pancreatic cancer, non-small cell lung cancer, or renal cell carcinoma, naptumomab estafenatox was used either alone or in combination with

Table 8 Targeting antigens and immunomodulatory signals in pancreatic adenocarcinoma

Genetic targets	Targeted agents	Pancreatic adenocarcinoma	Clinical trial	Comments	ClinicalTrials.gov Identifier	Reference
Mesothelin	SS1(dsFv)-PE38	Recurrent unresectable	Phase I	In patients previously treated with standard therapy or who refused prior standard therapy. Completed.	NCT00006981 NCT00066651	[120]
5T4	Naptumomab estafenatox (ABR-217620)	Advanced or metastatic, refractory to standard therapies	Phase I	Naptumomnab in patients with have received or declined at least one standard regimen. Completed.	NCT00056537	[123]
ASG-5	ASG-5ME	Metastatic	Phase I	As first-line therapy. Currently recruiting.	NCT01166490	–
CEA	Modified CEA (CAP1-6D)	Surgically resected or locally advanced	Phase II	As first-line therapy. Ongoing but not recruiting.	NCT00203892	–
CEA/CD3	MEDI-565	Refractory	Phase I	As second-line therapy in patients with tumor refractory to previous treatment. Currently recruiting.	NCT01284231	–
PSCA	AGS-1C4D4	Metastatic	Phase II	In combination with gemcitabine as first-line therapy. Ongoing but not recruiting.	NCT00902291	–
Telomerase	Telomerase peptide vaccine (GV1001)	Locally advanced unresectable or borderline resectable	Phase I	In combination with gemcitabine, sargramostim, tadalafil, and radiation as first-line therapy. Currently recruiting.	NCT01342224	–
Telomerase	Telomerase peptide vaccine (GV1001)	Locally advanced or metastatic	Phase III	As first-line therapy, gemcitabine capecitabine without or with GV1001 (either given sequentially or concurrently). Recruitment status unknown.	NCT00425360	–

(continued)

Table 8 (continued)

Genetic targets	Targeted agents	Pancreatic adenocarcinoma	Clinical trial	Comments	ClinicalTrials.gov Identifier	Reference
Telomerase	Telomerase peptide vaccine (GV1001)	Locally advanced or metastatic	Phase III	GV1001 with or without gemcitabine as first-line therapy. Terminated as preliminary data showed no survival benefit in the patients receiving GV1001 and gemcitabine as compared to those in the gemcitabine group.	NCT00358566	–
CD40	CP-870,893	Unresectable (90% with metastastic disease)	Phase I	In combination with gemcitabine as first-line therapy. Median overall survival 7.4 months. Median progression-free survival 5.6 months.	–	[131]
CTLA-4	Ipilimumab	Locally advanced or metastatic	Phase II	No responder except one subject showing a significant delayed response.	–	[134]
CTLA-4	Ipilimumab	Locally advanced and unresectable, or metastatic	Phase I	Ipilimumab is administered either alone or in combination with allogenic pancreatic tumor cells transfected with a GM-CSF gene. Ongoing but not recruiting.	NCT00836407	–
PD-1	CT-011	Resected	Phase II	In combination with gemcitabine. Currently recruiting.	NCT01313416	–
DPP-IV or CD26	Talabostat	Metastatic	Phase II	In combination with gemcitabine as first-line therapy. Terminated because of FDA hold.	NCT00116389	–

CEA carcinoembryonic antigen, *PSCA* prostate stem cell antigen, *CTLA-4* cytotoxic T-lymphocyte associated antigen-4, *DPP-IV* dipeptidyl peptidase IV

docetaxel (Table 8). Naptumomab estafenatox has been shown to be well tolerated with evidence of immunological activity and anti-tumor activity [123]. Further studies are planned for evaluation of the therapeutic efficacy of this immunotoxin.

ASG-5

ASG-5, also known as solute carrier family 44, member 4 (SLC44A4), is transmembrane transporter with an anion exchange motif, and its expression is up-regulated in a number of solid tumors such as pancreatic carcinoma. ASG-5ME is an antibody-drug conjugate composed of a human monoclonal antibody directed to SLC44A4 (AGS-5) and linked to a synthetic drug monomethyl auristatin E (MMAE). Upon binding to tumor cells, ASG-5ME is endocytosed and MMAE is released into the cytosol through enzymatic cleavage of the linker. MMAE then binds to tubulin and induces cell cycle arrest at G_2/M phases, resulting in apoptotic cell death [124]. The safety and the maximum tolerated dose of ASG-5ME are being evaluated in patients with metastatic pancreatic adenocarcinoma (Table 8).

Carcinoembryonic Antigen (CEA)

CEA, also known as CD66e, belongs to a family of cell-surface glycoprotein, and it plays a role in cell adhesion, signal transduction, and innate immunity [125]. CEA is selectively expressed in normal epithelial tissues and it tends to be over-expressed in carcinomas of a variety of organs including pancreas. The potential of CEA as a tumor-specific antigen has been explored in a variety of malignancies including pancreatic adenocarcinoma. CEA is expressed in more than 90% of pancreatic cancer, and it has been targeted for vaccination. In a clinical trial, patients with resected or locally advanced pancreatic adenocarcinoma are immunized with a modified CEA peptide and addition of GM-CSF (Table 8). It is expected to recruit dendritic cells and expand CEA-reactive cytotoxic T-lymphocytes, resulting in controlling tumor growth.

In another clinical trial for patients with refractory pancreatic adenocarcinoma, the therapeutic efficacy of the bispecific antibody called MEDI-565 that recognizes CEA and CD3 is being investigated (Table 8). The deimmunized anti-CD3 antibody component helps prevent competitive inhibition by soluble CEA in the serum and redirect cytotoxic T cells toward CEA-expressing tumors. The bispecific anti-CEA/CD3 antibodies have been shown to cause tumor cell lysis in pre-clinical models [126].

Prostate Stem Cell Antigen (PSCA)

PSCA, a glycosylphosphatidyl-inositol (GPI)-linked cell surface antigen, is found to be over-expressed in pancreatic cancer as compared with chronic pancreatitis and normal pancreas [127, 128]. In xenograft model, monoclonal anti-PSCA antibodies

(1G8) reduces initiation and progression of pancreatic tumor [127]. Using CD3/CD28-activated T lymphocytes engineered to express a chimeric antigen receptor targeting PSCA, the potential efficacy and safety of this approach is indicated [128]. AGS-1C4D4 is a fully human monoclonal antibody that selectively binds to PSCA, inducing complement-dependent cell lysis and antibody-dependent cell-mediated cytotoxicity in PSCA-expressing tumor cells. The efficacy of AGS-1C4D4 in combination with gemcitabine is being investigated in patients with metastatic pancreatic adenocarcinoma (Table 8).

Telomerase

Telomerase is a reverse transcriptase that catalyzes the formation of telomeres at chromosomal ends, and its expression is repressed in normal cells [129]. Telomerase is over- expressed in most tumor cell types and it contributes to their proliferative capacity. CV1001 is a synthetic vaccine that consists of a peptide derived from human telomerase reversible transcriptase. CV1001 binds to HLA class II molecules and activates a cytotoxic T-lymphocyte response against telomerase-expressing cancer cells. Preliminary data suggest no clinical benefit by using CV1001 to target telomerase (Table 8).

CD40

CD40 is a member of the TNF receptor superfamily and its expression on antigen presenting cells mediates tumor-specific priming and expansion of T lymphocytes. Targeting CD40 can activate immune cells and produce cytotoxic effects on cancer cells [130]. In a clinical trial with patients with unresectable pancreatic adenocarcinoma, combination of the monoclonal antibodies (CP-870,893) that agonize CD40 and gemcitabine induced a measurable anti-tumor response [131]. In a parallel effort using genetically engineered mice that express mutant K-Ras and p53, it was demonstrated that the agonist CD40 antibodies produced tumor regression independent of T cell but it involves macrophage infiltration into tumors with degradation of tumor-associated stroma [131]. Results of this study support future development of therapy that targets inflammatory cells and stroma in pancreatic tumor.

Cytotoxic T-Lymphocyte Associated Antigen-4 (CTLA-4)

CTLA-4 is a member of the immunoglobulin superfamily and it plays an important role in immune tolerance in the peripheral tissues by modulating activation of T cells through regulating T cell receptor/CD28 signaling [132, 133]. Ipilimumab is a recombinant human monoclonal antibody that binds to CTLA4 and blocks its interaction with CD80 or CD86 on antigen presenting cells, resulting in augmentation of T cell activation and proliferation. In a phase II study of locally advanced or

metastatic pancreatic adenocarcinoma, no objective response is demonstrated [134]. A phase I trial is ongoing to determine the safety and efficacy of ipilimumab either alone or in combination with GM-CSF-transfected tumor cells in patients with advanced pancreatic adenocarcinoma (Table 8).

Programmed Death-1 Receptor (PD-1)

PD-1 is a B7 family member that is expressed on activated T lymphocytes, and it is involved in immune suppression [135]. Interaction between the programmed death ligand (PDL)-1 or PDL-2 with PD-1 results in down-regulation of T-cell receptor mediated signaling, leading to anergy and apoptosis of T-lymphocytes. Expression of PDL-1 is found to be up-regulated in a variety of malignancies including pancreatic cancer [136]. CT-011 is a recombinant monoclonal antibody directed against PD-1 receptor and it blocks the interaction of PD-L1 with PD-1 on the immune responder cells, thus attenuating of the processes that lead to apoptotic cell death. CT-011 has been shown to inhibit tumor growth by generating tumor-specific immune response. A phase II trial is ongoing to determine the safety and efficacy of the combination of CT-011 and gemcitabine in patients following resection of pancreatic adenocarcinoma (Table 8).

Dipeptidyl Peptidase-IV (DPP-IV)

DPP-IV, also known as T cell activation antigen CD26, is a member of the subfamily of integral membrane serine proteases. It is involved in processing soluble factors and in degradation of extracellular matrix components. Its actions are essential for cell migration and matrix invasion, which are important events during tumor invasion, metastasis, and angiogenesis [137]. Talabostat (valine-proline-boronic acid) is a competitive inhibitor of DPP-IV, and it stimulates anti-tumor immune response via up-regulation of cytokines [138]. The efficacy of talabostat in combination with gemcitabine is being investigated in metastatic pancreatic adenocarcinoma (Table 8).

Multiple Genetic Targets

Some of the small molecules being investigated in clinical trials are known to act on more than one genetic targets (Table 9). These agents are expected to produce enhanced anti- tumor effects by targeting multiple components involved in the uncontrolled growth of a variety of malignancies including pancreatic adenocarcinoma. The results of several clinical trials using some of these small molecules including axitinib, sunitinib, and enzastaurin suggest that they do not provide any additional benefit on survival in patients with locally advanced or metastatic

Table 9 Targeting multiple kinases in pancreatic adenocarcinoma

Genetic targets	Targeted agents	Tumor stage	Clinical trial	Results comments	Clinical trials.gov identifier	Reference
VEGFR1,2,3	Axitinib	Unresectable, locally advanced or metastatic	Phase II	As first-line therapy in combination with gemcitabine. Median progression-free survival 4.2 months (axitinib + gemcitabine) versus 3.7 months (gemcitabine). Median overall survival 6.9 months (axitinib+gemcitabine) versus 5.6 months (gemcitabine), hazard ratio 0.71.	NCT00219557	[139]
VEGFR1,2,3	Axitinib	Metastatic or locally advanced	Phase III	As first-line therapy alone or in combination with gemcitabine. Median overall survival 8.5 months (axitinib+gemcitabine) versus 8.3 months (gemcitabine+placebo), $P=0.5436$.	NCT00471146	[140]
PDGFR, VEGFR, KIT, RET, CSF-1R, FLT3	Sunitinib	Metastatic	Phase II	As second-line therapy following tumor progression with gemcitabine-based therapy. Progression-free survival 1.31 months. Overall survival 3.68 months.	NCT00397787	[141]
PDGFR, VEGFR, KIT, RET, CSF-1R, FLT3	Sunitinib	Metastatic	Phase I	In combination with gemcitabine as first-line therapy. Completed.	NCT00462553	–
PDGFR, VEGFR, KIT, RET, CSF-1R, FLT3	Sunitinib	Metastatic	Phase II	Sunitinib versus observation as maintenance therapy, following 6 months of standard chemotherapy and without disease progression, Currently recruiting.	NCT00967603	–

PKCβ, PI3K/AKT	Enzastaurin (LY317615)	Locally advanced or metastatic	Phase II	Enzastaurin with or without gemcitabine as first-line therapy. Median overall survival 5.6 months (enzastaurin+gemcitabine) versus 5.1 months (gemcitabine). Median progression-free survival 3.4 months (enzastaurin+gemcitabine) versus 3.0 months (gemcitabine).		[142]
SRC, ABL	Dasatinib	Metastatic	Phase II	As first-line therapy. Ongoing but not recruiting.	NCT00474812	–
SRC, ABL	Dasatinib	Resected	Phase II	In combination with gemcitabine versus gemcitabine alone as adjuvant therapy. Currently recruiting.	NCT01234935	–
SRC, ABL	Bosutinib	Resected	Phase I/II	As post-operative adjuvant therapy. In combination with gemcitabine. Currently recruiting.	NCT01025570	–
SRC, ABL	Saracatinib (AZD0530)	Unresectable, locally advanced or metastatic	Phase I/II	In combination with gemcitabine as first-line therapy. Ongoing but not recruiting.	NCT00265876	–
SRC, ABL	Saracatinib (AZD0530)	Metastatic	Phase II	As second-line therapy in patients previously treated with chemotherapy, preferably gemcitabine-based regimen. Currently recruiting.	NCT00735917	–
RAF, VEGFR, mTOR	Sorafenib, Everolimus	Metastatic	Phase II	As second-line therapy following tumor progression through gemcitabine. Currently recruiting.	NCT00981162	–
RAF, VEGFR	Sorafenib	Locally advanced unresectable	Phase I	As first-line therapy. In combination with gemcitabine and radiotherapy. Ongoing but not recruiting.	NCT00375310	–

(continued)

Table 9 (continued)

Genetic targets	Targeted agents	Tumor stage	Clinical trial	Results comments	Clinical trials.gov identifier	Reference
RAF, VEGFR	Sorafenib	Locally advanced or metastatic	Phase II	In combination with gemcitabine as first-line therapy. Recruitment status unknown.	NCT00095966	–
RAF, VEGFR	Sorafenib	Unresectable	Phase II	In combination with erlotinib as first-line therapy. Ongoing but not recruiting.	NCT00837876	–
RAF, VEGFR	Sorafenib	Locally advanced or metastatic	Phase III	Sorafenib+gemcitabine versus placebo+gemcitabine as first-line therapy. Recruitment status unknown.	NCT00541021	–
FGFR-1, FGFR-2, VEGFR-2	Brivanib	Advanced, refractory	Phase II	Brivanib versus placebo. Currently recruiting.	NCT00633789	–
VEGFR-2, EGFR, RET	Vandetanib	Metastatic	Phase I	As first-line therapy. In combination with gemcitabine and capecitabine. Completed.	NCT00681798	–
ABL, PDGFR, KIT	Imatinib	Locally advanced or metastatic	Phase II	In combination with gemcitabine as first-line therapy. Completed.	NCT00161213	–
VEGFR, KIT, PDGFRβ	Vatalanib (PTK787 / ZK222584)	Metastatic or advanced	Phase II	As second-line therapy in gemcitabine-refractory pancreatic cancer	NCT00226005	–
VEGFR, KIT, PDGFRβ	Vatalanib (PTK787 / ZK222584)	Unresectable	Phase I/II	As first-line therapy in combination with gemcitabine. Completed.	NCT00185588	–

VEGFR vascular endothelial growth factor receptor, *PDGFR* platelet-derived growth factor receptor, *PKCβ* protein kinase C-β, *CSF-1R* colony stimulating factor-1 receptor, *ABL* Abelson proto-oncogene, *FGFR* fibroblast growth factor

pancreatic adenocarcinoma (Table 9). In a phase II trial, addition of axitinib to gemcitabine produces a small but statistically non-significant survival benefits [139]. The subsequent phase III double-blind randomized trial using axitinib in combination with either gemcitabine or placebo indicates that addition of axitinib to gemcitabine does not improve overall survival [140]. A phase II study in gemcitabine-refractory metastatic pancreatic adenocarcinoma, shows that sunitinib produces minimal objective response associated with moderate toxic side effects [141]. In a phase II trial of patients, addition of enzastaurin to gemcitabine does not improve survival [142].

Genetic Alterations as Clinical Biomarkers to Guide Therapy

Besides the utility as therapeutic targets, the genetic alterations in pancreatic cancer have begun to be exploited as molecular biomarkers for optimizing therapy. The development of biomarkers that can predict therapeutic response is expected to help select the optimal regimen for the individual patient. This principle can be illustrated by the following examples. Loss-of-function mutation in *BRCA2* sensitizes tumor cells to the therapeutic effects of DNA damaging agents by preventing DNA repair [143]. Germ-line mutations in *BRCA2* have been revealed in both familial and sporadic pancreatic adenocarcinoma. As BRCA2 functions in homology-directed repair of DNA mismatch, mutation in *BRCA2* impairs the ability to repair DNA double-strand breaks and leads to genomic instability of tumor cells. The contribution of BRCA2 to pancreatic carcinogenesis has been recently revealed in transgenic mouse models [144–146]. These data provide the molecular basis to support the use of DNA damaging agents (such as cisplatin and mitomycin C) or DNA repair inhibiting agents (such as iniparib) for treatment of pancreatic adenocarcinoma with loss-of-function mutation in *BRCA2* as illustrated by several recent reports.

A patient with metastatic pancreatic adenocarcinoma carrying a *BRCA2* mutation shows dramatic treatment response when treated with cisplatin-based therapy [147]. In another patient with a germ-line mutation in *BRCA2* mutation, the associated pancreatic cancer exhibited a complete pathologic response to iniparib (BSI-201), a poly(ADP-ribose) polymerase inhibitor that prohibits DNA repair [148]. A patient with pancreatic adenocarcinoma carrying inactivating mutations in the *PALB2* gene, which encodes the binding partner of BRCA2, showed a durable clinical response to treatment using the DNA damaging agent mitomycin C [149]. A phase II study was designed to use mitomycin-C, a DNA damaging agent, in patients with advanced or recurrent pancreatic cancer with *BRCA2* mutation (NCT00386399). However, these data support utilization of genetic targets of the individual pancreatic tumor for personalizing cancer treatment using targeted agents.

The expression level of human equilibrative nucleoside transporter-1 (hENT1) in pancreatic adenocarcinoma can help predict the anti-tumor response to gemcitabine in patients and thus select the use of gemcitabine or its derivative gemcitabine elaidate (CO-1.01). Clinical observations suggest that patients with low levels of

tumor cell hENT1 expression benefit less from gemcitabine treatment than those with high levels of tumor cell hENT1 expression [150]. These observations led to the hypothesis that pancreatic tumors expressing low levels of hENT1 will respond to CO-1.01 but not gemcitabine. This is because CO-1.01 enters tumor cells in a hENT1-independent manner. To test this hypothesis, two phase II clinical trials are designed by comparing CO-1.01 with gemcitabine as first-line therapy in patients with metastatic pancreatic adenocarcinoma (NCT01124786), or by evaluating the anti-tumor efficacy of CO-1.01 as a second-line therapy for patients with gemcitabine- refractory metastatic pancreatic adenocarcinoma with no expression of hENT1 in tumor (NCT01233375).

The potential of profiling the genetic targets of the individual pancreatic tumor for selecting cytotoxic and targeted agents has been explored. Amplification of *EGFR* and the mutational status of *K-RAS* correlate with the anti-tumor response to erlotinib. In addition, expression of thymidylate synthase, thymidine phosphorylase, ERCC-1 and topoisomerase I has been related to responsiveness to 5-fluorouracil, capecitabine, oxaliplatin and irinotecan, respectively. In a phase II clinical trial (NCT01394120) for patients with metastatic pancreatic adenocarcinoma, the efficacy of selected treatment using FOLFIRINOX, FOLFOX, FOLFIRI, gemcitabine/capecitabine, gemcitabine/erlotinib, or gemcitabine alone based on determination of those genetic targets will be evaluated.As a guide to personalized therapy, analysis for biomarker targets by molecular profiling of the tumor from the individual patient is commercially available. The data generated from the analysis help predict treatment response with the goal of improving the chance of response by cytotoxic drugs and targeting agents.

Impact of Pancreatic Cancer Stem Cells

The recent advances in the identification and characterization of pancreatic cancer stem cells have created new opportunity of genetic targeting for therapeutic applications. Pancreatic cancer stem cells have been identified, and these cancer stem cells potentially contribute to multiple aspects of pancreatic tumorigenesis. Various cellular markers including CD44, CD24, ESA, CD133, aldehyde dehydrogenase, and c-Met have been employed to isolate and characterize pancreatic cancer stem cells [24, 151, 152]. It has been postulated that varying proportions of cancer stem cells with distinct genetic mutations are present in multiple genetic subclones of a pancreatic tumor. Such intra-tumor genetic heterogeneity may account for different growth rates, tumor-initiating abilities, metastasizing capability, and therapeutic resistance of pancreatic tumors. Moreover, reactivation of developmental pathways including Sonic hedgehog [151] and possibly Wnt/β-catenin [153] may contribute to the malignant properties of pancreatic cancer stem cells. As in other types of malignant tumors, epithelial mesenchymal transition involving deregulated expression of E-cadherin, Twist1, and Zeb1, appears to play a key role in therapeutic resistance of pancreatic cancer stem cells [154–157]. Preclinical studies suggest

that the PI3K/AKT/mTOR pathway, Hedgehog signaling, as well as c-Met, are potential targets for therapy that preferentially depletes the population of pancreatic cancer stem cells. Conceivably, combination of a cytotoxic chemotherapeutic agent (e.g. gemcitabine) and a small molecule inhibitor directed against cancer stem cells and the stromal microenvironment (e.g. Smoothened antagonist) will produce maximal anti-tumor efficacy in pancreatic cancer.

Conclusions and Prospective

In recent years, the knowledge of the molecular genetics of pancreatic carcinogenesis has been translated into therapeutic strategies by developing chemicals and antibodies directed against multiple genetic targets. Many of these newly developed agents are still under clinical investigation, and many more are being tested in pre-clinical studies using pancreatic cancer cells, mouse xenografts, and genetically engineered mice. Thus far, the completed clinical trials testing the efficacy of new agents directed against genetic targets in pancreatic cancer have not shown significant impact on the clinical outcome in patients. The lack of efficacy of many of the targeted agents may be explained by the intrinsic complexity of pancreatic cancer cells, multiple redundant signaling pathways involved, the negative feedback loop of signal transduction, and the relatively avascular stroma associated with pancreatic tumor. Indeed, accumulating clinical data provide support for the critical necessity of fundamental understanding of the mechanisms that underlie pancreatic tumorigenesis through continued basic research in the normal and cancerous development of pancreas.

Embryological and post-natal studies of pancreas in animal models particularly rodents and fish have led to discovery of the genetic and epigenetic regulation of the biological processes during vertebrate development [80, 158, 159]. These studies have generated insights into how genetic and epigenetic mechanisms become aberrant and lead to the initiation and development of pancreatic neoplasia. Of note, recent preclinical evidence suggests that targeting developmental regulators such as the Hedgehog pathway can be an effective approach to eliminate pancreatic cancer stem cells. Indeed, developmental studies of exocrine pancreas using zebrafish as the model organism in conjunction with human pancreatic adenocarcinoma have led to identification of novel genetic and epigenetic targets [60, 80, 160–167]. These emerging targets include RNA polymerase III [162], the transient receptor potential (TRP) ion channels melastatin-subfamily members TRPM7 [163, 165] and TRPM8 [164, 166], and HDAC1 and its target genes [60]. Continued pre-clinical studies in genetically engineered animals and personalized tumor grafts using pancreatic adenocarcinoma cells or pancreatic cancer stem cells are expected to facilitate investigation of the molecular mechanisms of pancreatic carcinogenesis and screen/test/validate these genetic and epigenetic targets for therapy [5, 80].

Realization of the goal of personalized therapy in pancreatic cancer is still possible by concerted efforts in making advancement of knowledge in pancreatic development and cancer and applying novel technologies to design cancer-specific

strategies and improve anti-cancer therapeutic efficacy. With the hope of producing a clinically meaningful and significant impact on the morbidity and mortality of pancreatic adenocarcinoma, an integrated approach by targeting the molecular phenotype of pancreatic cancer is required by: (1) debulking tumor by cancer-specific targeted delivery of cytotoxic drugs and targeted agents, (2) eradicating cancer stem cells using agents that target developmental regulators, (3) targeting the tumor-associated stroma for improving delivery of therapeutic agents to tumor cells, (4) augmenting the anti-tumor immune response, (5) molecular profiling the tumor from the individual patient by genomic sequencing and mutational analysis for the purpose of guiding selection of cancer-specific and efficacious therapeutic agents.

References

1. Siegel R, Ward E, Brawley O, et al. Cancer statistics, 2011. CA Cancer J Clin. 2011;61:212–36.
2. Vincent A, Herman J, Schulick R, et al. Pancreatic cancer. Lancet. 2011;378:607–20.
3. Hong S-M, Park JY, Hruban RH, et al. Molecular signatures of pancreatic cancer. Arch Pathol Lab Med. 2011;135:716–27.
4. Hingorani SR. From inception to invasion: modeling pathways to pancreatic cancer. In: Lowy AM et al., editors. Pancreatic cancer. New York: Springer; 2008. p. 159–79.
5. Lonardo E, Hermann PC, Heeschen C. Pancreatic cancer stem cells—update and future perspectives. Mol Oncol. 2010;4:431–42.
6. Neesse A, Michl P, Frese KK, et al. Stromal biology and therapy in pancreatic cancer. Gut. 2011;60:861–8.
7. Hruban RH, Goggins M, Parsons J, et al. Progression model for pancreatic cancer. Clin Cancer Res. 2000;6:2969–72.
8. Yee NS, Furth EE, Pack M. Clinicopathologic and molecular features of pancreatic adenocarcinoma associated with Peutz-Jeghers syndrome. Cancer Biol Ther. 2003;2:38–47.
9. Koorstra J-BM, Hustinx SR, Offerhaus GJA, et al. Pancreatic carcinogenesis. Pancreatology. 2008;8:110–25.
10. Chun SG, Yee NS. Werner's syndrome as a hereditary risk factor for exocrine pancreatic cancer: potential role of WRN in pancreatic tumorigenesis and patient-tailored therapy. Cancer Biol Ther. 2010;10:430–7.
11. Hackel PO, Zwick E, Prenzel N, et al. Epidermal growth factor receptors: critical mediators of multiple receptor pathways. Curr Opin Cell Biol. 1999;11:184–9.
12. Sanvito F, Herrera PL, Huarte J, et al. TGF-beta 1 influences the relative development of the exocrine and endocrine pancreas in vitro. Development. 1994;120:3451–62.
13. Cras-Meneur C, Elghazi L, Czernichow P, et al. Epidermal growth factor increases undifferentiated pancreatic embryonic cells in vitro: a balance between proliferation and differentiation. Diabetes. 2001;50:1571–9.
14. Korc M, Friess H, Yamanaka Y, et al. Overexpression of the epidermal growth factor receptor in human pancreatic cancer is associated with concomitant increases in the levels of epidermal growth factor and transforming growth factor alpha. J Clin Invest. 1992;90:1352–60.
15. Lemoine NR, Hughes CM, Barton CM, et al. The epidermal growth factor receptor in human pancreatic cancer. J Pathol. 1992;166:7–12.
16. Yamanaka Y, Friess H, Kobrin MS, et al. Coexpression of epidermal growth factor receptor and ligands in human pancreatic cancer is associated with enhanced tumor aggressiveness. Anticancer Res. 1993;13:565–9.

17. Xiong HQ, Rosenberg A, LoBuglio A, et al. Cetuximab, a monoclonal antibody targeting the epidermal factor receptor, in combination with gemcitabine for advanced pancreatic cancer: a multicenter phase II trial. J Clin Oncol. 2004;22:2610–6.
18. Cascinu S, Berardi R, Labianca R, et al. Cetuximab plus gemcitabine and cisplatin compared with gemcitabine and cisplatin alone in patients with advanced pancreatic cancer: a randomised, multicentre, phase II trial. Lancet Oncol. 2008;9:39–44.
19. Philip PA, Benedetti J, Corless CL, 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;28:3605–10.
20. Moore M, Goldstein D, Hamm J, 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;25:1960–6.
21. Beltran PJ, Mitchell P, Chung Y-A, et al. AMG 479, a fully human anti-insulin-like growth factor receptor type I monoclonal antibody, inhibits the growth and survival of pancreatic carcinoma cells. Mol Cancer Ther. 2009;8:1095–105.
22. Birchmeier W, Brinkmann V, Niemann C, et al. Role of HGF/SF and c-Met in morphogenesis and metastasis of epithelial cells. CIBA Found Symp. 1997;212:230–40.
23. Perugini RA, McDade TP, Vittimberga FJ, et al. The molecular and cellular biology of pancreatic cancer. Crit Rev Eukaryot Gene Expr. 1998;8:377–93.
24. Li C, Wu J-J, Hynes M. c-Met is a marker of pancreatic cancer stem cells and therapeutic target. Gastroenterology. 2011;141:2218–27.
25. Kim ST, Lim DH, Jang K-T, et al. Impact of KRAS mutations on clinical outcomes in pancreatic cancer patients treated with first-line gemcitabine-based chemotherapy. Mol Cancer Ther. 2011;10:1993–9.
26. Pylayeva-Gupta Y, Grabocka E, Bar-Sagi D. RAS oncogenes: weaving a tumorigenic web. Nature Rev Cancer. 2011;11:761–74.
27. Almoguera C, Shibata D, Forrester K, et al. Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. Cell. 1988;53:549–54.
28. Fernandez-Medarde A, Santos E. Ras in cancer and developmental diseases. Genes Cancer. 2011;2:344–58.
29. Berndt N, Hamilton AD, Sebti SM. Targeting protein prenylation for cancer therapy. Nature Rev Cancer. 2011;11:775–91.
30. Cohen SJ, Ho L, Ranganathan S, et al. Phase II and pharmacodynamic study of the farnesyltransferase inhibitor R115777 as initial therapy in patients with metastatic pancreatic adenocarcinoma. J Clin Oncol. 2003;21:1301–6.
31. Macdonald JS, McCoy S, Whitehead RP, et al. A phase II study of farnesyl transferase inhibitor R115777 in pancreatic cancer: a southwest oncology group (SWOG 9924) study. Inves New Drug. 2005;23:485–7.
32. Van Cutsem E, van de Velde H, Karasek P, et al. Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer. J Clin Oncol. 2004;22:1430–8.
33. Martin NE, Brunner TB, Kiel KD, et al. A phase I trial of the dual farnesyltransferase and geranylgeranyltransferase inhibitor L-778,123 and radiotherapy for locally advanced pancreatic cancer. Clin Cancer Res. 2004;10:5447–54.
34. Lobell RB, Liu D, Buser CA, et al. Preclinical and clinical pharmacodynamic assessment of L-778,123, a dual inhibitor of farnesyl:protein transferase and geranylgeranyl:protein transferase type-1. Mol Cancer Ther. 2002;1:747–58.
35. Green J, Lipton A. Anticaner properties of zoledronic acid. Cancer Invest. 2010;28:944–57.
36. Tassone P, Tagliaferri P, Viscomi C, et al. Zoledronic acid induces antiproliferative and apoptotic effects in human pancreatic cancer cells in vitro. Brit J Cancer. 2003;88:1971–8.
37. Märten A, Lilienfeld-Toal M, Büchler MW, et al. Zoledronic acid has direct antiproliferative and antimetastatic effect on pancreatic carcinoma cells and acts as an antigen for delta2 gamma/delta T cells. J Immunother. 2007;30:370–7.

38. Gjertsen MK, Buanes T, Rosseland AR, et al. Intradermal ras peptide vaccination with granulocytemacrophage colony-stimulating factor as adjuvant: clinical and immunological responses in patients with pancreatic adenocarcinoma. Int J Cancer. 2001;92:441–50.
39. Weden S, Klemp M, Gladhaug IP, et al. Long-term follow-up of patients with resected pancreatic cancer following vaccination against mutant K-ras. Int J Cancer. 2011;128:1120–8.
40. Toubaji A, Achtar M, Provenzano M, et al. Pilot study of mutant ras peptide-based vaccine as an adjuvant treatment in pancreatic and colorectal cancers. Cancer Immunol Immunother. 2008;57:1413–20.
41. Carbone DP, Ciernik IF, Kelley MJ, et al. Immunization with mutant p53- and K-ras-derived peptides in cancer patients: immune response and clinical outcome. J Clin Oncol. 2005;23:5099–107.
42. Seufferlein T, Van Lint J, Liptay S, et al. Transforming growth factor alpha activates Ha-Ras in human pancreatic cancer cells with Ki-ras mutations. Gastroenterology. 1999;116:1441–52.
43. Geary RS, Leeds JM, Henry SP, et al. Antisense oligonucleotide inhibitors for the treatment of cancer: 1. Pharmacokinetic properties of phosphorothioate Oligodeoxynucleotides. Anticancer Drug Des. 1997;12:383–93.
44. Alberts SR, Schroeder M, Erlichman C, et al. Gemcitabine and ISIS-2503 for patients with locally advanced or metastatic pancreatic adenocarcinoma: a north central cancer treatment group phase II trial. J Clin Oncol. 2004;24:2944–50.
45. Strong JE, Coffey MC, Tang D, et al. The molecular basis of viral oncolysis: usurpation of the Ras signaling pathway by reovirus. EMBO J. 1998;17:3351–62.
46. Etoh T, Himeno Y, Matsumoto T, et al. Oncolytic viral therapy for human pancreatic cancer cells by reovirus. Clin Cancer Res. 2003;9:1218–23.
47. Friday BB, Adjei AA. Advances in targeting the Ras/Raf/MEK/Erk mitogen-activated protein kinase cascade with MEK inhibitors for cancer therapy. Clin Cancer Res. 2008;14:342–6.
48. LoRusso PM, Adjei AA, Varterasian M, et al. Phase I and pharmacodynamic study of the oral MEK inhibitor CI-1040 in patients with advanced malignancies. J Clin Oncol. 2005;23:5281–93.
49. Bodoky G, Timcheva C, Spigel DR, et al. A phase II open-label randomized study to assess the efficacy and safety of selumetinib (AZD6244 [ARRY-142886]) versus capecitabine in patients with advanced or metastatic pancreatic cancer who have failed first-line gemcitabine therapy. Invest New Drug. 2011;30:1272–3.
50. Courtney KD, Corcoran RB, Engelman JA. The PI3K pathway as drug target in human cancer. J Clin Oncol. 2010;28:1075–83.
51. Semba C, Moriya T, Kimura W, et al. Phosphorylated Akt/PKB controls cell growth and apoptosis in intraductal papillary-mucinous tumor and invasive ductal adenocarcinoma of the pancreas. Pancreas. 2003;26:250–7.
52. Yamamoto S, Tomita Y, Hoshida Y, et al. Prognostic significance of activated Akt expression in pancreatic ductal adenocarcinoma. Clin Cancer Res. 2004;10:2846–50.
53. Stanger BZ, Stiles B, Lauwers GY, et al. Pten constrains centroacinar cell expansion and malignant transformation in the pancreas. Cancer Cell. 2005;8:185–95.
54. Gupta AK, Cerniglia GJ, Mick R, et al. HIV protease inhibitors block Akt signaling and radiosensitize tumor cells both In vitro and In vivo. Cancer Res. 2005;65:8256–65.
55. Brunner TB, Geiger M, Grabenbauer GG, et al. Phase 1 trial of the human immunodeficiency virus protease inhibitor nelfinavir and chemoradiation for locally, advanced pancreatic cancer. J Clin Oncol. 2008;26:2699–706.
56. Schmelzle T, Hall MN. TOR, a central controller of cell growth. Cell. 2000;103:253–62.
57. Ito D, Fujimoto K, Mori T, et al. In vivo antitumor effect of the mTOR inhibitor CCI-779 and gemcitabine in xenograft models of human pancreatic cancer. Int J Cancer. 2006;118:2337–43.
58. Javle MM, Shroff RT, Xiong H, et al. Inhibition of the mammalian target of rapamycin (mTOR) in advanced pancreatic cancer: results of two phase II studies. BMC Cancer. 2010;10:368.
59. Marks PA, Xu W-S. Histone deacetylase inhibitors: potential in cancer therapy. J Cell Biochem. 2009;107:600–8.
60. Zhou W, Liang IC, Yee NS. Histone deacetylase 1 is required for exocrine pancreatic epithelial proliferation in development and cancer. Cancer Biol Ther. 2011;11:659–70.

61. Kumagai T, Wakimoto N, Yin D, et al. Histone deacetylase inhibitor, suberoylanilide hydroxamic acid (Vorinostat, SAHA) profoundly inhibits the growth of human pancreatic cancer cells. Int J Cancer. 2007;121:656–65.
62. Arnold NB, Arkus N, Gunn J, et al. The histone deacetylase inhibitor suberoylanilide hydroxamic acid induces growth inhibition and enhances gemcitabine-induced cell death in pancreatic cancer. Clin Cancer Res. 2007;13:18–26.
63. Chun SG, Zhou W, Yee NS. Combined targeting of histone deacetylases and hedgehog signaling enhances cytotoxicity in pancreatic cancer. Cancer Biol Ther. 2009;8:1328–39.
64. Jackman M, Lindon C, Nigg EA, et al. Active cyclin B1-Cdk1 first appears on centrosomes in prophase. Nat Cell Biol. 2003;5:143–8.
65. Gray Jr PJ, Bearss DJ, Han H, et al. Identification of human polo-like kinase 1 as a potential therapeutic target in pancreatic cancer. Mol Cancer Ther. 2004;3:641–6.
66. Weichert W, Schmidt M, Jacob J, et al. Overexpression of polo-like kinase 1 is a common and early event in pancreatic cancer. Pancreatology. 2005;5:259–65.
67. Gumireddy K, Reddy MV, Cosenza SC, et al. ON01910, a non-ATP-competitive small molecule inhibitor of Plk1, is a potent anticancer agent. Cancer Cell. 2005;7:275–86.
68. Attia S, Kolesar J, Mahoney MR, et al. A phase 2 consortium (P2C) trial of 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP) for advanced adenocarcinoma of the pancreas. Invest New Drugs. 2008;26:369–79.
69. Rosen ED, et al. PPARγ. A nuclear regulator of metabolism, differentiation, and cell growth. J Biol Chem. 2001;276:37731–4.
70. Takeuchi Y, Takahashi M, Sakano K, et al. Suppression of N-nitrosobis(2-oxopropyl)amine-induced pancreatic carcinogenesis in hamsters by pioglitazone, a ligand of peroxisome proliferator-activated receptor γ. Carcinogenesis. 2007;28:1692–6.
71. Conroy T, Desseigne F, Ychou M, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. New Engl J Med. 2011;364:1817–25.
72. Clark JW. Rubitecan. Expert Opin Investig Drugs. 2006;15:71–9.
73. Abou-Alfa GK, Letourneau R, Harker G, et al. Randomized phase III study of exatecan and gemcitabine compared with gemcitabine alone in untreated advanced pancreatic cancer. J Clin Oncol. 2006;24:4441–7.
74. Joshi KS, Rathos MJ, Joshi RD, et al. In vitro antitumor properties of a novel cyclin-dependent kinase inhibitor, P276-00. Mol Cancer Ther. 2007;6:918–25.
75. Wang LM, Ren DM. Flavopiridol, the first cyclin-dependent kinase inhibitor: recent advances in combination chemotherapy. Mini Rev Med Chem. 2010;10:1058–70.
76. Carvajal RD, Tse A, Shah MA, et al. A phase II study of flavopiridol (Alvocidib) in combination with docetaxel in refractory, metastatic pancreatic cancer. Pancreatology. 2009;9:404–9.
77. Wiezorek J, Holland P, Graves J. Death receptor agonists as a targeted therapy for cancer. Clin Cancer Res. 2010;16:1701–8.
78. Murdaca G, Colombo BM, Cagnati P, et al. Update upon efficacy and safety of TNF-α inhibitors. Expert Opin Drug Saf. 2012;11:1–5. Epub ahead of print.
79. Ozawa F, Friess H, Kleeff J, et al. Effects and expression of TRAIL and its apoptosis-promoting receptors in human pancreatic cancer. Cancer Lett. 2001;163:71–81.
80. Yee NS. Zebrafish as a biological system for identifying and evaluating therapeutic targets and compounds. In: Han H, Grippo PJ, editors. Drug discovery in pancreatic cancer: models and techniques. New York: Springer; 2010. p. 95–112.
81. Thayer SP, di Magliano MP, Heiser PW, et al. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. Nature. 2003;425:851–6.
82. Berman DM, Karhadkar S, Maitra A, et al. Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours. Nature. 2003;425:846–51.
83. Morton JP, Mongeau ME, Klimstra DS, et al. Sonnic hedgehog acts at multiple stages of pancreatic tumorigenesis. Proc Natl Acad Sci USA. 2007;104:5103–8.
84. Rudin CM, Hann CL, Laterra J, et al. Treatment of medulloblastoma with hedgehog pathway inhibitor GDC-0449. N Engl J Med. 2009;36:1173–8.
85. Von Hoff DD, LoRusso PM, Rudin CM, et al. Inhibition of the hedgehog pathway in advanced basal-cell carcinoma. N Engl J Med. 2009;36:1164–72.

86. Olive KP, Jacobetz MA, Davidson CJ, et al. Inhibition of hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science. 2009;324:1457–61.
87. Miele L, Golde T, Osborne B. Notch signaling in cancer. Curr Mol Med. 2006;6:905–18.
88. Wang Z, Zhang Y, Li Y, et al. Down-regulation of notch-1 contributes to cell growth inhibition and apoptosis in pancreatic cancer cells. Mol Cancer Ther. 2006;5:483–93.
89. Wang Z, Banerjee S, Li Y, et al. Down-regulation of notch-1 inhibits invasion by inactivation of nuclear factor-kappaB, vascular endothelial growth factor, and matrix metalloproteinase-9 in pancreatic cancer cells. Cancer Res. 2006;66:2778–84.
90. Puolakkainen PA, Brekken RA, Muneer S, et al. Enhanced growth of pancreatic tumors in SPARC-null mice is associated with decreased deposition of extracellular matrix and reduced tumor cell apoptosis. Mol Cancer Res. 2004;2:215–24.
91. Von Hoff DD, Ramanathan RK, Borad MJ, et al. Gemcitabine plus nab-paclitaxel is an active regimen in patients with advanced pancreatic cancer: a phase I/II trial. J Clin Oncol. 2011;29:4548–54.
92. Bloomston M, Zervos EE, Rosemurgy II AS, et al. Matrix metalloproteinases and their role in pancreatic cancer: a review of preclinical studies and clinical trials. Ann Surg Oncol. 2002;9:668–74.
93. Bramhall SR, Neoptolemos JP, Stamp GW, et al. Imbalance of expression of matrix metalloproteinases (MMPs) and tissue inhibitors of the matrix metalloproteinases (TIMPs) in human pancreatic carcinoma. J Pathol. 1997;182:347–55.
94. Zervos EE, Norman JG, Gower WR, et al. Matrix metalloproteinase inhibition attenuates human pancreatic cancer growth in vitro and decreases mortality and tumorigenesis in vivo. J Surg Res. 1997;69:367–71.
95. Haq M, Shafii A, Zervos EE, et al. Addition of matrix metalloproteinase inhibition to conventional cytotoxic therapy reduces tumor implantation and prolongs survival in a murine model of human pancreatic cancer. Cancer Res. 2000;60:3207–11.
96. Bramhall SR, Rosemurgy A, Brown PD, et al. Marimastat as first-line therapy for patients with unresectable pancreatic cancer: a randomized trial. J Clin Oncol. 2001;19:3447–55.
97. Bramhall SR, Schulz J, Nemunaitis J, et al. A double-blind placebo-controlled, randomized study comparing gemcitabine and marimastat with gemcitabine and placebo as first line therapy in patients with advanced pancreatic cancer. Br J Cancer. 2002;87:161–7.
98. Moore MJ, Hamm J, Dancey J, et al. Comparison of gemcitabine versus the matrix metalloproteinase inhibitor BAY 12–9566 in patients with advanced or metastatic adenocarcinoma of the pancreas: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol. 2003;21:3296–302.
99. Williams CS, Mann M, DuBois RN. The role of cyclooxygenases in inflammation, cancer, and development. Oncogene. 1999;18:7908–16.
100. Molina MA, Sitja-Arnau M, Lemoine MG, et al. Increased cyclooxygenase-2 expression in human pancreatic carcinomas and cell lines: growth inhibition by Nonsteroidal anti-inflammatory drugs. Cancer Res. 1999;59:4356–62.
101. Yip-Schneider MT, Barnard DS, Billings SD, et al. Cyclooxygenase-2 expression in human pancreatic adenocarcinomas. Carcinogenesis. 2000;21:139–46.
102. Merati K, Said Siadaty M, Andea A, et al. Expression of inflammatory modulator COX-2 in pancreatic ductal adenocarcinoma and its relationship to pathologic and clinical parameters. Amer J Clin Oncol. 2001;24:447–52.
103. El-Rayes BF, Ali S, Sarkar FH, et al. Cyclooxygenase-2-dependent and—independent effects of celecoxib in pancreatic cancer cell lines. Mol Cancer Ther. 2004;3:1421–6.
104. El-Rayes BF, Zalupski MM, Shields AF, et al. A phase II study of celecoxib, gemcitabine, and cisplatin in advanced pancreatic cancer. Invest New Drugs. 2005;23:583–90.
105. 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;23:8033–40.
106. 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;28:3617–22.

107. Javle M, Yu J, Garrett C, et al. Bevacizumab combined with gemcitabine and capecitabine for advanced pancreatic cancer: a phase II study. Br J Cancer. 2009;100:1842–5.
108. Crane CH, Winter K, Regine WF, et al. Phase II study of Bevacizumab with concurrent capecitabine and radiation followed by maintenance gemcitabine and Bevacizumab for locally advanced pancreatic cancer: Radiation Therapy Oncology Group RTOG 0411. J Clin Oncol. 2009;27:4096–102.
109. Ko AH, Dito E, Schillinger B, et al. A phase II study evaluating bevacizumab in combination with fixed-dose rate gemcitabine and low-dose cisplatin for metastatic pancreatic cancer: is an anti-VEGF strategy still applicable? Invest New Drugs. 2008;26:463–71.
110. Ko AH, Venook AP, Bergsland EK, et al. A phase II study of bevacizumab plus erlotinib for gemcitabine-refractory metastatic pancreatic cancer. Cancer Chemother Pharmacol. 2010;66:1051–7.
111. Howard OMZ, Ben-Baruch A, Oppenheim JJ. Chemokines: progress toward identifying molecular targets for therapeutic agents. Trends Biotechnol. 1996;14:46–51.
112. Monti P, Leone BE, Marchesi F, et al. The CC chemokine MCP-1/CCL2 in pancreatic cancer progression: regulation of expression and potential mechanisms of anti-malignant activity. Cancer Res. 2003;63:7451–61.
113. Hynes RO, Lively JC, McCarty JH, et al. The diverse roles of integrins and their ligands in angiogenesis. Cold Spring Harb Symp Quant Biol. 2002;67:143–53.
114. Lee BH, Ruoslahti E. alpha5beta1 integrin stimulates Bcl-2 expression and cell survival through Akt, focal adhesion kinase, and Ca2+/calmodulin-dependent protein kinase IV. J Cell Biochem. 2005;95:1214–23.
115. Mettouchi A, Meneguzzi G. Distinct roles of beta1 integrins during angiogenesis. Eur J Cell Biol. 2005;95:1214–23.
116. Bhaskar V, Zhang D, Fox M, et al. A function blocking anti-mouse integrin α5β1 antibody inhibits angiogenesis and impedes tumor growth in vivo. J Translat Med. 2007;5:61.
117. 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;7:3862–8.
118. Ryu B, Jones J, Blades NJ, et al. Relationships and differentially expressed genes among pancreatic cancers examined by large-scale serial analysis of gene expression. Cancer Res. 2002;62:819–26.
119. Pastan I, Hassan R, FitzGerald DJP, et al. Immunotoxin therapy of cancer. Nat Rev Cancer. 2006;6:559–65.
120. Kreitman RJ, Hassan R, Fitzgerald DJ, et al. Phase I trial of continuous infusion of anti-mesothelin recombinant immunotoxin SS1P. Clin Cancer Res. 2009;15:5274–9.
121. Hole N, Stern PL. A 72 kD trophoblast glycoprotein defined by a monoclonal antibody. Br J Cancer. 1988;57:239–46.
122. Southall PJ, Boxer GM, Bagshawe KD, et al. Immunohistological distribution of 5 T4 antigen in normal and malignant tissues. Br J Cancer. 1990;61:89–95.
123. Borghaei H, Alpaugh K, Hedlund G, et al. Phase I dose escalation, pharmacokinetic and pharmacodynamic study of naptumomab estafenatox alone in patients with advanced cancer and with docetaxel in patients with advanced non-small-cell lung cancer. J Clin Oncol. 2009;27:4116–23.
124. Arnett SO, Teillaud J-L, Wurch T. IBC's 21st annual antibody engineering and 8th annual antibody therapeutics international conferences and 2010 annual meeting of the antibody society. mAbs. 2011;3:133–52.
125. Hammarstrom S. The carcinoembryonic antigen (CEA) family: structures, suggested functions and expression in normal and malignant tissues. Seminars Cancer Biol. 1999;9:67–81.
126. Lutterbuese R, Raum T, Kischel R, et al. Potent control of tumor growth by CEA/CD3-bispecific single antibody constructs that are not competitively inhibited by soluble CEA. J Immunother. 2009;32:341–52.
127. Wente M, Jain A, Kono E, et al. Prostate stem cell antigen is a putative target for immunotherapy in pancreatic cancer. Pancreas. 2005;31:119–25.

128. Katari UL, Keiman JM, Worth AC, et al. Engineered T cells for pancreatic cancer treatment. HPB. 2011;13:643–50.
129. Brower V. Telomerase-based therapies emerging slowly. J Natl Cancer Inst. 2010;102:520–1.
130. Fonsatti E, Maio M, Altomonte M, et al. Biology and clinical applications of CD40 in cancer treatment. Semin Oncol. 2010;37:517–23.
131. Beatty GL, Chiorean EG, Fishman MP, et al. CD40 Agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. Science. 2011;331:1612–6.
132. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science. 1996;271:1734–6.
133. Salama AK, Hodi FS. Cytotoxic T-lymphocyte-associated antigen-4. Clin Cancer Res. 2011;17:4622–8.
134. Royal RE, Levy C, Turner K, et al. Phase 2 trial of single agent ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma. J Immunother. 2010;33:828–33.
135. Okazaki T, Honjo T. The PD-1-PD-L pathway in immunological tolerance. Trends Immunol. 2006;27:195–201.
136. 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;13:2151–7.
137. O'Brien P, O'Connor BF. Seprase: an overview of an important matrix serine protease. Biochim Biophys Acta. 2008;1784:1130–45.
138. Adams S, Miller GT, Jesson MI, et al. PT-100, a small molecule dipeptidyl peptidase inhibitor, has potent antitumor effects and augments antibody-mediated cytotoxicity via a novel immune mechanism. Cancer Res. 2004;64:5471–80.
139. Spano JP, Chodkiewicz C, Maurel J, et al. Efficacy of gemcitabine plus axitinib compared with gemcitabine alone in patients with advanced pancreatic cancer: an open-label randomised phase II study. Lancet. 2008;371:2101–8.
140. Kindler HL, Ioka T, Richel D, et al. Axitinib plus gemcitabine versus placebo plus gemcitabine in patients with advanced pancreatic adenocarcinoma: a double-blind randomized phase 3 study. Lancet. 2011;12:256–62.
141. O'Reilly EM, Niedzwiecki D, Hall M, et al. A cancer and leukemia group B phase II study of sunitinib malate in patients with previously treated metastatic pancreatic adenocarcinoma (CALGB 80603). Oncologist. 2010;15:1310–9.
142. Richards DA, Kuefler PR, Becerra C, et al. Gemcitabine plus enzastaurin or single-agent gemcitabine in locally advanced or metastatic pancreatic cancer: results of a phase II, randomized, noncomparative study. Invest New Drugs. 2011;29:144–53.
143. Yee NS. Brca2 deficiency and Trp53 deregulation in pancreatic cancer. Implications for therapeutic targeting. Cancer Biol Ther. 2011;11:969–73.
144. Skoulidis F, Cassidy LD, Pisupati V, et al. Germline Brca2 heterozygosity promotes Kras (G12D)-driven carcinogenesis in a murine model of familial pancreatic cancer. Cancer Cell. 2010;18:499–509.
145. Rowley M, Ohashi A, Mondal G, et al. Inactivation of Brca2 promotes Trp53-associated but inhibits KrasG12D-dependent pancreatic cancer development in mice. Gastroenterology. 2011;140:1303–13.
146. Feldmann G, Karikari C, dal Molin M, et al. Inactivation of Brca2 cooperates with $Trp53^{R172H}$ to induce invasive pancreatic ductal adenocarcinomas in mice: a mouse model of familial pancreatic cancer. Cancer Biol Ther. 2011;11:959–68.
147. Sonnenblick A, Kadouri L, Appelbaum L, et al. Complete remission, in BRCA2 mutation carrier with metastatic pancreatic adenocarcinoma, treated with cisplatin based therapy. Cancer Biol Ther. 2011;12:165–8.
148. Fogelman DR, Wolff RA, Kopetz S, et al. Evidence for the efficacy of iniparib, a PARP-1 inhibitor, in BRCA2-associated pancreatic cancer. Anticancer Res. 2011;31:1417–20.
149. Villarroel MC, Rajeshkumar NV, Garrido-Laguna I, et al. Personalizing cancer treatment in the age of global genomic analyses: PALB2 gene mutations and the response to DNA damaging agents in pancreatic cancer. Mol Cancer Ther. 2011;10:3–8.

150. Farrell JJ, Elsaleh H, Garcia M, et al. Human equilibrative nucleoside transporter 1 levels predict response to gemcitabine in patients with pancreatic cancer. Gastroenterology. 2009;136:187–95.
151. Li C, Heidt DG, Dalerba P, et al. Identification of pancreatic cancer stem cells. Cancer Res. 2007;67:1030–7.
152. Hermann PC, Huber SL, Herrler T, et al. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. Cell Stem Cell. 2007;1:313–23.
153. Al-Aynati MM, Radulovich N, Riddell RH, et al. Epithelial-cadherin and beta-catenin expression changes in pancreatic intraepithelial neoplasia. Clin Cancer Res. 2004;10:1235–40.
154. Shah AN, Summy JM, Zhang J, et al. Development and characterization of gemcitabine-resistant pancreatic tumor cells. Ann Surg Oncol. 2007;14:3629–37.
155. Arumugam T, Ramachandran V, Fournier KF, et al. Epithelial to mesenchymal transition contributes to drug resistance in pancreatic cancer. Cancer Res. 2009;69:5820–8.
156. Wellner U, Schubert J, Burk UC, et al. The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. Nat Cell Biol. 2009;11:1487–95.
157. Collisson EA, Sadanandam A, Olson P, et al. Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. Nat Med. 2011;17:500–3.
158. Gittes GK. Developmental biology of the pancreas: a comprehensive review. Dev Biol. 2009;326:4–35.
159. Tiso N, Moro E, Argenton F. Zebrafish pancreas development. Mol Cell Endocrinol. 2009;312:24–30.
160. Yee NS, Pack M. Zebrafish as a model for pancreatic cancer research. Methods Mol Med. 2004;103:273–98.
161. Yee NS, Lorent K, Pack M. Exocrine pancreas development in zebrafish. Dev Biol. 2005;284:84–101.
162. Yee NS, Gong W, Huang Y, et al. Mutation of RNA pol III subunit rpc2/polr3b leads to deficiency of subunit rpc11 and disrupts zebrafish digestive development. PLoS Biol. 2007;5:2484–92.
163. Yee NS, Zhou W, Liang IC. Transient receptor potential ion channel Trpm7 regulates exocrine pancreatic epithelial proliferation by Mg^{2+}-sensitive Socs3a signaling in development and cancer. Dis Model Mech. 2011;4:240–54.
164. Yee NS, Zhou W, Lee M. Transient receptor potential channel TRPM8 is over-expressed and required for cellular proliferation of pancreatic adenocarcinoma. Cancer Lett. 2010;297:49–55.
165. Yee NS, Zhou LM, et al. Targeted silencing of TRPM7 ion channel induces replicative senescence and produces enhanced cytotoxicity with gemcitabine in pancreatic adenocarcinoma. Cancer Lett. 2012;318:99–105.
166. Yee NS, Brown RD, Lee MS, et al. TRPM8 ion channel is aberrantly expressed and required for preventing replicative senescence in pancreatic adenocarcinoma: potential role of TRPM8 as a biomarker and target. Cancer Biol Ther. 2012;13:592–9.
167. Yee NS, Zhou W, Chun SG, et al. Targeting developmental regulators of zebrafish exocrine pancreas as a therapeutic approach in human pancreatic cancer. Biology Open 2012;1: 295–307.

Impact of Genetic Markers on Treatment of Non-small Cell Lung Cancer

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Abstract Non-small cell lung cancer represents a group of heterogeneous diseases. The last decade witnessed significant progress in improving our understanding of the biology of non-small cell lung cancer, which led to the identification of several genetic targets. Those genetic targets were utilized to explain clinical phenomena, such as the occurrence of non-small cell lung cancer in never-smokers, to predict response to conventional chemotherapy and biological agents, and to explain and predict resistance to therapy. The progress in the treatment of non-small cell lung cancer in the last few years was based on a new generation of population-enriched clinical trials that utilized genetic targets such as somatic EGFR mutations and ALK-EML4 mutations. In this review we will discuss the available information about the key genetic markers of non-small cell lung cancer and the pivotal clinical trials that validate the use of those genetic markers in non-small cell lung cancer patients.

Keywords Non-small cell lung cancer • EGFR • Erlotinib • Gefitinib • Affitinib • Cetuximab • ALK • Crizotinib • MET • Tivantinib • MetMab • ROS1 • VEGF • Bevacizumab

Introduction

Lung cancer is the leading cause of cancer-related mortality for both males and females in United States. In United States, in 2012, it is estimated that there are 226, 160 new cases of lung cancer diagnosed and 158,592 deaths from the disease [1].

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W.S. El-Deiry (ed.), *Impact of Genetic Targets on Cancer Therapy*, Advances in Experimental Medicine and Biology 779, DOI 10.1007/978-1-4614-6176-0_6,

Smoking remains the major risk factor for the development of NSCLC, however, 15% of men and 53% of women (25% of all cases worldwide) who develop lung cancer are never smokers [2]. Approximately 85% of new lung cancer cases are non-small cell lung cancer (NSCLC) which includes three major histological subtypes: adenocarcinoma, squamous cell carcinoma and large cell carcinoma. 40% of patients with NSCLC present with stage IV disease and the estimated 5-year survival is less than 1%. The primary goal of treatment of metastatic NSCLC is palliative, using systemic therapies which include conventional platinum-based chemotherapy and more recently biologic agents that target specific genetic markers. Patients who respond to first line of systemic therapy eventually develop resistance to treatment within 1 year. Second and third line treatments for NSCLC have shown a limited response rate of about 10%, improvement in progression free survival (PFS) of 2–3 months and a modest survival benefit. Clinical trials enrolling selected patient populations based on biological characteristics of the tumors have started to yield positive results, less toxicity and improved quality of life.

This review will provide an update on the role of genetic targets in the treatment of NSCLC and will focus on the epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), mesenchymal-epithelial transition factor (MET), and vascular endothelial growth factor (VEGF) as genetic targets with proven significant biological roles in NSCLC. Those genetic targets have therapeutic agents that are in developing stages or have been validated and approved for treatment.

Epidermal Growth Factor Receptor (EGFR)

EGFR is the most studied genetic marker in non-small cell lung cancer. It is the first genetic marker that has a targeted therapeutic agent approved for treatment of NSCLC in the USA. EGFR belongs to the ERB family of tyrosine kinase receptors, which consist of four members, ERB 1–4. The protein structure of EGFR is well studied and consists of an extracellular ligand binding domain, a transmembrane domain and an intracellular tyrosine kinase domain. EGFR exists as a monomer at the cell membrane. Activation of EGFR by ligand binding to the extracellular domain leads to homodimerization or heterodimerization of the EGFR receptor and subsequent autophosphorylation of the intracellular kinase domain. This process results in a cascade of activation of intracellular pathways that are involved in controlling cellular growth, apoptosis, invasion and angiogenesis. EGFR somatic gene mutations are present in 10–15% of European and North American NSCLC patients and in 25–30% of Asian NSCLC patients. The most common mutations involve exon 19 deletions or exon 21 point mutations (L858R). EGFR mutations provide autoactivation of the EGFR molecule without ligand binding with downstream signal activation of pro-survival pathways. This mechanism makes the EGFR mutated cell dependent on EGFR for survival [3]. These mutations are common in adenocarcinoma histology, in females and in never smokers. Presence of the EGFR mutation correlates with improvement in response rate and increased progression free survival with treatment with tyrosine kinase inhibitors (TKIs).

EGFR Inhibitors

There are two common approaches to inhibit the biologic effects of EGFR in NSCLC and other types of cancer. The first and most commonly studied in NSCLC involves the utilization of small molecules that inhibit the intracellular tyrosine kinase domain, such as gefitinib and erlotinib. The second approach consists of using a monoclonal antibody that competitively binds to the extracellular domain of the EGFR molecule such as cetuximab.

Gefitinib

Gefitinib is an oral reversible EGFR inhibitor that binds to the adenosine triphosphaste binding site and thereby prevents autophosphorylation of EGFR and activation of the downstream signaling pathway. Gefitinib was the first FDA approved anti EGFR therapy in the USA. The approval was based on two phase II studies, IDEAL-1 [4] and IDEAL-2 [5], in which unselected patients with refractory NSCLC were treated with either 250 mg or 500 mg of the drug. The response rate to gefitinib was about 10% with symptom improvement in 35–43% of the patients. However the subsequent phase III Study (ISEL study) that compared gefitinib to best supportive care in the same patient population did not show a significant survival benefit of gefitinib. Median survival did not differ significantly between the groups (5·6 months for gefitinib and 5·1 months for placebo; hazard ratio 0·89 (95% CI 0·77–1·02), p=0·087). Preplanned subgroup analyses showed significantly longer survival in the gefitinib group than the placebo group for never-smokers (n=375; 0·67 [0·49–0·92], p=0·012; median survival 8·9 and 6·1 months, respectively) and patients of Asian origin (n=342; 0·66 [0·48–0·91], p=0·01; median survival 9·5 for gefitinib vs. 5·5 months for placebo group) [6]. Based on the results of ISEL study the FDA withdrew the approval of gefitinib for treatment of the refractory NSCLC.

More recent studies of gefitinib in previously treated NSCLC patients showed non-inferiority to second line chemotherapy. The largest of those studies was an international phase III INTEREST trial which enrolled 1,433 patients who were randomized between gefitinib and docetaxel for second line treatment after failure of platinum based chemotherapy. The study results showed non-inferiority of gefitinib compared with docetaxel. Of note, patients on gefitinib arm had lower toxicity compared with docetaxel group [7].

In first line treatment adding gefitinib to platinum based chemotherapy showed no survival advantage over chemotherapy in 2 phase III studies, INTACT 1 and INTACT 2 [8, 9]. A possible explanation for the lack of synergy between an EGFR-TKI and chemotherapy may be the induction of G1 cell cycle arrest by EGFR TKIs, leading to increased resistance to chemotherapy. The understanding of the clinical/pathologic features of the responding patient in early clinical trials of anti-EGFR therapy, the discovery of EGFR mutations, and the sensitivity of the EGFR mutation to anti-EGFR therapy led to the design of a new generation of clinical trials using gefitinib in selected populations of lung cancer patients based on clinical, pathologic,

and molecular criteria such as Asian origin, adenocarcinoma histology, female gender, and known EGFR mutations. In a phase II study of never smokers in Korea, first line treatment with gefitinib was associated with a 69% response rate and 73% 1 year survival [10]. The phase III IPASS study included 1,217 selected patients with Asian origin, never-smoker or ex-light smoker, and adenocarcinoma histology. Patients were randomized between two groups, gefitinib or chemotherapy with a combination of carboplatin and paclitaxel. The 12-month progression-free survival was 24.9% with gefitinib and 6.7% with chemotherapy group,(hazard ratio for progression or death, 0.74; 95% confidence interval [CI], 0.65–0.85; P<0.001) [11]. EGFR mutations were the strongest predictive biomarker for PFS and tumor response to first-line gefitinib when compared with EGFR copy number and EGFR expression by immunohistochemistry. Overall survival was similar for gefitinib and carboplatin/paclitaxel with no significant difference between treatments in the study population (hazard ratio [HR], 0.90; 95% CI, 0.79–1.02; $P = .109$),in *EGFR* mutation–positive (HR, 1.00; 95% CI, 0.76–1.33; $P = .990$),in *EGFR* mutation–negative (HR, 1.18; 95% CI, 0.86–1.63; $P = .309$; and in treatment by *EGFR* mutation interaction $P = .480$) [12]. The results of the IPASS study were confirmed in other phase III studies in Japanese patients. The NEJ002 study prospectively randomized 230 patients with *EGFR* mutation-positive tumors to gefitinib or chemotherapy with carboplatin and paclitaxel. PFS favored gefitinib over carboplatin and paclitaxel (PFS HR, 0.30; 95% CI, 0.22–0.41; $P < .001$; median PFS, 10.8 vs. 5.4 months; tumor response rate, 73.7% vs. 30.7%, respectively; $P < .001$) [13]. The second study by The West Japan Thoracic Oncology Group 3405 (WJTOG3405) reported increased PFS with gefitinib over cisplatin and docetaxel in 172 patients with *EGFR* mutation-positive tumors (PFS HR, 0.49; 95% CI, 0.34–0.70; $P < .001$; median PFS, 9.2 vs. 6.3 months) [14].

Erlotinib

Erlotinib is another oral reversible EGFR inhibitor. An early phase II study of single agent erlotinib in previously treated patients with NSCLC documented its efficacy [15]. In a Phase III study (BR.21), 731 Patients previously treated with one or two lines of treatment were randomized to receive either erlotinib or placebo. Patients treated with erlotinib had statistically significant overall survival of 6.7 month versus 4.7 months in the placebo group (hazard ration 0.70; P<0.001). Progression free survival was 2.2 months in the erlotinib group versus 1.8 months in the placebo group (hazard ratio 0.61; p<0.001) [16]. All patients groups benefited from treatment with erlotinib but females, Asian ethnicity and never smoker patients had better outcomes with erlotinib treatment. Quality of life findings from the BR.21 study showed improvement of tumor related symptoms and better quality of life measurements in patients treated with erlotinib [17]. The results of BR.21 trial led to approval of erlotinib in the USA for second and third line treatment of NSCLC. In the phase III TITAN study, the efficacy and safety of erlotinib was compared to chemotherapy,

either docetaxel or pemetrexed, in patients with metastatic NSCLC who had disease progression after first line chemotherapy. The study closed prematurely after recruiting 424 patients. There was no difference in the efficacy between erlotinib and chemotherapy [18].

In first line treatment, combination of erlotinib and platinum based chemotherapy did not show any survival benefit in 2 phase III studies, TRIBUTE and TALENT [19, 20]. With the emergence of EGFR mutations as a predictor for response, recent studies compared erlotinib to chemotherapy in first line treatment. The OPTIMAL study compared erlotinib with gemcitabine and cisplatin in 154 Chinese patients with *EGFR* mutation-positive tumors reported a significant difference in PFS (HR, 0.16; 95% CI, 0.10–0.26; *P* = .001) [21]. A similarly designed European trial compared erlotinib versus chemotherapy (EURTAC) study showed a response rate of 54.5% in the erlotinib arm versus 10.7% on the chemotherapy arm and PFS of 9.7 months on the erlotinib arm versus 5.4 months on the chemotherapy arm; P < 0.0001 [22].

The role of erlotinib in maintenance therapy following first line chemotherapy has been tested in 2 phase III studies. In the SATURN study erlotinib was added as maintenance therapy versus placebo for patients with a partial response or stable disease following four cycles of platinum based chemotherapy, with PFS as the primary endpoint. At a median of 11.5 months follow up there was a significant difference in PFS with 12·3 weeks for patients in the erlotinib group versus 11·1 weeks for those in the placebo group (HR 0·71, 95% CI 0·62–0·82; p < 0·0001). The second maintenance study had a similar design but bevacizumab was added to the first line chemotherapy and was continued with erlotinib or placebo in patients without disease progression. The median PFS after randomization was 4.8 months for bevacizumab plus erlotinib while the patients received bevacizumab plus placebo had PFS of 3.7 months (HR = 0.722 (95% CI: 0.592–0.881), p = 0.0012) [23]. Despite this modest gain in PFS, erlotinib gained approval of the FDA for maintenance treatment for patients who responded to first line chemotherapy.

Resistance Mechanisms to Gefitinib and Erlotinib

Despite the ability of EGFR TKIs to achieve a significant improvement in PFS over platinum-doublet chemotherapy in the first-line treatment of advanced EGFR-positive NSCLC, patients eventually progress due to the emergence of resistance. Several mechanisms of TKI resistance had been identified but there are a significant number of cases that have no known mechanism for TKI resistance to date.

1. T790M
   The most common mechanism of resistance to EGFR TKIs is the development of the gatekeeper mutation T790M. Secondary T790M mutation has been determined to be responsible for 50% of the resistance cases resulting from EGFR TKIs [24]. The T790M mutation leads to steric hindrance of EGFR TKIs binding due to the presence of the bulkier methionine side chain [25]. Interestingly, T790M has also

been shown to increase the EGFR kinase affinity for ATP enhancing resistance by a second additional mechanism [26]. The T790M mutation has also been identified as a rare de novo mutation in EGFR [27]and it is associated with worse PFS than in patients without T790M. The T790M mutation seems to be dynamic in nature, meaning that once the selection pressure for T790M is abolished by discontinuing EGFR TKI therapy, the tumor loses the dependence on T790M for growth and T790M has been found to be lost in the same tumor sample [28]

2. MET amplification
   MET, a member of the insulin receptor tyrosine kinase family, encodes the receptor for hepatocyte growth factor (HGF) and triggers diverse intracellular signaling pathways. MET amplification has been shown to confer resistance to EGFR TKIs by activating the HER3/ERBB3 pathway or by resulting in secondary KRAS amplification. Secondary MET amplification has been identified in about 5–20% of the cases of acquired resistance to EGFR TKIs with or without the concurrent generation of T790M.
3. Phenotypic transformation
   There are two forms of phenotypic transformation described in cases of mutated EGFR NSCLC which developed resistance to TKI. The first is Small-cell lung cancer (SCLC) transformation. These transformed SCLC tumor cells retained the original EGFR mutation and they responded to SCLC-based platinum-etoposide therapy. The second form of transformation is epithelial-to-mesenchymal transition (EMT). In patients with EMT transformation the biopsy obtained after development of resistance to TKI showed acquired vimentin expression and loss of E-cadherin expression X. EMT has been shown in vitro to confer resistance to EGFR TKIs in NSCLC cell lines.

Afatinib (BIBW 2992)

Irreversible EGFR TKIs have a higher affinity for the EGFR kinase domain, and irreversible tyrosine kinase blockade may result in longer suppression of ERBB signaling than that resulting from reversible inhibitors. Second generation TKIs have modest in vitro activity against the T790M gatekeeper mutation and other rare mutations that render the first-generation reversible EGFR TKIs ineffective. Afatinib is an oral second generation irreversible HER family inhibitor that targets EGFR/HER-1, HER-2, and HER-4 with preclinical data supporting a role in overcoming resistance to reversible EGFR TKIs [29].

The role of afatinib in patients with NSCLC resistant to reversible TKIs has been explored in a number of clinical trials. LUX-Lung 1 was a phase IIb/III, randomized, double-blinded trial in patients with stage IIIB/IV lung adenocarcinoma who failed one or two chemotherapy treatments and progressed following ≥12 weeks of treatment with either erlotinib or gefitinib. LUX-Lung 1 patients ($N=585$) were randomized in a 2:1 ratio to best supportive care (BSC) plus afatinib (50 mg/day) or BSC plus placebo; the primary endpoint was overall survival. Majority of the patient population had clinical criteria for EGFR-activating mutations, with 58% Asian and

60% female patients. Prospective sequencing was not performed. Additionally, 81% of patients were previously treated with erlotinib or gefitinib for ≥24 weeks, with 45% having responded (PR or CR) to prior treatment. Primary analysis revealed median OS of 10.8 months for afatinib plus BSC and 12.0 months for placebo plus BSC (HR, 1.08; 95% CI, 0.86–1.35). Despite the lack of survival benefit, afatinib provided significantly better results in the secondary endpoints of PFS time (3.3 months vs. 1.1 months; HR, 0.38; $p < .0001$), disease control rate (DCR) at 8 weeks (58% vs. 19%; $p < .0001$), and objective RR (7.4% vs. 0.5% by independent analysis; $p < .01$) than with placebo [30].

Afatinib has also been evaluated as first-line and second-line therapy in patients who have not received a first-generation EGFR TKI. LUX-Lung 2 is a single-arm, multicenter, phase II trial evaluating the efficacy of afatinib (50 mg/day or 40 mg/day) in patients with stage IIIB/IV adenocarcinoma with EGFR mutations who had no more than one previous chemotherapy and no prior EGFR-targeted therapy. Of 129 patients who received treatment (first line, $n=61$; second line, $n=68$), 54 had L858R *EGFR* mutations, 52 had exon 19 deletions in *EGFR*, and 23 had other *EGFR* mutations. The objective ORR, median PFS interval, and median OS time were 61%, 10.1 months, and 24.8 months, respectively, for all patients. Afatinib had same level of activity in patients with exon 19 deletions and L858R mutations. The ORR, and median PFS were 63%, and 13.7 months, respectively, for patients with L858R mutations and 69%, and 13.7 months, respectively, for patients with exon 19 deletions [31].

Most recently, afatnib was tested in a phase III trial (LUX-Lung3) in patients with EGFR mutations and stage IIIB/IV chemo-naïve adenocarcinoma of the lung. Patients were randomized 2:1 to daily afatinib 40 mg or pemetrexed 500 mg/m^2 and cisplatin 75 mg/m^2 every 21 days up to 6 cycles. The primary endpoint was progression-free survival. Sixty-five percent of the study population were females, 72% Asian and 68% never-smoker. Forty-nine percent of the patients participated in the study had exon 19 deletions, 40% had L858R mutation and 11% had other mutations, Treatment with afatinib led to a significantly prolonged PFS versus chemotherapy, (median 11.1 versus 6.9 months; HR 0.58 [0.43–0.78]; $p=0.0004$). In 308 patients with the two common EGFR mutations exon 19 deletion and L858R, median PFS was 13.6 for patients treated with afatinib versus 6.9 months for the chemotherapy group, respectively (HR = 0.47 [0.34–0.65]; $p<0.0001$). Objective response rate was significantly higher for the afatinib group than the chemotherapy treated patients (56% vs. 23%; $p<0.0001$) [32]. This study was the first study to show improvement of PFS of first line treatment with EFGR TKI over cisplatin and pemetrexed.

Cetuximab

Cetuximab is a chimeric IgG1 monoclonal antibody that inhibits EGFR by binding to the extracellular domain, with more affinity than the natural ligands. Cetuximab has been studied extensively in NSCLC. In two phase II studies, combining cetuximab with chemotherapy (gemcitabine plus a platinum agent or vinorelbine plus cisplatin) resulted in increased response rates and PFS [33, 34]. Those promising results led

to two phase III trials for first line treatment of advanced NSCLC with standard chemotherapy with or without cetuximab. BMS099 recruited patients from the US and compared treatment with carboplatin and a Taxane (docetaxel or paclitaxel) chemotherapy with or without cetuximab. There was improvement of response rate (25.7% with cetuximab group versus 17.2% in chemotherapy only group). There was no significant difference between the two groups with regard to progression free survival (4.4 months vs. 4.24 months, HR 0.9, p=0.236) with slightly better but not statistically significant overall survival (9.69 months vs. 8.38 months with HR 0.890; p=0.169) [35]. The FLEX study is a second phase III study examining cisplatin and gemcitabine with or without cetuximab. The group of patients who received cetuximab in addition to chemotherapy had better overall survival than patients treated with chemotherapy, but only by about 1 month. Median survival was 11.3 months versus 10.1 months, respectively, hazard ratio, 0.87 [95% CI 0.762–0.996]; p=0.044 [36]. Because of the modest increase in survival, the cost associated with treatment and another negative phase III study, the results of the FLEX trial did not lead to FDA approval of cetuximab for treatment of NSCLC in the USA. A recent update of the FLEX study was presented at the 14th world conference on lung cancer in July 2011. Analysis of tumor EGFR expression (assessed with immunohistochemistry) showed that patients with high EGFR expression had better overall survival on cetuximab plus chemotherapy than chemotherapy alone. The median overall survival in the high expression group treated with cetuximab and chemotherapy was 12 months, compared with 9.6 months for patients treated with chemotherapy alone (hazard ratio, 0.73; p=0.011). One year survival was higher in the combination group than the chemotherapy group (50% vs. 37%) and 2-year survival was (24% vs. 15%). Patients with low EGFR expression showed no benefit from the addition of cetuximab to chemotherapy [37]. Although this is an important observation, a prospective validation is needed before it considering a standard of care.

Anaplastic Lymphoma Kinase (ALK)

The fusion between echinoderm microtubules-associated protein like 4 (EML 4) and anaplastic lymphoma kinase (ALK) as a result of a small inversion of the short arm of chromosome 2 has recently emerged as an important genetic marker in subset of NSCLC patients. EML4-ALK was identified in Japanese patients in 2007 [38], and reported to be present in 3–13% according to patient population ethnic origin. EML4-ALK has a characteristic epidemiologic and histological pattern. It is more common in never smoker patients than smokers. In Asian patients with EML4-Alk positive NSCLC the predominant histological type is an acinar pattern of adenocarcinoma while signet cell histology is reported in majority of western patients [39]. EML4-ALK tyrosine kinase activity is highly oncogenic and can induce cellular transformation [40]. EML4-ALK inhibition in vitro leads to reduced proliferation

and increased apoptosis in lung cancer cell lines harboring EML4-Alk; also it causes significant reduction in tumor size in pre-clinical models [41, 42].

Crizotinib (Formally, PF-02341066) is the most studied ALK inhibitor. It has a kinase inhibitory effect against ALK and MET. In a phase I trial crizotinib was studied in patients with advanced NSCLC and other solid tumors with ALK or MET activation. In the NSCLC group the response rate was 53% and disease control rate was 79% at 8 weeks [43]. A phase I trial dedicated to NSCLC included 82 patients with NSCLC harboring ALK mutations were treated with crizotinib 250 mg bid, based on the results of the lead dose escalation phase of the same study. At a mean treatment duration of 6.4 months, the overall response rate was 57% (47 of 82 patients, with 46 confirmed partial responses and 1 confirmed complete response); 27 patients (33%) had stable disease. A total of 63 of 82 patients (77%) were continuing to receive crizotinib at the time of study publication, and the estimated probability of 6-month progression-free survival was 72%, and no median survival was reached [44]. A recent update of the same study presented in abstract form, showed that the 1 year survival for the same cohort of patients was 74% and 2-year survival was 54%, and the median survival has not yet been reached at time of presentation. Overall survival did not differ based on age, sex, smoking history or ethnicity. There was a significant difference in survival in NSCLC patients with ALK mutations treated with crizotinib in comparison with a selected historical control group of patients with ALK mutations not treated with crizotinib ($p = 0.004$) [45].

Another presentation for expanded data from the same study included 119 patients with median follow up of 11 months. The overall response rate in that group of patients was 61% with median response duration of 48 weeks and disease control rates at 8 weeks was 79% and at 16 weeks was 67%. Median time to response was 8 weeks but responses were seen as early as 2 weeks. Median progression free-survival reported to be 10 months. Median survival has not reached at the time of the presentation but the 6 months probability of survival was 90% (95% CI 82.7–94.4%) and 12 months probability of survival was 81% (95% CI 70.9–87.2) [46].

The promising results from early phase studies for EML4-ALK positive NSCLC patients led to the design of phase II and III clinical trials. PROFILE 1005 is a large phase II trial that will enroll 400 patients who received two or more prior lines of treatment. The preliminary data from the PROFILE 1005 trial consisted of 136 patients from 12 countries who had progressed on prior treatments and included patients with brain metastases. Eighty-eight percent of the patients are still on the treatment after a median of 9 weeks of treatment. Overall response rate was 83%, with 50% having had a partial response and 33% with stable disease [47]. Other ongoing studies are PROFILE 1007 which will include patients who progressed after first line of chemotherapy. Those patients are randomized between standard second line treatment with pemetrexed or docetaxel versus crizotinib. PROFILE 1008 is a phase III study to test crizotinib as first line treatment versus a combination of pemetrexed and cisplatin or carboplatin. In August 2011 the FDA granted accelerated approval to crizotinib for patients with metastatic and locally advanced non-small cell lung cancer that tested positive for ALK.

Resistance to Crizotinib

In vitro resistance to crizotinib developing after exposure of cell lines to high concentrations of crizotinib has been described [48]. Two different mechanisms of resistance were identified, which include amplification of EML4-ALK and development of a gatekeeper mutation, L1196M within the kinase domain. Novel ALK inhibitors (NVP-TAE684 and AP26113) were able to overcome resistance to crizotinib in vitro. Interestingly, adding the heat shock protein 90 (HSP90) inhibitor 17-AAG to crizotinib restored sensitivity of resistant cells to crizotinib [48]. This observation of the sensitivity of ALK positive lung cancer to HSP90 inhibitor was confirmed in a recent clinical trial [49]. Other mechanism of crizitonib resistance have been identified including ALK copy number gain, novel mutation in the ALK domain encoding a G1269A amino acid substitution, and development of new driver mutations such as EGFR and Kras [50].

Mesenchymal-Epithelial Transition Factor (MET)

MET is a receptor tyrosine kinase encoded by the proto-oncogene, *c-MET*. Once the extracellular domain of MET is bound by its high affinity ligand, hepatocyte growth factor (HGF), the MET signaling pathway is activated and involved in a variety of physiologic processes with direct or indirect involvement in oncogenesis. Those processes include angiogenesis, tumor cell proliferation, survival, resistance to apoptosis, aggressive cellular invasion, and metastasis [51]. A subset of NSCLC patients harbor deregulated MET (including its overexpression, constitutive activation, gene amplification, ligand-dependent activation, or mutation) [52]. Acquired resistance to epidermal growth factor receptor (EGFR) inhibitors in NSCLC may be achieved through *c-MET* gene amplification, leading to MET activation and MET-dependent phosphorylation of HER3. Phosphorylated HER3 recruits phosphoinositide 3-kinase (PI3K) and stimulates PI3K-based survival pathways, causing resistance to EGFR inhibitors [53]. Inhibition of MET signaling in these resistant cells may potentially restore sensitivity to EGFR inhibitors.

Several agents have been developed to target MET in lung cancer and other types of tumors. In NSCLC most of the clinical trials aimed to combine both MET and EGFR inhibition in efforts to overcome resistance to anti-EGFR therapy. Tivantinib (Formally, ARQ197) and MetMab are the two agents that are in advanced stages of clinical trials. Tivantinib is a small molecule which specifically bind to inactive MET and prevent its activation. In dose finding phase I clinical trial in combination with erlotinib, the tivantinib dose was escalated to 360 mg bid in addition to the approved dose of erlotinib at 150 mg per day without significant dose limiting toxicity and that dose was recommended for phase II study [54]. A double-blind phase II clinical trial evaluated EGFR inhibitor naive patients comparing erlotinib plus tivantinib with erlotinib plus placebo in previously treated unselected patients with

chemotherapy. The primary study endpoint was PFS, results demonstrated that median time PFS was 3.8 months in the combination arm versus 2.3 months in the erlotinib and placebo arm (hazard ratio, 0.81; 95% CI, 0.57–1.16; $P = .24$). Although the study did not meet its primary end point, subset analysis showed a benefit for patients with nonsquamous histology, KRAS mutations and increased MET gene copy number. The findings from this phase II study led to a Phase III trial of tivantinib in combination with erlotinib, for patients diagnosed with non-squamous, NSCLC who have received one or two prior systemic therapies [55].

MetMab is a monovalent monoclonal antibody designed to bind specifically to MET and to inhibit the downstream signaling pathway. A combination of MetMab and erlotinib showed synergism and in overcoming resistance to erlotinib in cell lines. A phase II study was conducted, comparing a combination of MetMab plus erlotinib to erlotinib plus placebo in unselected patients who had received one or two prior treatments. PFS and OS were not statistically different between the two study arms. MET overexpression by immunohistochemistry is known as a negative prognostic factor and it correlated with worse outcomes in the erlotinib and placebo arms. In patients with MET overexpression who were treated with erlotinib and MetMAb, there was improvement inPFS and OS [56]. A Phase III study for selected patients with MET overexpression is in the planning stages.

ROS1

ROS1 rearrangement is the most recent identified driver mutation in adenocarcinoma of the lung. ROS1 is a receptor tyrosine kinase that belongs to a subfamily of insulin receptor genes, it is encoded by *ROS1* gene on chromosome 6. Chromosomal rearrangements involving the *ROS1* gene were originally described in glioblastoma multiforme, where *ROS1* (chromosome 6q22) is fused to the *FIG* (*F*used *i*n *G*lioblastoma) gene (chromosome 6q22 immediately adjacent to *ROS1*) [57]. These chromosomal rearrangements produce fusion proteins that lead to constitutive kinase activity with ability to transform cell in transgenic mice [58]. *ROS1* fusions were identified as potential driver mutations in NSCLC cell line and an NSCLC patient sample [59].

In a recent study ROS1 rearrangements have been found in 18 out of 1,073 patients (incidence of 1.7%) with adenocarcinoma of the lung using fluorescence in situ hybridization. Patients with lung cancer whose tumors harbored *ROS1* rearrangements were more likely to have adenocarcinoma, to be Asian, younger and never smokers. Those clinical features are similar to the clinical features seen in patients with both *EGFR* mutations and *ALK* rearrangements [60].

Currently there is no ROS1 targeted agents in clinical trials but preclinical studies have shown ability of the ALK inhibitor TAE684 to inhibit ROS1 as an off-target effect. Crizotinib used to treat a patient with NSCLC whose tumor had a *ROS1* rearrangement; the patient had a remarkable clinical response and near-complete radiographic response to crizotinib [60]. This case report confirms the role of ROS1

rearrangements in development of NSCLC and the therapeutic benefit of specific targeted ROS1 therapy. However, additional studies with larger numbers of patients will be required before a definitive conclusion can be reached regarding crizotinib treatment for *ROS1*-rearranged NSCLC.

Impact of the Tumor Microenvironment and Cancer Stem Cell

The tumor microenvironment is composed of a mixture of connective tissue elements, blood and lymphatic vessels, fibroblasts and infiltrating immune cells. Complex interactions between tumor cells and microenvironment promote tumor cells survival by regulating tumor growth, progression, metastasis and angiogenesis. Tumor growth and metastasis formation are dependent on the growth of newly formed blood vessels into the tumor mass. Targeting angiogenesis by inhibiting vascular endothelial growth factor (VEGF) has been proven to be an effective treatment for lung cancer and other types of cancer. VEGF, a 45 kDa glycoprotein encoded for by a gene located at chromosome 6 (6p21) [61]. At least five members of the VEGF family have been described. These are VEGF-A to E, where VEGF-A is the most important VEGF for angiogenesis. All VEGF isoforms are capable of binding to the receptor tyrosine kinases VEGFR1 and VEGFR2. VEGF is one of the most potent mitogenic factors for endothelial cells; it increases their proliferation, migration and vascular permeability High level of VEGF correlated with poor prognosis in patients with NSCLC. Bevacizumab is a humanized monoclonal antibody directed against VEGF [62]. VEGF gene polymorphisms influence VEGF functional expression and molecular VEGF phenotype and intratumoral angiogenesis in non-small cell lung cancer. The effect of VEFG polymorphisms has been explored in a recent retrospective review of survival date for a group of 462 patients, patients carrying variant C allele of VEGF gene (+ 405 G>C) polymorphism had 61% 5-year survival (95% CI, 54–67%) versus 51% (95%CI, 43–59%) for patients who had wild-type variant [63].

Bevacizumab

Bevacizumab is humanized IgG1monoclonal antibody that binds to all biologically active isoforms of VEGF. Preclinical work with bevacizumab showed that neutralization of VEGF led to inhibition of tumor cell proliferation in vitro and decrease in microvessel count in tumor xenograft. In a phase II study 99 patients with non-small cell lung cancer were randomized between three arms, a control arm assigned to chemotherapy with carboplatin and paclitaxel and two experimental arms using bevacizumab at two different doses 7.5 mg/kg and 15 mg/kg. Treatment with chemotherapy plus bevacizumab at dose of 15 mg/kg resulted in higher response rate compared with control arm (31.5% vs. 18.8%), longer time to progression

(7.4 m vs. 4.2 m) and a modest increase in survival (17.7 m vs. 14.9 m). Patients on the control arm were allowed to receive bevacizumab at a dose of 15 mg/kg on disease progression. Of the 19 patients who crossed over to bevacizumab therapy, five patients experienced stable disease, and the 1-year survival rate was 47% [64]. E4599 was the first study to show increase of overall survival and time to progression for patient with metastatic or locally advanced non-small cell lung cancer by adding biological agent to chemotherapy. In that study 878 patients were randomized for treatment with six cycles of carboplatin and paclitaxel as the standard arm and patients on the experimental arm were treated with six cycles of carboplatin and paclitaxel plus bevacizumab, then they continued with bevacizumab as a maintenance therapy for 1 year or until progression. The group of patients received bevacizumab had median survival of 12.3 months, as compared to 10.6 months in the standard chemotherapy group; hazard ratio 0.79; $p=0.003$, median disease progression free survival in the two groups was 6.2 and 4.5 months respectively with a hazard ratio for progression 0.66; $p<0.001$. There were increased number of grade three and four toxicities associated with bevacizumab treatment, hematological toxicities (neutropenia and thrombocytopenia) were significantly higher in the bevacizumab group, hypertension was higher in bevacizumab group 0.7% versus 7%; $p<0.001$, proteinuria none versus 3.1%; $p<0.001$, and bleeding 0.7% versus 4.4%; $p<0.001$. Seven patients on the study died from bleeding, five from hemoptysis and two from hematemesis [65]. Recently published data from the AVAiL trial confirmed clinical efficacy of bevacizumab in combination with cisplatin and gemcitabine in patients with stage IIIB/IV NSCLC; however, OS benefit favoring bevacizumab did not reach statistical significance [66].

Other strategies to target angiogenesis in lung cancer are under study. Small molecules targeting VEGFR or VEGF trap such as aflibercept are in different stages in clinical trials.

Other Targets in Tumor Microenvironment

T regulatory cells (Tregs) FOXP3+ CD4+ are a subgroup of the immune cells that infiltrate lung cancer. These cells are a unique subset of T lymphocytes that play an critical function in maintaining immune homeostasis and protecting the host against autoimmune diseases. Tregs suppress antitumor cytotoxic T-cell response in cancer patients. Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) is an immune checkpoint molecule that down-regulates pathways of T-cell activation . Ipiliumumab a humanized monoclonal antibody that targets CTLA-4 results in augmenting T-cell activation and proliferation [67]. Ipilimumab monotherapy has shown improvement in survival of patients with metastatic malignant melanoma [68]. In phase II study administration of phased ipilimumab regimen (two cycles of paclitaxel and carboplatin followed by four doses of ipilimumab plus paclitaxel and carboplatin) has shown promising results with improvement of immune-related progression-free survival [69]. Another promising immunotherapeutic approach for patients with

NSCLC is by targeting Programmed death 1 (PD-1) which is a key immune-checkpoint receptor expressed by activated T cells, and it mediates immunosuppression. Monoclonal antibodies directed to PD-1 or it ligand PD-L1 have shown activity for NSCLC in phase I studies [70, 71]. Further studies are needed to validate the benefit of those novel immune therapeutic in treatment of NSCLC.

Future Directions

The identification of genetic targets in NSCLC allowed us to have a better understanding of their biological role and to develop targeted therapeutic agents. The recent clinical trials that showed benefits for selected populations based on genetic targets represent a step toward delivering personalized therapy. Besides the genetic targets reviewed above there are other targets that are mutated, amplified or translocated in NSCLC such as Kras, HER2/neu, BRAF, PI3K, PDGFR and others [72]. A more global approach to identify genetic targets in newly diagnosed NSCLC patients was reported by the lung cancer mutation consortium. Fifty-four percent of the patients on the study had identifiable driver mutations at time of diagnosis. Those patients were placed on clinical trials targeting their driver mutations. Identification of the unknown biological characteristics of the remaining 46% is an area of active research [73]. Those efforts can lead us to understand the full mutational spectrum of oncogenes and tumor suppressor genes in NSCLC and offer specific treatment for every patient based on their tumor genotype.

Our current technology used to identify genetic targets such as in situ hybridization is sensitive but it is time consuming, not widely available, and not cost effective. There is a need to develop strategies to detect genetic targets that are rapid and cost-effective. The development of novel antibodies that are reliable to detect an abnormal protein in the tumor tissue which is not normally expressed in the lung tissue. This could be applied to ALK and any other abnormal proteins that will be identified in the future. Such test could be integrated in the immunohistochemistry panel used as a routine for new case of NSCLC. Advances in technology will lead to the development tools to screen cancer specimens simultaneously for a variety of genomic alterations including mutations, copy number changes, and chromosomal rearrangements.

Identification of mechanisms of resistance to targeted therapy and developing novel strategies to overcome that resistance and restore sensitivity to targeted agents is another area of intense research with several promising agents in various stages in development such as second generation EGFR TKIs, MEK inhibitors and other ALK inhibitors.

Despite the remarkable advances in treatment of adenocarcinoma of the lung in the last few years, little progress has been achieved in the other subtypes of the NSCLC. Treatment of squamous cell carcinoma (SCC) of the lung has lagged behind, because of a lack of understanding of the oncogenes driving SCC. There are several mutations identified in SCC such as FGFR1, PI3K and DDr2 and early

phase clinic trails with targeted agents are undergoing. The emerging data from the cancer genome atlas project [74] that characterize the genetic and epigenetic changes in SCC are promising and will help to advance our understanding of the genomic changes in SCC.

Conclusions

Identification of genetic targets and designing specific therapeutic agents had great impact on the treatment of patients with NSCLC by improving PFS, OS and quality of life. The newly developed therapeutic agents that target mutated EGFR and ALK translocation offer great help to about 20% of newly diagnosed case of NSCLC, approximately 40,000 patients, in the USA every year [75].

Despite the significant advances in understanding the biology of NSCLC, treatment of NSCLC remains challenging and more novel therapeutic approaches are needed.

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics. CA Cancer J Clin. 2012;62:10–29.
2. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005;55:74–108.
3. Sharma SV, Bell DW, Settleman J, Haber DA. Epidermal growth factor receptor mutations in lung cancer. Nat Rev. 2007;7:169–81.
4. Fukuoka M, Yano S, Giaccone G, Tamura T, Nakagawa K, Douillard JY, Nishiwaki Y, Vansteenkiste J, Kudoh S, Rischin D, Eek R, Horai T, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial) [corrected]. J Clin Oncol. 2003;21:2237–46.
5. Kris MG, Natale RB, Herbst RS, Lynch Jr TJ, Prager D, Belani CP, Schiller JH, Kelly K, Spiridonidis H, Sandler A, Albain KS, Cella D, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. JAMA. 2003;290:2149–58.
6. Thatcher N, Chang A, Parikh P, Rodrigues Pereira J, Ciuleanu T, von Pawel J, Thongprasert S, Tan EH, Pemberton K, Archer V, Carroll K. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). Lancet 2005;366:1527–37.
7. Kim ES, Hirsh V, Mok T, Socinski MA, Gervais R, Wu YL, Li LY, Watkins CL, Sellers MV, Lowe ES, Sun Y, Liao ML, et al. Gefitinib versus docetaxel in previously treated non-small-cell lung cancer (INTEREST): a randomised phase III trial. Lancet. 2008;372:1809–18.
8. Giaccone G, Herbst RS, Manegold C, Scagliotti G, Rosell R, Miller V, Natale RB, Schiller JH, Von Pawel J, Pluzanska A, Gatzemeier U, Grous J, et al. Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial–INTACT 1. J Clin Oncol. 2004;22:777–84.
9. Herbst RS, Giaccone G, Schiller JH, Natale RB, Miller V, Manegold C, Scagliotti G, Rosell R, Oliff I, Reeves JA, Wolf MK, Krebs AD, et al. Gefitinib in combination with paclitaxel and

carboplatin in advanced non-small-cell lung cancer: a phase III trial–INTACT 2. J Clin Oncol. 2004;22:785–94.
10. Lee DH, Han JY, Lee HG, Lee JJ, Lee EK, Kim HY, Kim HK, Hong EK, Lee JS. Gefitinib as a first-line therapy of advanced or metastatic adenocarcinoma of the lung in never-smokers. Clin Cancer Res. 2005;11:3032–7.
11. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, Han B, Margono B, Ichinose Y, Nishiwaki Y, Ohe Y, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med. 2009;361:947–57.
12. Fukuoka M, Wu YL, Thongprasert S, Sunpaweravong P, Leong SS, Sriuranpong V, Chao TY, Nakagawa K, Chu DT, Saijo N, Duffield EL, Rukazenkov Y, et al. Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). J Clin Oncol. 2011;29:2866–74.
13. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, Gemma A, Harada M, Yoshizawa H, Kinoshita I, Fujita Y, Okinaga S, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med. 2010;362:2380–8.
14. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, Seto T, Satouchi M, Tada H, Hirashima T, Asami K, Katakami N, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol. 2010;11:121–8.
15. Perez-Soler R, Chachoua A, Hammond LA, Rowinsky EK, Huberman M, Karp D, Rigas J, Clark GM, Santabarbara P, Bonomi P. Determinants of tumor response and survival with erlotinib in patients with non–small-cell lung cancer. J Clin Oncol. 2004;22:3238–47.
16. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Thongprasert S, Campos D, Maoleekoonpiroj S, Smylie M, Martins R, van Kooten M, Dediu M, et al. Erlotinib in previously treated non-small-cell lung cancer. N Engl J Med. 2005;353:123–32.
17. Bezjak A, Tu D, Seymour L, Clark G, Trajkovic A, Zukin M, Ayoub J, Lago S, de Albuquerque Ribeiro R, Gerogianni A, Cyjon A, Noble J, et al. Symptom improvement in lung cancer patients treated with erlotinib: quality of life analysis of the National Cancer Institute of Canada Clinical Trials Group Study BR.21. J Clin Oncol 2006; 24:3831–7.
18. Ciuleanu T, Stelmakh L, Cicenas S, Miliauskas S, Grigorescu AC, Hillenbach C, Johannsdottir HK, Klughammer B, Gonzalez EE. Efficacy and safety of erlotinib versus chemotherapy in second-line treatment of patients with advanced, non-small-cell lung cancer with poor prognosis (TITAN): a randomised multicentre, open-label, phase 3 study. Lancet Oncol. 2012;13(3):300–8.
19. Herbst RS, Prager D, Hermann R, Fehrenbacher L, Johnson BE, Sandler A, Kris MG, Tran HT, Klein P, Li X, Ramies D, Johnson DH, et al. TRIBUTE: a phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small-cell lung cancer. J Clin Oncol. 2005;23:5892–9.
20. Gatzemeier U, Pluzanska A, Szczesna A, Kaukel E, Roubec J, De Rosa F, Milanowski J, Karnicka-Mlodkowski H, Pesek M, Serwatowski P, Ramlau R, Janaskova T, et al. Phase III study of erlotinib in combination with cisplatin and gemcitabine in advanced non-small-cell lung cancer: the Tarceva Lung Cancer Investigation Trial. J Clin Oncol. 2007;25:1545–52.
21. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, Zhou S, Ren S, Lu S, Zhang L, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol. 2012;12(8):735–42.
22. R. Rosell RG, Vergnenegre A, Massuti B, Felip E, Cardenal F, Garcia Gomez R, Pallares C, Sanchez JM, Porta R, Cobo M, Di Seri M, Garrido Lopez P, Insa A, De Marinis F, Corre R, Carreras M, Carcereny E, Taron M, Paz-Ares LG. Erlotinib versus chemotherapy (CT) in advanced non-small cell lung cancer (NSCLC) patients (p) with epidermal growth factor receptor (EGFR) mutations: Interim results of the European Erlotinib Versus Chemotherapy (EURTAC) phase III randomized trial. Lancet Oncolo. 2102; 13(3):239–46.
23. Miller VA, O'Connor P, Soh C, Kabbinavar F, for the ATLAS Investigators; Memorial Sloan-Kettering Cancer Center, New York, NY; Genentech, Inc., South San Francisco, CA; University

of California, Los Angeles, Los Angeles, CA. A randomized, double-blind, placebo-controlled, phase IIIb trial (ATLAS) comparing bevacizumab (B) therapy with or without erlotinib (E) after completion of chemotherapy with B for first-line treatment of locally advanced, recurrent, or metastatic non-small cell lung cancer (NSCLC). J Clin Oncol. 2009;27:18s. suppl; abstr LBA8002.
24. Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, Kris MG, Varmus H. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS medicine. 2005;2:e73.
25. Engelman JA, Janne PA. Mechanisms of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. Clin Cancer Res. 2008;14:2895–9.
26. Yun CH, Mengwasser KE, Toms AV, Woo MS, Greulich H, Wong KK, Meyerson M, Eck MJ. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. Proc Nat Acad Sci USA. 2008;105:2070–5.
27. Inukai M, Toyooka S, Ito S, Asano H, Ichihara S, Soh J, Suehisa H, Ouchida M, Aoe K, Aoe M, Kiura K, Shimizu N, et al. Presence of epidermal growth factor receptor gene T790M mutation as a minor clone in non-small cell lung cancer. Cancer Res. 2006;66:7854–8.
28. Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, Bergethon K, Shaw AT, Gettinger S, Cosper AK, Akhavanfard S, Heist RS, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med. 2011;3:75ra26.
29. Li D, Ambrogio L, Shimamura T, Kubo S, Takahashi M, Chirieac LR, Padera RF, Shapiro GI, Baum A, Himmelsbach F, Rettig WJ, Meyerson M, et al. BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models. Oncogene. 2008;27:4702–11.
30. Miller VA, Hirsh V, Cadranel J, Chen YM, Park K, Kim SW, Zhou C, Su WC, Wang M, Sun Y, Heo DS, Crino L, et al. Afatinib versus placebo for patients with advanced, metastatic non-small-cell lung cancer after failure of erlotinib, gefitinib, or both, and one or two lines of chemotherapy (LUX-Lung 1): a phase 2b/3 randomised trial. Lancet Oncol. 2012;13:528–38.
31. Yang JC, Shih JY, Su WC, Hsia TC, Tsai CM, Ou SH, Yu CJ, Chang GC, Ho CL, Sequist LV, Dudek AZ, Shahidi M, et al. Afatinib for patients with lung adenocarcinoma and epidermal growth factor receptor mutations (LUX-Lung 2): a phase 2 trial. Lancet Oncol. 2012;13:539–48.
32. James Chih-Hsin Yang MHS, Yamamoto N, O'Byrne KJ, Hirsh V, Mok T, Geater SL, Orlov SV, Tsai C-M, Boyer MJ, Su W-C, Bennouna J, Kato T, Gorbunova V, Lecia V. LUX-Lung 3: a randomized, open-label, phase III study of afatinib versus pemetrexed and cisplatin as first-line treatment for patients with advanced adenocarcinoma of the lung harboring EGFR-activating mutations. J Clin Oncol. 2012;30 (suppl; abstr LBA7500).
33. Butts CA, Bodkin D, Middleman EL, Englund CW, Ellison D, Alam Y, Kreisman H, Graze P, Maher J, Ross HJ, Ellis PM, McNulty W, et al. Randomized phase II study of gemcitabine plus cisplatin or carboplatin [corrected], with or without cetuximab, as first-line therapy for patients with advanced or metastatic non small-cell lung cancer. J Clin Oncol. 2007;25:5777–84.
34. Rosell R, Robinet G, Szczesna A, Ramlau R, Constenla M, Mennecier BC, Pfeifer W, O'Byrne KJ, Welte T, Kolb R, Pirker R, Chemaissani A, et al. Randomized phase II study of cetuximab plus cisplatin/vinorelbine compared with cisplatin/vinorelbine alone as first-line therapy in EGFR-expressing advanced non-small-cell lung cancer. Ann Oncol. 2008;19:362–9.
35. Lynch TJ, Patel T, Dreisbach L, McCleod M, Heim WJ, Hermann RC, Paschold E, Iannotti NO, Dakhil S, Gorton S, Pautret V, Weber MR, et al. Cetuximab and first-line taxane/carboplatin chemotherapy in advanced non-small-cell lung cancer: results of the randomized multicenter phase III trial BMS099. J Clin Oncol. 2010;28:911–7.
36. Pirker R, Pereira JR, Szczesna A, von Pawel J, Krzakowski M, Ramlau R, Vynnychenko I, Park K, Yu CT, Ganul V, Roh JK, Bajetta E, et al. Cetuximab plus chemotherapy in patients with advanced non-small-cell lung cancer (FLEX): an open-label randomised phase III trial. Lancet. 2009;373:1525–31.
37. Pirker R, Pereira JR, von Pawel J, Krzakowski M, Ramlau R, Park K, de Marinis F, Eberhardt WE, Paz-Ares L, Storkel S, Schumacher KM, von Heydebreck A, 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. 2012;13:33–42.
38. Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, Fujiwara S, Watanabe H, Kurashina K, Hatanaka H, Bando M, Ohno S, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature. 2007;448:561–6.
39. Shaw AT, Yeap BY, Mino-Kenudson M, Digumarthy SR, Costa DB, Heist RS, Solomon B, Stubbs H, Admane S, McDermott U, Settleman J, Kobayashi S, et al. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. J Clin Oncol. 2009;27:4247–53.
40. Soda M, Takada S, Takeuchi K, Choi YL, Enomoto M, Ueno T, Haruta H, Hamada T, Yamashita Y, Ishikawa Y, Sugiyama Y, Mano H. A mouse model for EML4-ALK-positive lung cancer. Proc Natl Acad Sci USA. 2008;105:19893–7.
41. Settleman J. Cell culture modeling of genotype-directed sensitivity to selective kinase inhibitors: targeting the anaplastic lymphoma kinase (ALK). Semin Oncol. 2009;36:S36–41.
42. McDermott U, Iafrate AJ, Gray NS, Shioda T, Classon M, Maheswaran S, Zhou W, Choi HG, Smith SL, Dowell L, Ulkus LE, Kuhlmann G, et al. Genomic alterations of anaplastic lymphoma kinase may sensitize tumors to anaplastic lymphoma kinase inhibitors. Cancer Res. 2008;68:3389–95.
43. Kwak DRC EL, Clark J, Shapiro GI, Maki RG, Ratain MJ, Solomon B, Bang Y, S.Ou R, et al. Clinical activity observed in a phase I dose escalation trial of an oral c-met and ALK inhibitor, PF-02341066. J Clin Oncol. 2009;27:15s (suppl; abstr 3509).
44. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, Ou SH, Dezube BJ, Janne PA, Costa DB, Varella-Garcia M, Kim WH, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med. 2010;363:1693–703.
45. AT Shaw BYY, Solomon BJ, Riely GJ, Iafrate AJ, Shapiro G, Costa DB, Butaney M, Ou SI, Maki RG, Bang Y, Varella-Garcia M, Salgia R, Wilner KD, Kulig K, Selaru P, Tang Y, Kwak EL, Clark JW, Camidge DR. Impact of crizotinib on survival in patients with advanced, ALK-positive NSCLC compared with historical controls. J Clin Oncol. 2011;29 (suppl; abstr 7507).
46. DR Camidge YB, Kwak EL, Shaw AT, Iafrate AJ, Maki RG, Solomon BJ, Ou SI, Salgia R, Wilner KD, Costa DB, Shapiro G, LoRusso P, Stephenson P, Tang Y, Ruffner K, Clark JW. Progression-free survival (PFS) from a phase I study of crizotinib (PF-02341066) in patients with ALK-positive non-small cell lung cancer (NSCLC). J Clin Oncol. 2011;29 (suppl; abstr 2501).CED.
47. L Crinò DK, Riely GJ, Janne PA, Blackhall FH, Camidge DR, Hirsh V, Mok T, Solomon BJ, Park K, Gadgeel SM, Martins R, Han J, De Pas TM, Bottomley A, Polli A, Petersen J, Tassell VR, Shaw AT. Initial phase II results with crizotinib in advanced ALK-positive non-small cell lung cancer (NSCLC): PROFILE 1005. J Clin Oncol. 2011;29 (suppl; abstr 7514).
48. Katayama R, Khan TM, Benes C, Lifshits E, Ebi H, Rivera VM, Shakespeare WC, Iafrate AJ, Engelman JA, Shaw AT. Therapeutic strategies to overcome crizotinib resistance in non-small cell lung cancers harboring the fusion oncogene EML4-ALK. Proc Natl Acad Sci USA. 2011;108:7535–40.
49. Wong K, Koczywas M, Goldman JW, Paschold EH, Horn L, Lufkin JM, Blackman F, Teofilovici G, Shapiro M. An open-label phase II study of the Hsp90 inhibitor ganetespib (STA-9090) as monotherapy in patients with advanced non-small cell lung cancer (NSCLC). J Clin Oncol. 2011;29 (suppl; abstr 7500).
50. Doebele RC, Pilling AB, Aisner DL, Kutateladze TG, Le AT, Weickhardt AJ, Kondo KL, Linderman DJ, Heasley LE, Franklin WA, Varella-Garcia M, Camidge DR. Mechanisms of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. Clin Cancer Res. 2012;18:1472–82.
51. Gentile A, Trusolino L, Comoglio PM. The met tyrosine kinase receptor in development and cancer. Cancer Metast Rev. 2008;27:85–94.
52. Ma PC, Jagadeeswaran R, Jagadeesh S, Tretiakova MS, Nallasura V, Fox EA, Hansen M, Schaefer E, Naoki K, Lader A, Richards W, Sugarbaker D, et al. Functional expression and

mutations of c-Met and its therapeutic inhibition with SU11274 and small interfering RNA in non-small cell lung cancer. Cancer Res. 2005;65:1479–88.
53. Bean J, Brennan C, Shih JY, Riely G, Viale A, Wang L, Chitale D, Motoi N, Szoke J, Broderick S, Balak M, Chang WC, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. Proc Nat Acad Sci USA. 2007;104:20932–7.
54. I Laux JG, Just R, Brady K, Li J, Schwartz B, Savage R, et al. Phase I dose escalation trial (ARQ 197-111) evaluating combination of selective c-Met inhibitor ARQ 197 and erlotinib. J Clin Oncol. 2009;27:15s (suppl; abstr 3549).
55. Sequist LV, von Pawel J, Garmey EG, Akerley WL, Brugger W, Ferrari D, et al. Randomized phase II study of erlotinib plus tivantinib versus erlotinib plus placebo in previously treated non-small-cell lung cancer. J Clin Oncol. 2011;29(24):3307–15.
56. Spigel DR, Ervin TJ, Ramlau R, Daniel DB, Goldschmidt JH, Blumenschein GR, et al. Final efficacy results from OAM4558g, a randomized phase II study evaluating MetMAb or placebo in combination with erlotinib in advanced NSCLC. J Clin Oncol. 2011;29 (suppl; abstr 7505).
57. Charest A, Lane K, McMahon K, Park J, Preisinger E, Conroy H, Housman D. Fusion of FIG to the receptor tyrosine kinase ROS in a glioblastoma with an interstitial del(6)(q21q21). Gene Chromosome Canc. 2003;37:58–71.
58. Charest A, Wilker EW, McLaughlin ME, Lane K, Gowda R, Coven S, McMahon K, Kovach S, Feng Y, Yaffe MB, Jacks T, Housman D. ROS fusion tyrosine kinase activates a SH2 domain-containing phosphatase-2/phosphatidylinositol 3-kinase/mammalian target of rapamycin signaling axis to form glioblastoma in mice. Cancer Res. 2006;66:7473–81.
59. Rikova K, Guo A, Zeng Q, Possemato A, Yu J, Haack H, Nardone J, Lee K, Reeves C, Li Y, Hu Y, Tan Z, et al. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell. 2007;131:1190–203.
60. Bergethon K, Shaw AT, Ou SH, Katayama R, Lovly CM, McDonald NT, Massion PP, Siwak-Tapp C, Gonzalez A, Fang R, Mark EJ, Batten JM, et al. ROS1 rearrangements define a unique molecular class of lung cancers. J Clin Oncol. 2012;30:863–70.
61. Ferrara N, Henzel WJ. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochem Bioph Res Co. 1989;161:851–8.
62. Salven P, Ruotsalainen T, Mattson K, Joensuu H. High pre-treatment serum level of vascular endothelial growth factor (VEGF) is associated with poor outcome in small-cell lung cancer. Int J Cancer. 1998;79:144–6.
63. Heist RS, Zhai R, Liu G, Zhou W, Lin X, Su L, Asomaning K, Lynch TJ, Wain JC, Christiani DC. VEGF polymorphisms and survival in early-stage non-small-cell lung cancer. J Clin Oncol. 2008;26:856–62.
64. Johnson DH, Fehrenbacher L, Novotny WF, Herbst RS, Nemunaitis JJ, Jablons DM, Langer CJ, De Vore 3rd RF, Gaudreault J, Damico LA, Holmgren E, Kabbinavar F. Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. J Clin Oncol. 2004;22:2184–91.
65. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, Lilenbaum R, Johnson DH. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med. 2006;355:2542–50.
66. Reck M, Von Pawel J, Zatloukal P, Ramlau R, Gorbounova V, Hirsh V, Leighl N, Mezger J, Archer V, Moore N, Manegold C. Overall survival with cisplatin-gemcitabine and bevacizumab or placebo as first-line therapy for nonsquamous non-small-cell lung cancer: results from a randomised phase III trial (AVAiL). Ann Oncol. 2010;21:1804–9.
67. Melero I, Hervas-Stubbs S, Glennie M, Pardoll DM, Chen L. Immunostimulatory monoclonal antibodies for cancer therapy. Nat Rev. 2007;7:95–106.
68. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, Van Den Eertwegh AJ, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363:711–23.

69. Lynch TJ, Bondarenko I, Luft A, Serwatowski P, Barlesi F, Chacko R, Sebastian M, Neal J, Lu H, Cuillerot JM, Reck M. 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.
70. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366:2455–65.
71. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366:2443–54.
72. Sharma SV, Haber DA, Settleman J. Cell line-based platforms to evaluate the therapeutic efficacy of candidate anticancer agents. Nat Rev. 2010;10:241–53.
73. Kris MG, Johnson BE, Kwiatkowski DJ, Iafrate AJ, Wistuba II, Aronson SL, et al. Identification of driver mutations in tumor specimens from 1,000 patients with lung adenocarcinoma: The NCI's Lung Cancer Mutation Consortium (LCMC). J Clin Oncol. 2011;29 (suppl; abstr CRA7506).
74. Govindan R, Hammerman PS, Hayes DN, Wilkerson MD, Baylin S, Meyerson M. Comprehensive genomic characterization of squamous cell carcinoma of the lung. J Clin Oncol. 2012;30 (suppl; abstr 7006).
75. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics. CA Cancer J Clin. 2010;60:277–300.

Impact of Genetic Targets on Therapy in Head and Neck Squamous Cell Carcinoma

Irina Chaikhoutdinov and David Goldenberg

Abstract Despite advances in surgical technique, radiation therapy and chemotherapy, the mortality from head and neck squamous cell carcinoma (HNSCC) has not improved significantly. Squamous cell carcinoma is caused by tobacco use, alcohol consumption and infection with high-risk types of human papillomavirus. It is the 6th most common cancer in the world, with upwards of 45,000 new cases reported yearly in the United States alone.

In recent years, there has been a significant increase in the understanding of the molecular and genetic pathogenesis of head and neck cancer, shedding light on the unexpected heterogeneity of the disease. Genetic analysis has led to new classification schemes for HNSCC, with different subgroups exhibiting different prognoses. In addition, multiple targets in aberrant signaling pathways have been identified using increasingly sophisticated bio-informatics tools. Advances in technology have allowed for novel delivery mechanisms to introduce genetic material into cells to produce a therapeutic effect by targeting cancer cells via a number of different approaches.

A pressing need to develop novel therapies to augment current treatment modalities has led to a number of translational studies involving gene therapy in the treatment of HNSCC. This article will focus on a review of the most recent developments in molecular biology of head and neck squamous cell carcinoma in regards to possible targets for gene therapy, as well as the array of novel therapeutic strategies directed at these targets.

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W.S. El-Deiry (ed.), *Impact of Genetic Targets on Cancer Therapy*, Advances in Experimental Medicine and Biology 779, DOI 10.1007/978-1-4614-6176-0_7,

Keywords Gene therapy • Head and neck squamous cell carcinoma (HNSCC) • Genetic targets • HPV • P53

Introduction

Despite advances in surgical technique, radiation therapy and chemotherapy, the mortality from head and neck squamous cell carcinoma (HNSCC) has not improved significantly. Although the world-wide incidence of HNSCC has been steadily declining over the past 20 years, it is still the sixth most common cancer by incidence worldwide [1]. Yearly an estimated 600,000 new cases of HNSCC are reported worldwide, with upwards of 45,000 new cases reported in the United States alone [2].

Prognosis in HNSCC is largely determined by the stage at presentation. Current staging relies on the TNM (tumor, node, metastasis) system and dictates treatment planning. Although presentation at an early stage in the disease is associated with a favorable outcome, this prognosis applies to only about one third of HNSCC patients [2]. The majority of patients unfortunately present with advanced disease. The most important prognostic indicator at time of presentation is presence of lymph node metastases, which decreases long-term survival by 50% [3].

Current therapy consists of multimodality treatment. Early stage tumors, i.e. those that do not have nodal metastases, can be treated either with radiation or surgical resection alone. Advanced tumors with presence of nodal or distant metastases require a combination of surgery, chemotherapy and radiation. The most current multimodality treatments yield a 5 year survival rate of about 60% for invasive cancer of the oral cavity and pharynx according to the Surveillance Epidemiology and End Results (SEER) data [4]. Up to one third of patients experience tumor recurrence and survival rates drop dramatically with failure of first line treatment. Disease progression is the primary cause of death, causing approximately 50% of the mortality associated with HNSCC [5].

Given the continuing high mortality associated with HNSCC, new treatment innovations are imperative. Gene therapy—the introduction of RNA or DNA into diseased cells to effect genetic expression profiles—offers the option of targeting specific tissues and ideally avoiding toxic effects on surrounding healthy tissue. The recent advances in biotechnology and microarray gene analysis have given us insight into the gene expression profiles associated with HNSCC. This body of work has highlighted the unexpected heterogeneity of the disease and has also identified possible genetic targets that can be targeted using gene therapy.

Current Therapy for HNSCC

The organs involved in HNSCC effect speech, swallowing, breathing, taste and smell. Preservation of structure and function in an anatomically limited and complex space continues to be a challenge in treatment of HNSCC. Radical resection and radiation both have lasting negative effects on organ function and quality of life.

Unfortunately the majority of HNSCC patients present for treatment with locally advanced stage III or IV disease. Treatment consists of a combination of chemotherapy, radiation or surgery. Post-operative chemoradiotherapy is used after surgical resection when the patient is at an increased risk for local and distant recurrence. Pre-operative chemotherapy is considered when the risk of metastatic disease is high. Patients who present with stage I or stage II disease are often treated with either radiation or surgery alone and have an excellent prognosis.

Radiation therapy continues to be the mainstay of treatment for oropharyngeal, advanced hypopharyngeal and laryngeal cancer. Recent advances have focused on fractionation schedules and the use of intensity-modulated radiation therapy to increase delivery of radiation to target areas while sparing surrounding structures and tissues in the hopes of preserving the function of vital structures. Chemotherapy, often administered concurrently with radiation therapy, is an integral part of treatment of locally advanced disease. Compared to radiation therapy alone, chemoradiotherapy provides an 8% absolute benefit [6]. Recent agents such as cetuximab appear to show promise and will be discussed later in this review. Additionally, surgical and reconstructive techniques continue to advance and improve preservation of structure and function.

HPV-Positive HNSCC

HNSCC affects mucosal surfaces of the oral cavity, oropharynx, hypopharynx and larynx. Traditionally, the most notable risk factors identified for HNSCC are alcohol consumption and various forms of tobacco use, the combination of the two having a significantly synergistic effect on carcinogenesis. More recently, HNSCC caused by exposure to human papillomavirus (HPV) has been increasing in incidence and has been identified as an independent subgroup in HNSCC [7].

The pathogenesis and clinical course of HPV-positive tumors is appreciably different from that of non-HPV infected HNSCC. HPV-positive HNSCC more commonly affects the oropharynx, specifically tonsils and base of tongue. A meta-analysis has shown that HPV genomic DNA was detected in 26% of all HNSCC by polymerase chain reaction [8]. Oropharyngeal cancers have a higher incidence, with 36% shown to be positive for HPV [8]. Patients with HPV-positive HNSCC are generally younger and have better performance status as compared to those with HPV-negative HNSCC. They are also less likely to have a history significant for alcohol and tobacco use. However, history of multiple sexual partners is a major risk factor for HPV-positive HNSCC and is consistent with the known transmission pattern for HPV.

HPV is a double stranded DNA virus that is most notorious for causing cervical cancer. There are multiple subtypes of HPV, with HPV type 16 and 18 being the most virulent. HPV 16 has been shown to be present in over 90% of HPV-positive tumors of the head and neck [8]. Individuals who are sero-positive for HPV-16 are at an increased risk (15-fold) for developing HNSCC. Progression through phases of mitosis is necessary for HPV infection and gene expression [9]. HPV DNA codes for expression of two oncogenic proteins, E6 and E7, which affect cell cycle regulation in infected cells.

E6 inactivates the tumor suppressor p53 while E7 inhibits pRb (retinoblastoma). E6 induces the ubiquitination of p53 and targets it for degradation. Absence of p53 causes deregulation of cell cycle checkpoints and DNA repair mechanisms in the face of DNA damage, leading to genomic instability and inhibition of apoptosis, thus allowing the virus to replicate. E7 ubiquitinates pRb and targets it for degradation. Absence of pRb releases E2F transcription factors and allows for S phase to proceed unchecked. Studies have shown that inhibition of E6 and E7 induces apoptosis and decreases cell viability, thus indicating that their expression is necessary for tumor survival [10]. Nonetheless, expression of E6 an E7 alone is not enough to cause malignant transformation and other genetic alterations may be required.

In addition to having a different molecular pathogenesis from that of non-HPV related HNSCC, HPV-positive HNSCC has been shown to have more favorable clinical outcomes [11]. These tumors appear to respond to chemoradiation therapy better than their non-HPV counterparts. Patients have better overall and progression-free survival and less locoregional recurrence. The reasons for this are unclear, but several potential lines for further investigation have been identified. Overall, HPV-positive tumors have less genome wide DNA copy number alterations and less genome-wide hypomethylation than HPV-negative tumors. Another proposal involves the tumor suppressor p53, which is involved in the pathogenesis of both HPV-positive and HPV-negative HNSCC. Although p53 is inhibited by E6 as part of the HPV mechanism of transformation, tumors that express E6 and/or E7 have wild type TP53 [12]. HPV-negative HNSCC, in contrast, frequently has TP53 mutations, and mutations in TP53 have been shown to be associated with a poor prognosis [13].

Given the difference in clinical outcomes as well as the pathophysiology responsible for disease in HPV-positive and HPV-negative HNSCC, it is prudent that HPV infection be identifiable in studies that evaluate the efficacies of new treatment modalities as it may serve as a confounder. Additionally, the presence or absence of HPV infection should serve to tailor future treatment planning. The gold standard for identifying HPV infection is based on identifying expression of E6 and E7 proteins. Immunohistochemical staining of tumors for p16 expression has also been shown to be a reliable surrogate for identifying HPV infection. A mediator of senesce and differentiation, p16 normally inhibits cyclins and cyclin-dependent kinases which regulate cell cycle progression. A hallmark of HPV infection is over-expression of p16 secondary to the effects of E7.

Non-HPV HNSCC

The most significant risk factors for non-HPV related HNSCC are tobacco use and alcohol consumption. Although the incidence of non-HPV related HNSCC has been decreasing, the disease carries an overall worse prognosis as compared to HPV-positive HNSCC. Non-HPV related HNSCC patients are generally older, have a worse performance status, and respond less well to treatment modalities.

It has been well documented that HNSCC progresses through a series of well-defined clinical and histopathological stages and that it typically arises in areas of preneoplastic change. In 1953, a seminal article by Slaughter et al. introduced the term 'field cancerization,' a concept describing the presence of histologically abnormal tissue surrounding oral squamous cell carcinoma [14]. Field cancerization was intended to explain several key observations made by the authors. It was noted that oral cancer develops in areas of pre-cancerous change and that similar histologically abnormal tissue surrounds the tumor. Additionally, abnormal tissue was noted to be present after tumor resection. It was thus postulated that the histological abnormalities present in the area surrounding tumor contributed to the high rate of second primary tumors as well as to local tumor recurrence.

Studies have shown that not all significant stages of the disease process in HNSCC are reflected in the histopathology. At the level of the genome, there appear to be an accumulation of genetic changes that are responsible for transforming normal squamous cell epithelium to invasive squamous cell carcinoma. Several key molecular biology concepts were used to recognize and organize these genetic changes into a meaningful model of disease progression. Loss of heterozygosity (LOH) is a genetic alteration that results in the loss or change in an allele of a gene whose other allele has already undergone a change or a loss of function. LOH can happen via multiple mechanisms including gene deletion, point mutation, chromosome loss, mitotic recombination and promoter methylation. LOH generally leads to inactivation of a gene, typically a tumor suppressor, such as TP53. In many tumor types, including HNSCC, p53 inactivation occurs in the transition from the preinvasive to invasive state [15]. To map the progression to LOH in key genes, microsatellites- or tandem repeat sequences were used as gene markers and analyzed by PCR.

Using the above concepts, recent studies have elucidated the multistep process of epithelial carcinogenesis in respect to the spectrum of chromosomal loss and alteration that propels benign hyperplasia to dysplasia, and eventually to invasive carcinoma and metastases. Califano et al. used microsatellite marker analysis to delineate a genetic progression model for HNSCC based on frequency of genetic changes in pre-invasive lesions and invasive tumors [16]. Several genetic events are required for this process. Alteration of chromosomal region 9p21 appears to be an early event and is found in 70–80% of cases of squamous cell dysplasia in HNSCC, making it the most prevalent genetic change [17]. In the Califano model, LOH at chromosomal region 9p21 is responsible for transition of normal mucosa to hyperplastic mucosa, and it is found in 30% of squamous cell hyperplasia [16]. The gene locus at 9p21 encodes for proteins p16 and p14, which are responsible for G1 cell cycle regulation and MDM2 mediated degradation of p53. Inactivation of p16 leads to an increase in phosphorylation of Rb, and thus progression from G1 to S phase of the cell cycle. In the progression from hyperplasia to dysplasia, it appears that LOH at 3p is the next most common genetic alteration, although the specific gene responsible is not yet known. LOH at 17p13 is responsible for inactivation of the tumor suppressor p53. Mutations in TP53 are common in HNSCC, with one study demonstrating that mutations are present in greater than 50% of tumors [13]. Alterations in tumor suppressor p53 are believed to be early events in transformation, with evidence

of these alterations being present in histologically normal mucosa adjacent to tumors. Notably, studies have shown that patients with p53 mutations have a poorer response to chemotherapy and an overall worse prognosis [13]. The next step in progression of HNSCC is transformation of dysplastic epithelium to carcinoma in situ, and this has been associated with amplification of 11q13 and over-expression of cyclin D1. This is noted in 30–60% of HNSCC and has been associated with poor prognosis, including an increased rate of lymph node metastases [18]. Cyclin D1 is responsible for phosphorylation of Rb and progression of cell cycle to S phase. The altered cells resulting from these numerous genetic changes carry a survival advantage and eventually displace or replace surrounding mucosa, leading to clonal expansion and field cancerization.

Improved understanding of the molecular pathogenesis of HNSCC, specifically as it relates to field cancerization, has raised concerns about the management of surgical margins after tumor resection. The high rate of recurrence has propelled several recent studies into examining the genetic changes in tumor margins. The concepts of clonal expansion and field cancerization dictate that the para-neoplastic field is also genetically altered, but likely in earlier stages of cancerization as compared to tumor. Ideally, the ability to identify these changes at surgical resection would allow for identification of patients who are at higher risk for recurrence and perhaps result in tailored treatment plans. Molecular analysis of surgical margins based on LOH showed that greater than one-third of lesions sampled contained genetic abnormalities associated with tumor progression in surgical margins that appeared to be histopathologically normal [19]. Additionally, in almost all cases, the genetic alterations between tumor and genetically altered mucosa left in the patient contained similarities [19]. This has raised significant concern for ability to provide clean margins at time of resection. Studies addressed the detection of genotypically abnormal cells at the surgical margins as this information can be used to identify cohorts with high-risk disease. Retrospective studies have shown that remaining surgical fields are a prevalent source of local recurrences and secondary primaries in HNSCC patients [20]. Brennan et al., using a sensitive TP53 plaque assay, demonstrated the presence of p53 mutations in 13 of 25 patients who appeared to have complete tumor resection on the basis of microscopically negative surgical margins [21]. Five of the 13 patients developed local recurrences whereas none of the 12 patients without mutations had recurrence of their tumor. More recently Van Houten et al. showed that p53 mutations were present in surgical margins regarded as negative by histopathology in 50 of 76 patients [22]. Nine of the patients developed recurrence and four patients developed locoregional disease. However, in the group of patients without p53 mutations, none developed recurrence and only one developed regional disease [22]. In a study published in 2009, Poeta et al. used TP53 LigAmp to assay for residual disease in tumor margins in 95 patients with HSNCC and identified TP53 mutations in an attempt to identify high-risk patients and predict recurrence [23]. Unfortunately due to a small cohort and limited number of recurrences, no survival benefit was identified in this study. Nonetheless, using molecular analysis of tumor margins to identify disease during the surgical procedure is ideal, and with future advances in technology and possibly additional biomarkers, this technique would serve as welcome adjunct to the treatment of HNSCC patients.

Epigenetics

Epigenetics, or the study of heritable changes in gene expression caused by mechanisms other than changes in the underlying genetic code, has greatly contributed to our understanding of cancer biology in recent years. Heritable changes to the DNA molecule occur through several mechanisms that alter gene expression, mainly DNA methylation and post-translational modification of histone proteins. Hypermethylation of promoters is a method primarily associated with inactivation of tumor suppressor genes by gene silencing, and combined with quantitative PCR it is a powerful tool in gene profiling. Recently, these tools have been evaluated in several studies for their diagnostic and therapeutic uses. Given that prognosis in HNSCC is poor when disease is diagnosed at advance stages, HNSCC screening modalities have been investigated. Carvalho et al. designed a study to evaluate for epigenetic changes that may be useful in identifying patients with early disease [24]. Using quantitative PCR, they evaluated promoter hypermethylation in a panel of 21 candidate tumor suppressor genes in a cohort of 211 HNSCC patients compared to normal controls. They designed several panels with a range of specificities and sensitivities that were able to improve rates of identification of early disease as compared to single marker analyses [24]. In another study, Goldenberg et al. used rapid quantitative methylation-specific PCR to analyze tumor margins intraoperatively in 13 patients with HNSCC [25]. The tumors underwent molecular analysis at time of biopsy for promoter hypermethylation of two target genes, and qualified patients underwent intraoperative tumor margin analysis by rapid PCR. The analysis required on average 5 h, which is an acceptable time period for complex tumor resection and reconstruction, and was as sensitive as standard PCR analysis.

Impact of the Tumor Microenvironment and Cancer Stem Cells

Stem cells play a major role in the biology of HNSCC. Unlike embryonic stem cells, adult stem cells are undifferentiated cells with a limited capacity for regeneration. They play a major role in tissue homeostasis and regeneration, however they have recently also been shown to play a major role in the biology of cancer [26]. Cancer stem cells are defined as a subset of tumor cells that exhibit the ability of self-renewal and multi-potency, serving as progenitor cancer cells [27].

In HNSCC, evidence suggests the existence of a small group of cells with distinct tumorogenic potential. Prince et al. showed that CL44 expression discriminates a sub-population of progenitor cells [28]. In a follow up study, they showed that aldehyde dehydrogenase activity also identifies a group of highly tumorogenic cells [29]. The combination of both markers revealed that 1–3% of cells from primary human HNSCC are capable of generating tumors [30]. Given that such a specific subpopulation of cells exists, the ability to identify cancer stem cells should lead to more specific and targeted cancer treatment.

Epithelial-to-mesenchymal (EMT) transition is the process that allows a polarized cell to assume a phenotype characterized by enhanced motility and invasiveness.

This process plays a role in embryogenesis and is involved in several pathologies, including cancer. Recent studies suggest that EMT is involved in the acquisition of cancer stem cell properties [31]. The loss of cell polarity is a critical step in EMT and is mediated by the loss of E-cadherin, which appears to be correlated with tumor progression [32]. The tumor microenvironment appears to contribute to the survival of stem cells. In head and neck cancer, the stem cells are found in close proximity to blood vessels, suggesting the existence of a perivascular niche [30]. Studies demonstrate that this niche is functionally relevant in HNSCC, suggesting that disruption of these communications may be used in the treatment.

Gene Expression Profiles in HNSCC

Gene profiling uses high throughput techniques such as cDNA microarrays and comparative genomic hybridization to perform genome wide analysis of specimens. These techniques allow us to compare the genetic profiles of a multitude of tumors or patients in attempts to identify differences in expression patterns. Correlating this information to clinical outcomes can set up paradigms that can be used to guide treatment selection.

Two studies have attempted to identify a group of genes that can be used to predict clinical outcome. A study by Chung et al. used a 75 gene profile to identify two clusters of patients with significant difference in recurrence free survival [33]. Additionally, they noted that genes involved in epithelial-to-mesenchymal transition were associated with high-risk tumors. Alternatively, Mendez et al. used an expression profile of 108 genes that were selected based on differences in expression between oral squamous cell carcinoma and normal epithelium, and identified a cluster of genes that correlated with a poor disease-specific and overall survival [34].

Gene expression profiling has also been used to predict lymph node metastasis and distant metastasis. In 2005, Roepman et al. reported a set of 102 genes that offered predictive value for lymph node metastases [35]. The series included 82 oral and oropharyngeal carcinomas that were separated based on absence or presence of lymph node metastases in lymph node dissection specimens. The predictive value of this profile was better than the clinical diagnosis and has been validated in an independent series.

Angiogenesis in HNSCC

Angiogenesis is the process that leads to the formation of new blood vessels and is the hallmark of tumor progression. The role of angiogenesis has been studied in many cancers and many agents are available and useful in the treatment of certain cancers. In the treatment of HNSCC however few trials have shown promising results.

Vascular endothelial growth factor A (VEGF-A) is the most well known agent associated with induction of angiogenesis. It is part of the platelet derived growth factor (PDGF) super family and its expression is induced by hypoxemia and mediated via hypoxia-inducible factor (HIF-1a) [36]. The VEGF family signals through several surface receptor tyrosine kinases, known as VEGFR. Through these receptors VEGF exerts its mitogenic, chemotactic and vascular permeabilizing effects on endothelial cells.

Angiogenesis has been shown play an important role in HNSCC. Expression of VEGF in HNSCC is associated with more advanced disease, increased resistance to cytotoxic agents, and poor prognosis [37]. A meta-analysis of 12 studies revealed that VEGF expression was associated with a twofold higher risk of death at 2 years [38].

There are at least two different molecular pathways for inducing angiogenesis in HNSCC [39]. Studies have focused on targeting these specific molecular pathways in attempts to block angiogenesis and consequently tumor growth. The best results against tumor growth were obtained with triple combination of two drugs such as an anti-EGFR agent (cetuximab), anti-VEGFR agent (sunitinib) and radiotherapy [40]. Other in-vivo studies have suggested that inhibition of angiogenesis alone is not enough to suppress growth of HNSCC and these treatments need to be integrated with different approaches [41].

Sorafenib and sunitinib are two tyrosine kinase inhibitors with activity against VEGF and PDGF receptors that have been tested in clinical trials in HNSCC. Sunitibib has been shown to have no significant activity in monotherapy against HNSCC in two studies [42, 43]. Another study showed limited activity of sunitinib at higher doses against recurrent HNSCC however this was associated with significant risk of hemorrhage [44]. Sorafenib, although well tolerated, also did not show a significant response rate, however when used in chemonaive patients, overall survival was comparable to that achieved with more toxic regimens [45]. Lastly, an anti-angiogenic agent in combination with an EGFR inhibitor showed response to therapy. This study also identified a molecular biomarker that could predict greater likelihood of response to anti-angiogenic treatment: an exciting idea that warrants further study [46].

Gene Therapy Approaches

Although several different gene therapy approaches have been investigated in attempts to augment treatment of HNSCC, the most notable and successful therapy has been aimed at epidermal growth factor receptor (EGFR). EGFR is a tyrosine kinase that signals through the Ras-MAPK, PI3K-PTEN-AKT and phospholipase G pathways. These pathways are associated with cellular proliferation, apoptosis, angiogenesis and invasion (Fig. 1). EGFR dysregulation can induce cell proliferation and block apoptosis, and it has been identified as a key component in invasion and metastases [47]. EGFR is over-expressed in more than 80% of HNSCC [48].

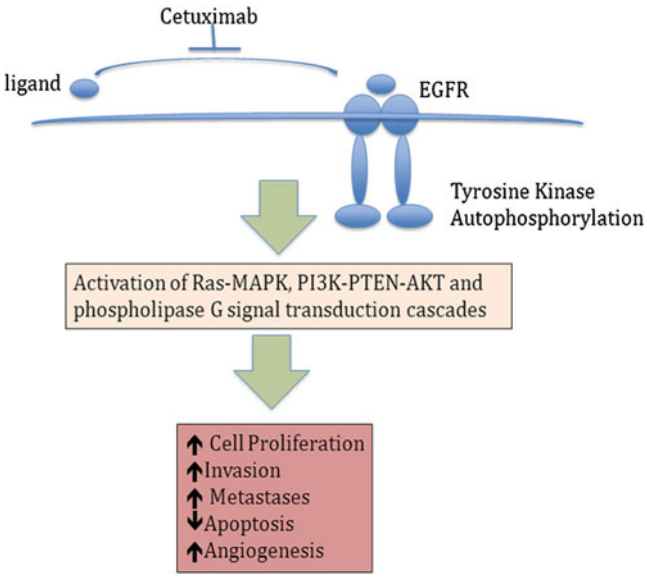

Fig. 1 Cetuximab is a monoclonal antibody that targets EGFR, a tyrosine kinase receptor which signals through the Ras-MAPK, PI3K-PTEN-AKT and phospholipase G pathways

An EGFR-associated expression profile has been noted to show a poor prognosis [49]. Mutations in the EGFR gene are rare; protein over expression is often responsible for activation of the pathway in HNSCC [50].

Cetuximab, a monoclonal EGFR specific antibody, has recently been the focus of multiple trials as a treatment modality for HNSCC. Cetuximab has been approved by the FDA for use in patients with platinum resistant disease [51]. When used as a single agent in patients with recurrent or metastatic head and neck cancer that was resistant to platinum, cetuximab resulted in a 46% rate of disease control—including complete or partial response or stable disease. As first line therapy in patients with metastatic disease or in patients with recurrent HNSCC, cetuximab plus fluorouracil and cisplatin prolonged progression free survival [51].

Cetuximab has also been approved for use in combination with radiation therapy in previously untreated patients [52]. In combination with radiation therapy, cetuximab improved locoregional control and overall and progression-free survival in patients with locally advanced disease [52]. The risk of death was decreased by 26%. The rate of metastases was unaffected [52].

Conclusion

Given the high mortality rate in HNSCC with current treatment and the associated side effects of aggressive treatment, new options for treatment are imperative. Gene therapy offers the option of targeting specific tissues and ideally avoiding toxic

effects on surrounding healthy tissue. Advances in molecular biology and biomolecular technology will help us diagnose HNSCC disease earlier and expand our scope of options for treatment.

References

1. 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(14):2137–50.
2. Jemal A, et al. Global cancer statistics. CA Cancer J Clin. 2011;61(2):69–90.
3. Sanderson RJ, Ironside JA. Squamous cell carcinomas of the head and neck. BMJ. 2002;325(7368):822–7.
4. Howlader N. SEER Cancer Statistics Review, 1975–2008. National Cancer Institute, 2011.
5. Argiris A, et al. Competing causes of death and second primary tumors in patients with locoregionally advanced head and neck cancer treated with chemoradiotherapy. Clin Cancer Res. 2004;10(6):1956–62.
6. Pignon JP, et al. Chemotherapy added to locoregional treatment for head and neck squamous-cell carcinoma: three meta-analyses of updated individual data. MACH-NC collaborative group. Meta-analysis of chemotherapy on head and neck cancer. Lancet. 2000;355(9208):949–55.
7. Gillison ML, et al. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. J Natl Cancer Inst. 2008;100(6):407–20.
8. Kreimer AR, et al. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev. 2005;14(2):467–75.
9. Pyeon D, et al. Establishment of human papillomavirus infection requires cell cycle progression. PLoS Pathog. 2009;5(2):e1000318.
10. Rampias T, et al. E6 and e7 gene silencing and transformed phenotype of human papillomavirus 16-positive oropharyngeal cancer cells. J Natl Cancer Inst. 2009;101(6):412–23.
11. Ragin CC, Taioli E. Survival of squamous cell carcinoma of the head and neck in relation to human papillomavirus infection: review and meta-analysis. Int J Cancer. 2007;121(8):1813–20.
12. Wiest T, et al. Involvement of intact HPV16 E6/E7 gene expression in head and neck cancers with unaltered p53 status and perturbed pRb cell cycle control. Oncogene. 2002;21(10):1510–7.
13. Poeta ML, et al. TP53 mutations and survival in squamous-cell carcinoma of the head and neck. N Engl J Med. 2007;357(25):2552–61.
14. Slaughter DP, Southwick HW, Smejkal W. Field cancerization in oral stratified squamous epithelium; clinical implications of multicentric origin. Cancer. 1953;6(5):963–8.
15. Boyle JO, et al. Gene mutations in saliva as molecular markers for head and neck squamous cell carcinomas. Am J Surg. 1994;168(5):429–32.
16. Califano J, et al. Genetic progression model for head and neck cancer: implications for field cancerization. Cancer Res. 1996;56(11):2488–92.
17. Mao L, et al. Frequent microsatellite alterations at chromosomes 9p21 and 3p14 in oral premalignant lesions and their value in cancer risk assessment. Nat Med. 1996;2(6):682–5.
18. Meredith SD, et al. Chromosome 11q13 amplification in head and neck squamous cell carcinoma. Association with poor prognosis. Arch Otolaryngol Head Neck Surg. 1995;121(7):790–4.
19. Tabor MP, et al. Persistence of genetically altered fields in head and neck cancer patients: biological and clinical implications. Clin Cancer Res. 2001;7(6):1523–32.
20. Tabor MP, et al. Genetically altered fields as origin of locally recurrent head and neck cancer: a retrospective study. Clin Cancer Res. 2004;10(11):3607–13.
21. Brennan JA, et al. Molecular assessment of histopathological staging in squamous-cell carcinoma of the head and neck. N Engl J Med. 1995;332(7):429–35.
22. van Houten VM, et al. Mutated p53 as a molecular marker for the diagnosis of head and neck cancer. J Pathol. 2002;198(4):476–86.

23. Poeta ML, et al. The ligamp TP53 assay for detection of minimal residual disease in head and neck squamous cell carcinoma surgical margins. Clin Cancer Res. 2009;15(24):7658–65.
24. Carvalho AL, et al. Evaluation of promoter hypermethylation detection in body fluids as a screening/diagnosis tool for head and neck squamous cell carcinoma. Clin Cancer Res. 2008;14(1):97–107.
25. Goldenberg D, et al. Intraoperative molecular margin analysis in head and neck cancer. Arch Otolaryngol Head Neck Surg. 2004;130(1):39–44.
26. Al-Hajj M, et al. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA. 2003;100(7):3983–8.
27. Reya T, et al. Stem cells, cancer, and cancer stem cells. Nature. 2001;414(6859):105–11.
28. Prince ME, et al. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. Proc Natl Acad Sci USA. 2007;104(3):973–8.
29. Clay MR, et al. Single-marker identification of head and neck squamous cell carcinoma cancer stem cells with aldehyde dehydrogenase. Head Neck. 2010;32(9):1195–201.
30. Krishnamurthy S, et al. Endothelial cell-initiated signaling promotes the survival and self-renewal of cancer stem cells. Cancer Res. 2010;70(23):9969–78.
31. Mani SA, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell. 2008;133(4):704–15.
32. Perl AK, et al. A causal role for E-cadherin in the transition from adenoma to carcinoma. Nature. 1998;392(6672):190–3.
33. Chung CH, et al. Gene expression profiles identify epithelial-to-mesenchymal transition and activation of nuclear factor-kappa B signaling as characteristics of a high-risk head and neck squamous cell carcinoma. Cancer Res. 2006;66(16):8210–8.
34. Ang KK, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med. 2010;363(1):24–35.
35. Roepman P, et al. An expression profile for diagnosis of lymph node metastases from primary head and neck squamous cell carcinomas. Nat Genet. 2005;37(2):182–6.
36. Maxwell PH, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature. 1999;399(6733):271–5.
37. Tse GM, et al. Strong immunohistochemical expression of vascular endothelial growth factor predicts overall survival in head and neck squamous cell carcinoma. Ann Surg Oncol. 2007;14(12):3558–65.
38. Kyzas PA, Cunha IW, Ioannidis JP. Prognostic significance of vascular endothelial growth factor immunohistochemical expression in head and neck squamous cell carcinoma: a meta-analysis. Clin Cancer Res. 2005;11(4):1434–40.
39. Hasina R, et al. Angiogenic heterogeneity in head and neck squamous cell carcinoma: biological and therapeutic implications. Lab Invest. 2008;88(4):342–53.
40. Bozec A, et al. Combination of sunitinib, cetuximab and irradiation in an orthotopic head and neck cancer model. Ann Oncol. 2009;20(10):1703–7.
41. Myoung H, et al. Evaluation of the anti-tumor and anti-angiogenic effect of paclitaxel and thalidomide on the xenotransplanted oral squamous cell carcinoma. Cancer Lett. 2001;163(2):191–200.
42. Fountzilas G, et al. A phase II study of sunitinib in patients with recurrent and/or metastatic nonnasopharyngeal head and neck cancer. Cancer Chemother Pharmacol. 2010;65(4):649–60.
43. Choong NW, et al. Phase II study of sunitinib malate in head and neck squamous cell carcinoma. Invest New Drugs. 2010;28(5):677–83.
44. Machiels JP, et al. Phase II study of sunitinib in recurrent or metastatic squamous cell carcinoma of the head and neck: GORTEC 2006–01. J Clin Oncol. 2010;28(1):21–8.
45. Williamson SK, et al. Phase II evaluation of sorafenib in advanced and metastatic squamous cell carcinoma of the head and neck: southwest oncology group study S0420. J Clin Oncol. 2010;28(20):3330–5.
46. Cohen EE, et al. Erlotinib and bevacizumab in patients with recurrent or metastatic squamous-cell carcinoma of the head and neck: a phase I/II study. Lancet Oncol. 2009;10(3):247–57.

47. Ciardiello F, Tortora G. EGFR antagonists in cancer treatment. N Engl J Med. 2008;358(11):1160–74.
48. Ang KK, et al. Impact of epidermal growth factor receptor expression on survival and pattern of relapse in patients with advanced head and neck carcinoma. Cancer Res. 2002;62(24):7350–6.
49. Chung CH, et al. Molecular classification of head and neck squamous cell carcinomas using patterns of gene expression. Cancer Cell. 2004;5(5):489–500.
50. Sheikh Ali MA, et al. Expression and mutation analysis of epidermal growth factor receptor in head and neck squamous cell carcinoma. Cancer Sci. 2008;99(8):1589–94.
51. Vermorken JB, et al. Platinum-based chemotherapy plus cetuximab in head and neck cancer. N Engl J Med. 2008;359(11):1116–27.
52. Bonner JA, et al. Radiotherapy plus cetuximab for locoregionally advanced head and neck cancer: 5 year survival data from a phase 3 randomised trial, and relation between cetuximab-induced rash and survival. Lancet Oncol. 2010;11(1):21–8.

Tyrosine Kinase Targeted Treatment of Chronic Myelogenous Leukemia and Other Myeloproliferative Neoplasms

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Abstract Myeloproliferative neoplasms (MPNs) include Philadelphia chromosome positive (Ph+) chronic myeloid leukemia (CML) and the Ph− diseases primary myelofibrosis (PMF), polycythemia vera (PV) and essential thrombocythemia (ET). Since FDA approval of imatinib in 2001, CML treatment has been focused on tyrosine kinase inhibitors. With these targeted therapies, imatinib-resistant CML has emerged as a major problem. Second generation tyrosine kinase inhibitors (TKIs) have allowed for effective treatment of some patients with imatinib resistance, but bcr-abl mutants such as T315I remain problematic. Additional agents are in development and are discussed here. New clinical issues with TKI treatment include premature termination of therapy due to adverse-effects, the cost of therapy, and the apparently indefinite duration of treatment in patients who have achieved complete molecular response (CMR). In contrast to Ph+ CML, targeted therapy for Ph− MPNs is novel and of less clear therapeutic potential. New insights into Ph− MPNs include alterations in the JAK-STAT signaling pathway, particularly as mediated by the JAK2 V617F mutation. The recent development of multiple JAK2 inhibitors has provided hope for the rational and effective management of these disorders. Recently, ruxolitinib was approved as therapy for PMF. Current data suggests, however, that given its vital cell signaling function, the therapeutic benefit of targeting Jak kinases in general, or JAK2 specifically may be less than that derived from ABL-directed TKI treatment of CML. This review focuses on the current treatment options for CML

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W.S. El-Deiry (ed.), *Impact of Genetic Targets on Cancer Therapy*, Advances in Experimental Medicine and Biology 779, DOI 10.1007/978-1-4614-6176-0_8,

and Philadelphia chromosome negative myeloproliferative neoplasms (MPNs) and limitations faced in current clinical practice.

Keywords CML • Philadelphia chromosome • Polycythemia vera • Imatinib • TKI • JAK2 • BCR-ABL • Blastic phase • Ruxolitinib • Myeloproliferative neoplasms • Nilotinib • Resistance • Cytogenetic response • OCT-1 • Dasatinib • QT-prolongation • Bosutinib • Bafetinib • Ponatinib • GNF-5 • PIGF • Thrombopoietin • STAT • DNA repair • Erythropoiesis • Hydoxyurea • Lenalidomide • Myelofibrosis • Cytopenia • Leukemia • TG101348 • Lestaurtinib • CYT387 • SB1518 • DCC-2036

Introduction

Chronic myeloid leukemia is now highly treatable with the advent of tyrosine kinase inhibitors (TKIs) providing therapy targeted at BCR-ABL. The development of major molecular response (MMR or >1,000 fold reduction of bcr-abl as quantitated by PCR study) and complete molecular response (CMR, BCR-ABL mRNA undetectable by RT-PCR) in many patients and the relatively limited toxicity profile of this therapy has provided a dramatic contrast to the historically toxic and ineffective interferon based therapies. Most striking has been the clear change in the natural history of the disease with imatinib therapy with greatly reduced incidence of accelerated and blastic phase of the disease [1]. However, imatinib resistance among patients with CML has emerged as a clinically significant problem [2]. Second generation TKIs including dasatinib and nilotinib, while addressing some resistance, remain inactive for many patients. Ongoing studies involving imatinib, nilotinib and dasatinib are discussed here [2–4]. Imatinib resistance mechanisms have been identified, including abl kinase domain mutations (e.g. the T315I mutation) that have been shown to be operative in many cases. Compounds targeting kinase domain mutations and other forms of TKI resistance are now in clinical development. Other concerns include the potential for long term side effects of abl inhibition as exemplified by the development of cytogenetic changes in Philadelphia chromosome negative cells in patients on long term tyrosine kinase inhibitor therapy.

In distinction to longstanding understanding of CML pathogenesis, the Philadelphia negative (Ph–) myeloproliferative neoplasms (MPNs) have only recently been associated with mutations involving Jak-2, other related tyrosine kinases, and the downstream STAT signal transduction pathway [5]. This finding, in 2005, has prompted development of a variety of oral Jak-2 inhibitors, some of which are advanced in clinical development. Ruxolitinib, a JAK1/JAK2 inhibitor, was recently approved for clinical use by the FDA. The biology of the JAK-STAT pathway and these Ph– MPNs contrasts in interesting and clinically significant ways with bcr-abl dependant Ph+ CML. These differences highlight different net outcomes and therapeutic expectations for these two groups of diseases.

In this review current clinical problems in the management of Ph+ CML and Ph– MPNs are discussed. Subsequently, targeted tyrosine kinase inhibitor agents in current clinical development for these diseases are examined, particularly from the standpoint of how they may allow the current clinical issues to be addressed. Management of Ph+ CML and the Ph– MPNs are addressed separately, but the relative potential benefits of TKI therapy of the two disease types is subsequently compared and contrasted.

Targeted Therapy of Philadelphia Chromosome Positive Chronic Myeloid Leukemia

Clinical Features of Ph+ CML

Based on a single center review of 430 patients with CML referred for bone marrow transplantation, approximately 20% of the cases are currently diagnosed incidentally. Fatigue and weight loss is present in patients with greater degree of anemia, leukocytosis and splenomegaly. Typically, middle-aged individuals are affected and younger patients may present with a more aggressive form of CML, such as in accelerated phase or blast crisis.

Ph+ CML progresses through chronic phase, accelerated phase, or blast crisis. Mature cells predominate in the chronic phase, cellular and additional cytogenetic abnormalities occur in the accelerated phase, and rapid proliferation of immature cells comprises the blast phase.

Current standard frontline therapy for newly diagnosed CML as outlined in the NCCN 2012 guidelines is imatinib 400 mg daily, nilotinib 300 mg BID, or dasatinib 100 mg daily. Some investigators prefer second generation TKIs nilotinib and dasatinib for patients with intermediate or high risk factors such as age, spleen size, number of peripheral blast cells, eosinophils and basophils. Therapy is changed to a higher dose imatinib or to a second generation TKI if complete hematologic response (CHR) is not achieved or if drug toxicity is a concern. Rare patients who are unable to tolerate imatinib, nilotinib or dasatinib are then considered for IFN/PEG-IFN, hematopoietic stem cell transplantation or enrollment in a clinical trial (see http://guidelines.nccn.org/published-guideline/EBA4F5EF-5A9B-E0B4-C6A4-BFDD8FA8BF78/guideline.pdf).

Current Clinical Problems in Ph+ CML

Tyrosine Kinase Inhibitor Resistance: TKI resistance is arguably the most important issue currently encountered in CML treatment. Given that persistence of active CML poses ongoing risk for disease progression, patients with imatinib resistance

manifest increased risks of progression to accelerated phase or blast crisis. Thus, there is strong data suggesting well established milestones for defining treatment success in CML patients during therapy [6]. Complete cytogenetic response (CcyR, no Ph+ metaphases detected in a sample of at least 20 metaphases) is well established as an endpoint and should be achieved by or close to 1 year of therapy. Major molecular response has been shown to be associated with stable response in TKI therapy and should be achieved at or close to 18 months of therapy [6, 7]. Approximately 20% of patients that are started on imatinib do not achieve CCyR and 15% of those initially responsive to imatinib therapy eventually lose response. When they are subsequently started on nilotinib or dasatinib, approximately 70% of those patients achieve CCyR with the second TKI [8]. Unfortunately, 15% of these patients eventually lose response. When a third TKI is used, CCyR occurs in only about 20% and is usually of short duration. Prognosis is poor for these patients. Currently, there is no standard therapy available for patients failing therapy with a third TKI. (Liunan Li. et al, ASH 2010 Abstract 1238). Their options include IFN/PEG IFN, hematopoietic stem cell transplantation or enrollment in a clinical trial.

Four main mechanisms of resistance to imatinib have been described. Mutations of the abl moiety kinase site, such as the T315I mutation; altered expression of multidrug transporter proteins, bcr-abl amplification, and alternative signaling pathways [9] have all been shown to produce meaningful cellular resistance to imatinib [10]. The first two mechanisms are briefly addressed below.

The best characterized form of imatinib resistance is that generated by the development of secondary abl kinase domain mutations [11]. These typically develop after imatinib or other TKI therapy and present highly variable degrees of TKI resistance [12]. The T315I mutation however presents particular difficulties clinically, as it renders the bcr-abl resistant to imatinib, dasatinib and nilotinib—i.e. all of the licensed abl directed TKIs. Thus, there is an urgent need to develop strategies to circumvent this resistance.

Imatinib resistance has been found in cells that lack or have decreased activity of the organic cation transporter 1(OCT-1) protein [13, 14]. Functional activity of OCT-1 predicts molecular response over the first 24 months of imatinib therapy for chronic phase CML patients. Intracellular uptake and retention of imatinib depends on OCT-1 activity and its mRNA expression. Nilotinib uptake is not OCT-1 dependent, allowing it to be more effective at targeting BCR-ABL activity [15].

Duration of TKI Therapy: Current treatment strategy in terms of duration from diagnosis remains unclear. Patients that achieve CCyR currently face a possibility of lifelong TKI therapy and reasonably inquire about long term side effects. Imatinib resistant CML precursor cells are thought to persist even in patients that achieve cytogenetic and molecular response. Currently the standard of care is to continue TKI therapy even for patients who achieve deep remission reflected by ongoing CMR. To identify the subset of patients that would need to continue TKI therapy indefinitely, Mahon et al conducted a prospective, multicenter trial involving 69 adult chronic-phase CML patients who had been treated with imatinib for ≥ 3 years and had remained in CMR for ≥ 2 years. Patients discontinued imatinib but were carefully followed. Peripheral blood analysis was conducted on a monthly basis for

the first 12 months of treatment, at treatment end, and every 2 months thereafter. If molecular relapse occurred, then they were restarted on imatinib therapy [16]. Relapse occurred in 42 (61%) of the patients. 40 of those 42 patients had relapsed less than 6 months from the end of treatment. Ongoing CMR was observed in 41% at 12 month and 38% at 24 months of follow-up. All of the patients that had relapsed responded to re-initiation of imatinib therapy. It is thus possible that patients that are intolerant to therapy may benefit from interruption and re-initiation if they achieve CMR. More studies are needed to gain insight on duration of TKI therapy.

Patient Adherence to therapy: Adherence (compliance) to therapy is crucial for success in CML treatment. Marin et al assessed whether adherence to once-daily oral imatinib treatment was associated with MMR and with CMR[17]. Drug adherence was defined based on self- reporting, frequency of repeat prescriptions, pill counts, drug plasma levels, and data from an electronic device fitted in the medication bottle cap. They studied 87 patients with chronic-phase CML who had achieved CCyR during treatment with imatinib. Initial dose was 400 mg daily. All patients had received therapy for at least 2 years (median duration was 5 years). Adherence was >90% in 64 patients and ≤90% in 23 patients. At 18 months, 58% of patients with >90% adherence achieved MMR versus 9% in the <90% adherence group ($p<0.001$). The 6-year probability of achieving CMR also was higher among those with >90% adherence (44% vs. 0%; $p=0.002$). Patients with ≤80% adherence did not achieve MMR at all. Lower rates of adherence were associated with higher dose of imatinib (600 mg daily), adverse effects (discussed later), and younger age. [17]

Secondary Cytogenetic Changes: The development of chromosomal abnormalities in Ph-negative metaphases has been recognized in patients with CML on imatinib treatment [18]. Additionally, Cytogenetic abnormalities related to second generation TKIs nilotinib, dasatinib and bosutinib were found in 41 of 453 patients in a study by Jabbour and colleagues. 72 chromosomal abnormalities were noted, most common of which consisted of –Y (n=7, 10%), trisomy 8 (n=6, 8%), and del 20q (n=5, 7%) in Ph-negative metaphases. These cytogenetic changes were found to be transient and had uncertain clinical significance with occasional reported exceptions [19]. Thus, many investigators choose to follow patients with CCyR with periodic marrow cytogenetics to identify and monitor this phenomenon.

Tyrosine Kinase Inhibitors

First Generation Tyrosine Kinase Inhibitor

Imatinib mesylate: The identification of imatinib as a selective inhibitor of BCR-ABL and its mutants has revolutionized the treatment of Ph+ CML [20]. Phase II studies have suggested that higher dose therapy might improve hematologic and molecular response rates. A phase III, multinational, randomized trial was conducted to assess hematologic, cytogenetic, and molecular responses to imatinib 400 mg versus 800 mg daily (which was given as 400 mg twice daily) in 476 patients with newly

diagnosed CML. The investigators also noted the difference in toxicity profile [21]. Higher dose imatinib was associated with more rapid MMR but MMR rates were unaffected by the starting dose after 12 months of therapy (40.1% in the 400-mg group and 46.4% in the 800-mg group). Similarly, CCyR at 12 months was 65.6% for the lower dose and 69.9% for the higher dose group. In this study, higher dose provoked more discontinuation of therapy, with 15.9% of patients in the 400 mg group versus 19.7% of patients in the 800 mg group. Toxicities included fatigue, edema, diarrhea, myalgias and skin rash (Table 1). 17.8% of patients in the 400 mg group required a dose reduction versus 61.4% of patients in the 800 mg group.

The issue of potential discontinuation of imatinib therapy continues to be explored. Investigators have recently studied the combination of imatinib and pegylated-interferon with a view to potential cure of the disease, allowing long term discontinuation [22].

Second Generation Tyrosine Kinase Inhibitors

Nilotinib: Second generation TKIs, nilotinib and dasatinib are FDA approved for newly diagnosed CML given their efficacy and favorable side-effect profiles. Nilotinib, like imatinib, binds to the kinase domain in the inactive conformation. It is active in imatinib refractory or intolerant patients [23–26]. It has been compared to imatinib in the phase 3, randomized, study of 846 patients with chronic-phase CML (the ENESTnd study). This study continues to demonstrate the superiority of nilotinib versus imatinib in yielding cytogenetic and molecular response at 12 months of follow-up [27]. The more frequent CMRs seen in newly diagnosed CML patients treated with nilotinib have led to discussion regarding approaches to ultimate discontinuation of TKI therapy. Main toxicities include QT-prolongation, fatigue, rash and hyperlipasemia (Table 1).

Dasatinib: Dasatinib is a multikinase inhibitor (it also inhibits sarc (SRC) family of kinases) which binds to the ABL kinase domain in the open conformation, addressing another mechanism of resistance. It, like nilotinib, is active in yielding CML responses in a proportion of imatinib refractory patients [28, 29]. The DASISION study comparing dasatinib to imatinib in newly diagnosed CML continues to confirm more rapid CCyR and MMR development in patients treated with dasatinib [30]. Dasatinib is associated with pleural and pericardial effusions, occurring in 5–10% of patients, but generally appearing within a year of initiation of therapy (Table 1).

Bosutinib: This compound is a dual inhibitor of SRC and ABL with clinical activity in imatinib refractory patients [31]. It also shows in vitro activity against imatinib resistant abl kinase domain mutants [12]. An 18-month follow-up in the BELA trial comparing bosutinib versus imatinib in patients with chronic phase CML revealed a significantly higher MMR rate at 1 year, significantly faster times to CCyR and MMR, lower transformation rate and an acceptable toxicity profile with bosutinib [31, 32]. Bosutinib is being evaluated as a third-line therapy for chronic-phase CML

Table 1 cAbl tyrosine kinase inhibitors in clinical development

Inhibitor	IC50 fold increase versus T315I mutation	Enzyme targets	Clinical dosing range/MTD	Relative potency versus imatinib for BCR-ABL1	Toxicities	Clinical efficacy	Stage of clinical study (trial)
Imatinib	17.50	BCR-ABL1, c-KIT, PDGFR, c-FMS	400 mg qd–400 mg bid	1	Fatigue, edema, myalgia, cytopenias, hypophosphatemia	CCR, much improved (IRIS) MMR, CMR seen	FDA approved, III (IRIS)
Nilotinib	39.41	BCR-ABL1, c-KIT, PDGFR, c-FMS	300–400 mg bid	10–30	Fatigue, QT-prolongation, hyperlipasemia, cytopenias	Faster response (CCyR + MMR) than Imat, Active in some resistant patients	FDA approved, III (ENESTnd)
Dasatinib	75.03	>100 kinases including BCR-ABL1, PDGFR, c-KIT, SRC family, +others	100–140 mg daily or 50–70 mg bid	200–300	Fatigue, pleural effusions, pericardial effusions, hyperlipasemia, immune-suppression, cytopenias, QT-prolongation	Faster response (CCyR + MMR) than Imat, Active in some resistant patients	FDA approved, III (DASISION)

(continued)

Table 1 (continued)

Inhibitor	IC50 fold increase versus T315I mutation	Enzyme targets	Clinical dosing range/MTD	Relative potency versus imatinib for BCR-ABL1	Toxicities	Clinical efficacy	Stage of clinical study (trial)
Bosutinib	45.42	ABL, SRC family	500 mg qd	1–10	GI disturbance, fatigue, cytopenia	Active in some resistant patients	FDA Approved, III (BELA)
Bafetinib (INNO-406)	NA	ABL, Src family kinases, Lyn	10–50 mg bid	NA	safety study is completed, results are pending. (www.clinicaltrials.gov)	Active in some resistant patients	I
Ponatinib (AP24534)	NA	BCR-ABL1 (including T315I mutation)	45 mg qd	NA	safety study is ongoing. (www.clinicaltrials.gov)	Active in T315I mutant bcr-abl	II (PACE)
DCC-2036	NA	BCR-ABL1(including T315I mutation)	NA	NA	NA	Active in T315I mutant bcr-abl	I–II

following failure with imatinib and then nilotinib or dasatinib. A phase I/II trial evaluated safety and efficacy of bosutinib as a third-line therapy in 114 patients. 73% of the patients achieved complete hematologic response (CHR, normalization of peripheral blood counts) and 32% achieved major cytogenetic response (MCyR, <35% Ph+ metaphases). 11 patients achieved CCyR and 22% achieved MMR. MCyR and CHR were observed across 11 different BCR-ABL kinase domain mutations, but T315I still remained resistant (2011 ASCO Annual Meeting, abstract 6535). Bosutinib was FDA approved on Sept 4, 2012 for Ph+ CML in chronic, accelerated, or blast phase, in patients with resistance or intolerance to prior therapy. (www.cancer.gov)

Bafetinib (**INNO-406**): Bafetinib is also a second-generation TKI in development. Its structure is a modified structure of imatinib with improved binding and potency against Bcr-Abl as well as Src family kinase Lyn, which has been associated with resistance to imatinib in CML [33]. Preclinical studies showed that it inhibits 12 of 13 most frequent imatinib-resistant Bcr-Abl point mutations, but again it is unable to inhibit the T315I mutation which accounts for 10–20% of mutations detected after failure of TKI therapy [33, 34].

Ponatinib (**AP24534**): A pivotal phase II trial (PACE trial) is currently ongoing for use of another second-generation TKI, ponatinib. This TKI actually showed antitumor activity against the T315I mutation in the phase I study and was effective against tumor cells that developed resistance to nilotinib and dasatinib [35]. (clinicaltrials.gov identifier NCT01207440).

DCC-2036: DCC-2036, is a potent broad spectrum inhibitor of BCR-ABL kinase that also inhibits the T315I mutation [36]. It is currently undergoing testing in a multicenter phase 1 clinical trial (clinicaltrials.gov identifier NCT00827138).

GNF-5: This agent targets and selectively inhibits BCR-ABL via an alternative binding site. Drug resistance was lowered exponentially when GNF 5 was used in combination with imatinib or nilotinib [37]. In a mouse model of CML that closely resembles human disease, treatment with both nilotinib and GNF-5 (compared with either drug alone) greatly prolonged survival [37]. 80% of mice that received the combination therapy had no signs of residual disease and no toxicity at 6 weeks post-treatment. The approach of simultaneously targeting multiple sites is proposed to decrease the incidence of new mutations.

Impact of the Tumor Microenvironment and Cancer Stem Cells

There is emerging evidence that highlights the importance of the tumor microenvironment, in particular the bone marrow stroma, for the growth and survival of TKI resistant leukemic cells. Placenta growth factor (PlGF), a VEGF family member, has recently been evaluated in imatinib-resistant CML. In vitro studies have revealed that PlGF promotes the survival of hematopoietic precursors. VEGFR-1 (Flt1) is also widely expressed in human CML cells. Preclinical studies have suggested that CML

cells signal the bone marrow stroma to produce increasing amounts of PlGF. Furthermore, murine models with genetic deletion of PlGF have prolonged survival of CML-bearing mice. This was confirmed by Schmidt and colleagues when they used a monoclonal antibody against PlGF (αPlGF) plus imatinib in an imatinib-sensitive CML mouse model and found a significantly longer survival rate [38]. These findings suggest targeting the microenvironment, in addition to the leukemic cell itself, would offer a multi-dimensional approach for the treatment of TKI-resistant CML.

Targeted Therapy in Philadelphia Chromosome Negative Myeloproliferative Neoplasms (MPNs)

Philadelphia Chromosome negative MPNs include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). Understanding of these disorders has lagged behind that for Philadelphia chromosome positive CML. In 1978, Prchal et al showed that PV is characterized by erythropoietin-independent erythroid colonies. In 2005 three groups demonstrated that the tyrosine kinase JAK-2 (Janus Kinase-2) is mutated at codon 617 in nearly all patients with PV, rendering this enzyme more active in signal transduction [39–42]. This mutation affects a non-kinase domain which appears active in kinase regulation (Fig. 1). Mutations that are now known to contribute to the pathogenesis of PV are JAK2 V617F, exon 12 JAK2 mutations, and somatic mutations of thrombopoietin receptor (cMPL) [43]. Mutations with gain of function include JAK2, MPL, CBL and loss of function include LNK and NF1 which activate the JAK-STAT pathway [44]. However, many patients with PMF and ET have no identifiable mutation in any of these genes. Thus, comprehensive understanding of the initial events in the development of MPNs remains elusive. Even PMF patients without known somatic mutations may be shown to have activation of the myeloid STAT signaling pathway, suggesting that these patients share at a minimum some common signal transduction pathology. The final phenotype of MPNs is then associated with inflammation, angiogenesis, myeloid proliferation and resistance to apoptosis.

While mutant JAK-2 may be compared to the BCR-ABL oncoprotein from drug-development standpoint, it's vital function in development and physiology separate it from the ABL derived target in CML. While the function of ABL appears related to cell cycle arrest, potentially as it relates to DNA repair, deletion of this gene is compatible with life, albeit with minor developmental differences [45, 46]. Thus, complete inhibition of this enzyme and its variants with targeted therapy yields (at least in the medium term) few serious toxicities. In distinction, wild-type JAK2 enzyme is involved in normal hematopoiesis (Fig. 1); therefore, complete inhibition of this enzyme may not be possible clinically. JAK-2 is necessary for erythropoiesis—knock-out mice lack definitive erythropoiesis [47]. Thus, targeting of this enzyme is limited by the tolerance for complete versus partial inhibition of this target. It has also been suggested that JAK-2 mutations do not seem to be the disease initiating or primary mutation in these diseases. This pathway is however the target of current JAK2 inhibitors [44, 48].

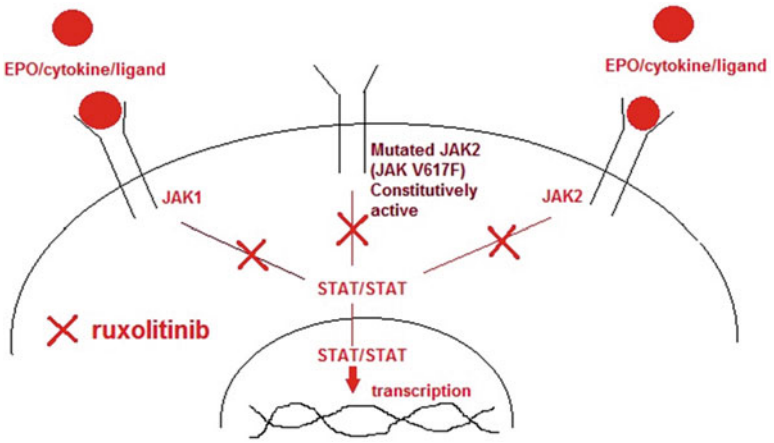

Fig. 1 Ruxolitinib acts as a JAK1 and JAK2 inhibitor and blocks normal activation as well as mutated JAK-STAT pathway

Clinical Problems in Ph− Myeloproliferative Neoplasms

Constitutional Symptoms and Splenomegaly: While initially often manifesting relatively benign behaviors, all patients with MPNs are at risk for evolving to highly symptomatic phases characterized by constitutional symptoms (sweating, fatigue, fever) and massive splenomegaly due to extramedullary hematopoesis. These are typically frequent earlier in the course of MF than in PV or ET, but patients with these latter conditions often become disabled by these symptoms late in their course. While hydroxyurea and corticosteroids yield modest palliation for some, many patients experience fatigue and sweating which is refractory to these agents. Splenectomy has been reported effective for anemia, but is highly morbid and not helpful for constitutional symptoms. Thalidomide [49] and lenalidomide have shown modest activity, but only for a minority of patients.

The most positive findings are reported in the COMFORT I and COMFORT II trials, leading to the FDA approval of ruxolitinib in November of 2011. The COMFORT I study is an ongoing double-blind, placebo-controlled study that showed that 42% of myelofibrosis patients treated with ruxolitinib had a 35% or more splenic size reduction on MRI at 24 weeks versus only 0.7% patients in the placebo group. 46% of patients in the ruxolitinib group also had a 50% or more improvement in the total symptom score at 24 weeks compared with only 5% of patients in the placebo group. Anemia and thrombocytopenia were the most common adverse events reported in the ruxolitinib group, but this rarely led to discontinuation of therapy [50].

The COMFORT II study compared ruxolitinib to best available therapy. At week 24, 32% of the patients in the ruxolitinib group had 35% or more reduction in spleen size on MRI compared to 0% of the patients in the best available therapy group. Patients in the ruxolitinib group had reduction in disease related symptoms compared to best available therapy. The impact of ruxolitinib on overall survival, tolerability profile and symptom reduction has been positive based on recent outcome of 107 patients treated with ruxolitinib compared to 310 matched historical controls [51, 52].

Marrow Fibrosis and cytopenias: As MPNs progress, marrow fibrosis becomes denser and net marrow hematopoietic cellularity falls. Often in this phase patients develop cytopenias, particularly anemia, but also thrombocytopenia. Bone marrow fibrosis is generally associated with atypical megakaryocytic hyperplasia and thickening and distortion of the bony trabeculae.

Terminal phase MPN—acute leukemic transformation: While many older patients with indolent Ph– MPNs may have chronic courses which are not life threatening, others, particularly those diagnosed at younger ages, will ultimately develop transformation of their MPN to an acute leukemic phase resembling acute myelogenous leukemia (AML). Such transformed diseases, seen in ~20% of patients with MF, are very resistant to cytotoxic chemotherapy, and the afflicted patients are in many cases so debilitated by their illness that they are unfit for intensive chemotherapy. Thus, while occasionally AML induction chemotherapy may yield remission of these acute transformations, these remissions are generally brief in the absence of allogeneic transplantation. In sum, the development of a therapy which may prevent acute transformation to a terminal phase would provide hope for a true change in the natural history of these disorders. No data has so far suggested that any existing therapy prevents this terminal transformation.

Early clinical trials of JAK2 inhibitors have mainly focused on MF or later stages of PV and ET because of the serious implications of these conditions. JAK2 inhibitors currently in development are discussed below and presented in Table 2.

Jak-2 Tyrosine Kinase Inhibitors

Ruxolitinib (**INCB018424—Incyte**): is arguably the most studied inhibitor of JAK 1 and JAK 2 [44, 53, 54]. It is being studied in phase I/II trials COMFORT I (compared to placebo) and COMFORT II (compared to best available treatment). These studies have revealed that ruxolitinib provides marked and sustained clinical benefit in spleen size and an acceptable safety profile relative to placebo and best available therapy [44, 55].

As a result of these findings, ruxolitinib became the first FDA approved treatment for myelofibrosis in November of 2011. An additional phase 1–2 trial was reported by Verstovsek and others, examining Ruxolitinib in 153 patients with primary myelofibrosis, post–essential thrombocythemia myelofibrosis, or post–polycythemia vera myelofibrosis [56]. The best responses as measured by reduced spleen size were in patients receiving 15 to 25 mg twice daily dosing. Sixty percent of those treated at

Table 2 Jak-2 kinase inhibitors in clinical development

Inhibitor	IC50 versus Jak2	IC50 cell targets	Enzyme targets	Clinical dosing range/MTD	Plasma Cmax/T1/2	Toxicities	Clinical efficacy (improvements)	Stage of clinical study
INCB018424 (Ruxolitinib)	2.8 nM	127 nM	Jak1, Jak2, Tyk2	10–25 mg bid	934 nM / 3.0 h	*Thrombocytopenia*, anemia, diarrhea	Splenomegally and const. symptoms	FDA approved III (COMFORT I, COMFORT II)
TG101348		NA	NA	680 mg daily	16–34 h	*Hyperamylasemia* nausea, diarrhea, anemia	Splenomegally and const. symptoms	II
CEP-701 (lestaurtinib)	2–3 nM (FLT3)	NA	FLT3, JAK2	40–60 mg bid	6.8–9.2 h	Nausea, vomiting, diarrhea	Splenomegally const. symptoms, anemia	II
CYT387	18 nM	0.5–1.5 uM	Jak1,Jak2 (Jak3)	NA	NA	*Hyperlipasemia, headache*	Splenomegally const. symptoms, anemia	II
XL019	2 nM	NA	NA	NA	NA	*Peripheral neuropathy*	Splenomegally and const. symptoms	I
SB1518	19–23 nM	160–340 nM	Jak2, TYK2,FLT3 (Jak1,Jk3)	NA	NA	*Diarrhea, nausea*	Splenomegally and const. symptoms	II
AZD1480		500–2,000 nM (myelomas)	Jak2	NA	NA	NA	NA	

the 25 mg bid dose required dose reductions for thrombocytopenia, suggesting the lower dose as an appropriate initial therapy. Importantly, constitutional symptoms including night sweats, itching and fatigue showed sustained improvement in patients in this study. Treatment emergent anemia is however seen with this agent [5]. Circulating levels of inflammatory markers (C reactive protein, IL1b, IL6) fell in patients in the study. Clinical and biochemical responses were independent of Jak-2 mutational status [56].

TG101348: This agent is an oral agent which, unlike Ruxolitinib, is selective for Jak-2 inhibitor with additional inhibitory activity for MPL. It was studied in high- or intermediate-risk primary or post–polycythemia vera/essential thrombocythemia myelofibrosis in a once daily, phase I trial [57]. The maximal tolerated dose was 680 mg daily, with modest amylase elevations identified as the dose limiting toxicity. While many patients experienced nausea, diarrhea and new onset anemia, most showed improvement in constitutional symptoms, leukocytosis and thrombocytosis, a substantial minority also showed reduced spleen size.

Lestaurtinib (**CEP710—Cephalon**): This agent is an orally bioavailable inhibitor of FLT-3 and Jak-2. This agent was administered to 22 patients with primary or post PV or ET myelofibrosis at a dose of 80 mg twice daily in a phase 2 trial reported in 2010 [58]. Diarrhea, nausea, and headache were frequently seen toxicities, but several patients also experienced grade 3 or 4 anemia or thrombocytopenia. Six patients (27%) showed disease response by IWG-MRT criteria.

CYT387 is a Jak-2 inhibitor with activity against Jak-1 and Tyk-2. CYT387 showed significant activity in a murine model of Jak-2 -V617F dependant MPN [59]. In this model the burden of the mutant clone was reduced by in-vivo treatment with this agent.

SB1518 exhibits inhibitory activity against Jak-2 and FLT3, and inhibited tumor growth in a murine Jak-2 V617F model [60].

Discussion

The availability of abl specific tyrosine kinase inhibitors has changed the natural history of Ph+ CML. Ongoing development of agents offers prospects for addressing the outstanding issues in CML care discussed above.

Imatinib and other TKI resistance via a variety of mechanisms poses serious ongoing obstacle for some patients. Second generation agents Dasatinib and Nilotinib yield faster CML responses and thus the prospect for less emergence of resistance. The development of agents potentially capable of treating kinase domain mutant bcr-abl, in particularly Ponatinib (AP24534) and DCC-2036, both of which show activity against the T315I mutation, offers prospects for management of this very serious complication. It may be expected, however, that some patients will continue to develop resistant disease under the selective pressure of highly active therapy. Thus, ongoing studies of novel agents are of great practical interest.

Long term use of TKIs remains expensive and not without toxicities for CML patients. The potential toxic impact of long term inhibition of tyrosine kinase targets is surely of vital interest. To date there is little data for serious long-term toxicity of imatinib and second generation abl kinase inhibitors. However, observations of secondary "myelodysplasia related" chromosome changes [19] in patients under treatment with these agents are thought provoking and merit ongoing study.

For Ph− MPNs the outlook is very different. JAK-STAT alterations, unlike BCR-ABL in CML, do not appear to be the driver of primary changes in these neoplasms [5]. The Jak-2 and related enzymes have essential functions in hematopoiesis [61], rendering them unsuitable for complete inhibition therapy. Thus, while the agents under clinical development appear to offer significant palliation to patients with advanced Ph− MPNs, it is unclear what the impact of these agents will be on the natural history of these patients. No data as yet suggests much improvement in marrow fibrosis or Jak-2 mutant (V617F) allelic burden with therapy with these agents. Their long term effects will be of great interest.

In summary, TKI directed therapy presents interesting contrasts for Ph+ and Ph− MPNs. Ph+ CML is dependent on mutant abl, an enzyme whose function appears optional for normal hematopoiesis. Complete inhibition of abl activity has limited toxicity, and the consequences of such therapy include in-vivo selection for resistant mutants. In distinction to Ph+ diseases, at this time no primary disease initiating mutation has been identified for Ph− MPNs. Jak-2 is a tyrosine kinase clearly essential for normal hematopoiesis, rendering its complete therapeutic inhibition problematic. Therefore, while Jak2 inhibitors currently under study are of great interest and may provide important palliation of symptoms, their potential to affect the natural history of the Ph− MPNs is uncertain.

References

1. Druker BJ, Guilhot F, O'Brien SG, Gathmann I, Kantarjian H, Gattermann N, et al. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Engl J Med. 2006;355(23):2408–17.
2. Soverini S, Colarossi S, Gnani A, Castagnetti F, Rosti G, Bosi C, et al. Resistance to dasatinib in Philadelphia-positive leukemia patients and the presence or the selection of mutations at residues 315 and 317 in the BCR-ABL kinase domain. Haematologica. 2007;92(3):401–4.
3. Talpaz M, Shah NP, Kantarjian H, Donato N, Nicoll J, Paquette R, et al. Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. N Engl J Med. 2006;354(24):2531–41.
4. Ottmann O, Dombret H, Martinelli G, Simonsson B, Guilhot F, Larson RA, et al. Dasatinib induces rapid hematologic and cytogenetic responses in adult patients with Philadelphia chromosome positive acute lymphoblastic leukemia with resistance or intolerance to imatinib: interim results of a phase 2 study. Blood. 2007;110(7):2309–15.
5. Pardanani A, Vannucchi AM, Passamonti F, Cervantes F, Barbui T, Tefferi A. JAK inhibitor therapy for myelofibrosis: critical assessment of value and limitations. Leukemia. 2011;25(2):218–25.
6. O'Brien S, Tefferi A, Valent P. Chronic myelogenous leukemia and myeloproliferative disease. Hematology Am Soc Hematol Educ Program. 2004;1:146–62.
7. Baccarani M, Saglio G, Goldman J, Hochhaus A, Simonsson B, Appelbaum F, et al. Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. Blood. 2006;108(6):1809–20.

8. Jabbour E, Kantarjian H, O'Brien S, Shan J, Garcia-Manero G, Wierda W, et al. Predictive factors for outcome and response in patients treated with second-generation tyrosine kinase inhibitors for chronic myeloid leukemia in chronic phase after imatinib failure. Blood. 2011;117(6):1822–7.
9. Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science. 2001;293(5531):876–80.
10. Tang C, Schafranek L, Watkins DB, Parker WT, Moore S, Prime JA, et al. Tyrosine kinase inhibitor resistance in chronic myeloid leukemia cell lines: investigating resistance pathways. Leuk Lymphoma. 2011;52:2139–47.
11. Soverini S, Hochhaus A, Nicolini FE, Gruber F, Lange T, Saglio G, et al. Bcr-Abl kinase domain mutation analysis in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors: recommendations from an expert panel on behalf of European LeukemiaNet. Blood. 2011;118:1208–15.
12. Redaelli S, Piazza R, Rostagno R, Magistroni V, Perini P, Marega M, et al. Activity of bosutinib, dasatinib, and nilotinib against 18 imatinib-resistant BCR/ABL mutants. J Clin Oncol. 2009;27(3):469–71.
13. Engler JR, Frede A, Saunders V, Zannettino A, White DL, Hughes TP. The poor response to imatinib observed in CML patients with low OCT-1 activity is not attributable to lower uptake of imatinib into their CD34+ cells. Blood. 2010;116(15):2776–8.
14. White DL, Dang P, Engler J, Frede A, Zrim S, Osborn M, et al. Functional activity of the OCT-1 protein is predictive of long-term outcome in patients with chronic-phase chronic myeloid leukemia treated with imatinib. J Clin Oncol. 2010;28(16):2761–7.
15. Engler JR, Frede A, Saunders VA, Zannettino AC, Hughes TP, White DL. Chronic myeloid leukemia CD34+ cells have reduced uptake of imatinib due to low OCT-1 activity. Leukemia. 2010;24(4):765–70.
16. Mahon FX, Rea D, Guilhot J, Guilhot F, Huguet F, Nicolini F, et al. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. Lancet Oncol. 2010;11(11):1029–35.
17. Marin D, Bazeos A, Mahon FX, Eliasson L, Milojkovic D, Bua M, et al. Adherence is the critical factor for achieving molecular responses in patients with chronic myeloid leukemia who achieve complete cytogenetic responses on imatinib. J Clin Oncol. 2010;28(14):2381–8.
18. Bumm T, et al. Restoration of polyclonal hematopoiesis in most cml patients in complete cytogenetic remission to imatinib but rapid emergence of clonal cytogenetic abnormalities in Ph-negative cells in a subset of patients.
19. Jabbour E, Kantarjian H, Cornelison AM, Kadia T, Welch M-A, Abruzzo LV, et al. Chromosomal abnormalities in Philadelphia chromosome (Ph)-negative metaphases appearing during second generation tyrosine kinase inhibitors (2nd TKI) therapy in patients (pts) with Chronic Myeloid Leukemia (CML). Blood. 2010;116(21):1232. (ASH Annual Meeting Abstracts), 19 Nov 2010.
20. Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med. 1996;2(5):561–6.
21. Cortes JE, Baccarani M, Guilhot F, Druker BJ, Branford S, Kim DW, et al. Phase III, randomized, open-label study of daily imatinib mesylate 400 mg versus 800 mg in patients with newly diagnosed, previously untreated chronic myeloid leukemia in chronic phase using molecular end points: tyrosine kinase inhibitor optimization and selectivity study. J Clin Oncol. 2010;28(3):424–30.
22. Simonsson B, Gedde-Dahl T, Markevarn B, Remes K, Stentoft J, Almqvist A, et al. Combination of pegylated interferon-{alpha}2b with imatinib increases molecular response rates in patients with low or intermediate risk chronic myeloid leukemia. Blood. 2011;118:3228–35.
23. Cortes JE, Hochhaus A, le Coutre PD, Rosti G, Pinilla-Ibarz J, Jabbour E, et al. Minimal cross-intolerance with nilotinib in patients with chronic myeloid leukemia in chronic or accelerated phase who are intolerant to imatinib. Blood. 2011;117(21):5600–6.

24. Giles FJ, Abruzzese E, Rosti G, Kim DW, Bhatia R, Bosly A, et al. Nilotinib is active in chronic and accelerated phase chronic myeloid leukemia following failure of imatinib and dasatinib therapy. Leukemia. 2010;24(7):1299–301.
25. Kantarjian HM, Giles FJ, Bhalla KN, Pinilla-Ibarz J, Larson RA, Gattermann N, et al. Nilotinib is effective in patients with chronic myeloid leukemia in chronic phase after imatinib resistance or intolerance: 24-month follow-up results. Blood. 2011;117(4):1141–5.
26. le Coutre P, Ottmann OG, Giles F, Kim DW, Cortes J, Gattermann N, et al. Nilotinib (formerly AMN107), a highly selective BCR-ABL tyrosine kinase inhibitor, is active in patients with imatinib-resistant or -intolerant accelerated-phase chronic myelogenous leukemia. Blood. 2008;111(4):1834–9.
27. Cortes JE, Jones D, O'Brien S, Jabbour E, Konopleva M, Ferrajoli A, et al. Nilotinib as front-line treatment for patients with chronic myeloid leukemia in early chronic phase. J Clin Oncol. 2010;28(3):392–7.
28. Cortes J, Rousselot P, Kim DW, Ritchie E, Hamerschlak N, Coutre S, et al. Dasatinib induces complete hematologic and cytogenetic responses in patients with imatinib-resistant or -intolerant chronic myeloid leukemia in blast crisis. Blood. 2007;109(8):3207–13.
29. Saglio G, Hochhaus A, Goh YT, Masszi T, Pasquini R, Maloisel F, et al. Dasatinib in imatinib-resistant or imatinib-intolerant chronic myeloid leukemia in blast phase after 2 years of follow-up in a phase 3 study: efficacy and tolerability of 140 milligrams once daily and 70 milligrams twice daily. Cancer. 2010;116(16):3852–61.
30. Kantarjian H, Shah NP, Hochhaus A, Cortes J, Shah S, Ayala M, et al. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2010;362(24):2260–70.
31. Abbas R, Hug BA, Leister C, Gaaloul ME, Chalon S, Sonnichsen D. A phase I ascending single-dose study of the safety, tolerability, and pharmacokinetics of bosutinib (SKI-606) in healthy adult subjects. Cancer Chemother Pharmacol. 2011;69:221–7.
32. Keller G, Schafhausen P, Brummendorf TH. Bosutinib: a dual SRC/ABL kinase inhibitor for the treatment of chronic myeloid leukemia. Expert Rev Hematol. 2009;2(5):489–97.
33. Santos FP, Kantarjian H, Cortes J, Quintas-Cardama A. Bafetinib, a dual Bcr-Abl/Lyn tyrosine kinase inhibitor for the potential treatment of leukemia. Curr Opin Investig Drugs. 2010;11(12):1450–65.
34. Leonetti F, Stefanachi A, Nicolotti O, Catto M, Pisani L, Cellamare S, et al. BCR-ABL inhibitors in chronic myeloid leukemia: process chemistry and biochemical profile. Curr Med Chem. 2011;18:2943–59.
35. O'Hare T, Shakespeare WC, Zhu X, Eide CA, Rivera VM, Wang F, et al. AP24534, a pan-BCR-ABL inhibitor for chronic myeloid leukemia, potently inhibits the T315I mutant and overcomes mutation-based resistance. Cancer Cell. 2009;16(5):401–12.
36. Prchal JF, Adamson JW, Murphy S, Steinmann L, Fialkow PJ. Polycythemia vera. The in vitro response of normal and abnormal stem cell lines to erythropoietin. J Clin Invest. 1978;61(4):1044–7.
37. Zhang J, Adrian FJ, Jahnke W, Cowan-Jacob SW, Li AG, Iacob RE, et al. Targeting Bcr-Abl by combining allosteric with ATP-binding-site inhibitors. Nature. 2010;463(7280):501–6.
38. Schmidt T, Kharabi Masouleh B, Loges S, Cauwenberghs S, Fraisl P, Maes C, et al. Loss or inhibition of stromal-derived PlGF prolongs survival of mice with imatinib-resistant Bcr-Abl1(+) leukemia. Cancer Cell. 2011;19(6):740–53.
39. Vainchenker W, Constantinescu SN. A unique activating mutation in JAK2 (V617F) is at the origin of polycythemia vera and allows a new classification of myeloproliferative diseases. Hematology Am Soc Hematol Educ Program. 2005:1:195–200.
40. Staerk J, Kallin A, Demoulin JB, Vainchenker W, Constantinescu SN. JAK1 and Tyk2 activation by the homologous polycythemia vera JAK2 V617F mutation: cross-talk with IGF1 receptor. J Biol Chem. 2005;280(51):41893–9.
41. Zhao R, Xing S, Li Z, Fu X, Li Q, Krantz SB, et al. Identification of an acquired JAK2 mutation in polycythemia vera. J Biol Chem. 2005;280(24):22788–92.

42. Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell. 2005;7(4):387–97.
43. Pardanani AD, Levine RL, Lasho T, Pikman Y, Mesa RA, Wadleigh M, et al. MPL515 Mutations in myeloproliferative and other myeloid disorders: a study of 1,182 patients. Blood. 2006;108(10):3472–6.
44. Passamonti F, Maffioli M, Caramazza D, Cazzola M. Myeloproliferative neoplasms: from JAK2 mutations discovery to JAK2 inhibitor therapies. Oncotarget. 2011;2(6):485–90.
45. Welch PJ, Wang JYJ. A C-terminal protein-binding domain in the retinoblastoma protein regulates nuclear c-Abl tyrosine kinase in the cell cycle. Cell. 1993;75:779–90.
46. Welch PJ, Wang JYJ. Abrogation of retinoblastoma protein function by c-Abl through tyrosine kinase-dependent and -independent mechanisms. Mol Cell Biol. 1995;15:5542–51.
47. Parganas E, Wang D, Stravopodis D, Topham DJ, Marine JC, Teglund S, et al. Jak2 is essential for signaling through a variety of cytokine receptors. Cell. 1998;93(3):385–95.
48. Prchal JT. Philadelphia chromosome-negative myeloproliferative disorders: an historical perspective. Hematology Am Soc Hematol Educ Program. 2008;1:68
49. Canepa L, Ballerini F, Varaldo R, Quintino S, Reni L, Clavio M, et al. Thalidomide in agnogenic and secondary myelofibrosis. Br J Haematol. 2001;115(2):313–5.
50. Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, DiPersio JF, et al. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. N Engl J Med. 2012;366(9): 799–807.
51. Harrison C, Kiladjian JJ, Al-Ali HK, Gisslinger H, Waltzman R, Stalbovskaya V, et al. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. N Engl J Med. 2012;366(9):787–98.
52. Verstovsek S, Kantarjian HM, et al. Long-term outcomes of 107 patients with myelofibrosis receiving JAK1/JAK2 inhibitor ruxolitinib: survival advantage in comparison to matched historical controls. Blood. 2012 Aug 9;120(6):1202–9. doi: 10.1182/blood-2012-02-414631. Epub 2012 Jun 20.
53. Mesa RA. Ruxolitinib, a selective JAK1 and JAK2 inhibitor for the treatment of myeloproliferative neoplasms and psoriasis. IDrugs. 2010;13(6):394–403.
54. Naqvi K, Verstovsek S, Kantarjian H, Ravandi F. A potential role of ruxolitinib in leukemia. Expert Opin Invest Drugs. 2011;20(8):1159–66.
55. Quintas-Cardama A. JAK2 inhibitors in polycythemia vera. Clin Adv Hematol Oncol. 2011;9(5):397–400.
56. Verstovsek S, Kantarjian H, Mesa RA, Pardanani AD, Cortes-Franco J, Thomas DA, et al. Safety and efficacy of INCB018424, a JAK1 and JAK2 inhibitor, in myelofibrosis. N Engl J Med. 2010;363(12):1117–27.
57. Pardanani A, Gotlib JR, Jamieson C, Cortes JE, Talpaz M, Stone RM, et al. Safety and efficacy of TG101348, a selective JAK2 inhibitor, in myelofibrosis. J Clin Oncol. 2011;29(7):789–96.
58. Santos FP, Kantarjian HM, Jain N, Manshouri T, Thomas DA, Garcia-Manero G, et al. Phase 2 study of CEP-701, an orally available JAK2 inhibitor, in patients with primary or post-polycythemia vera/essential thrombocythemia myelofibrosis. Blood. 2010;115(6):1131–6.
59. Tyner JW, Bumm TG, Deininger J, Wood L, Aichberger KJ, Loriaux MM, et al. CYT387, a novel JAK2 inhibitor, induces hematologic responses and normalizes inflammatory cytokines in murine myeloproliferative neoplasms. Blood. 2010;115(25):5232–40.
60. Hart S, Goh KC, Novotny-Diermayr V, Hu CY, Hentze H, Tan YC, et al. SB1518, a novel macrocyclic pyrimidine-based JAK2 inhibitor for the treatment of myeloid and lymphoid malignancies. Leukemia. 2011;25:1751–9.
61. Neubauer H, Cumano A, Muller M, Wu H, Huffstadt U, Pfeffer K. Jak2 deficiency defines an essential developmental checkpoint in definitive hematopoiesis. Cell. 1998;93(3):397–409.

Targeted Therapy of Multiple Myeloma

Nathan G. Dolloff and Giampaolo Talamo

Abstract Multiple myeloma (MM) is a plasma cell malignancy and the second most common hematologic cancer. MM is characterized by the accumulation of malignant plasma cells within the bone marrow, and presents clinically with a broad range of symptoms, including hypercalcemia, renal insufficiency, anemia, and lytic bone lesions. MM is a heterogeneous disease associated with genomic instability, where patients may express multiple genetic abnormalities that affect several oncogenic pathways. Commonly detected genetic aberrations are translocations involving immunoglobulin heavy chain (IgH) switch regions (chromosome 14q32) and oncogenes such as c-maf [t(14:16)], cyclin D1 [t(11:14)], and FGFR3/MMSET [t(4:14)]. Advances in the basic understanding of MM and the development of novel agents, such as the immunomodulatory drugs (IMiDs) thalidomide and lenalidomide and the proteasome inhibitor bortezomib, have increased therapeutic response rates and prolonged patient survival. Despite these advances MM remains incurable in the majority of patients, and it is therefore critical to identify additional therapeutic strategies and targets for its treatment. In this chapter, we review the underlying genetic components of MM and discuss the results of recent clinical trials that demonstrate the effectiveness of targeted agents in the management of MM. In addition, we discuss experimental therapies that are currently in clinical development along with their molecular rationale in the treatment of MM.

Keywords Clinical trials • Immunomodulatory agents • Molecular pathways • Multiple myeloma • Proteasome inhibitors • Targeted therapy

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W.S. El-Deiry (ed.), *Impact of Genetic Targets on Cancer Therapy*, Advances in Experimental Medicine and Biology 779, DOI 10.1007/978-1-4614-6176-0_9,

Introduction

Multiple myeloma (MM) accounts for 1% of all cancers, is the second most common hematologic cancer, and is responsible for approximately 10,000 deaths per year in the U.S. [1]. MM is characterized by the clonal proliferation of plasma cells within the bone marrow and presents clinically with a broad range of manifestations, such as hypercalcemia, renal insufficiency, anemia, lytic bone lesions, among other complications [2]. In the last 10 years, extensive research efforts have identified key molecular pathways that contribute to proliferation and survival of malignant plasma cells. This knowledge of MM pathogenesis at the molecular level has led to the development of novel agents with mechanisms of action different from conventional chemotherapy agents, which act by non-specifically poisoning DNA synthesis and the mitotic process. Clinical results obtained with these new agents have not only improved the duration of disease remission in MM, but have prolonged patient survival [3]. Despite these therapeutic options, the vast majority of MM patients are not cured, therefore emphasizing the need to continue the development of new therapeutic options with activity directed against MM-specific molecular targets and signaling pathways. The clinical armamentarium for treating MM has now expanded to include new IMiDs, new proteasome inhibitors, histone deacetylase inhibitors, and other targeted agents that we discuss in this review chapter.

Standard of Care for Multiple Myeloma

High dose melphalan in conjunction with autologous stem cell transplantation (ASCT) is the standard of care for newly diagnosed MM patients under 65 years of age [4]. Patients undergo an induction phase of therapy prior to high-dose therapy and ASCT, and then receive consolidation and maintenance therapies following ASCT. Numerous therapies and combinations are incorporated during the induction and consolidation/maintenance phases and include conventional chemotherapy agents (e.g., melphalan, cyclophosphamide, and liposomal doxorubicin), corticosteroids (e.g., dexamethasone and prednisone), the immunomodulatory drugs (IMiDs) thalidomide and lenalidomide, and the proteasome inhibitor bortezomib [5]. The same agents in various combinations are routinely used for transplant-ineligible patients. New generation IMiDs and proteasome inhibitors along with other classes of molecular targeted agents are now being incorporated into the therapy of MM, particularly in the refractory setting. In this review, we analyzed the evidence supporting therapeutic use of these novel agents and discuss the molecular pathways that contribute to MM progression and may be targeted for the development of future clinical trials. We focused our attention on the antineoplastic therapy of MM and did not review other aspects of MM treatment, such as the targeted therapy of bone disease. Osteolyticlesions, a clinical hallmark of MM, are the result of an imbalance between bone formation and resorption. Several molecules, such as osteoprotegerin, RANK (Receptor Activator of Nuclear factor-KappaB), RANKL (RANK ligand), and DKK1 have been identified as key mediators of the formation

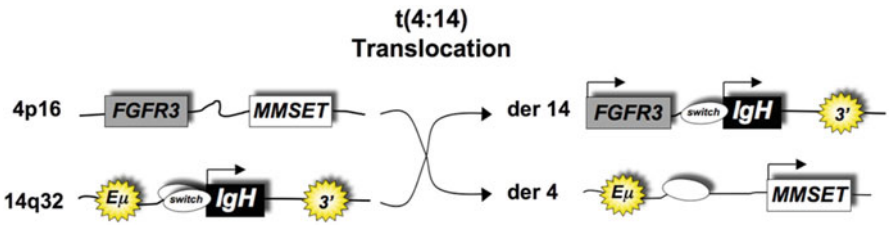

Fig. 1 The t(4:14) translocation in multiple myeloma. The immunoglobulin heavy chain (*IgH*) gene is highly expressed in normal plasma cells due to the influence of powerful enhancer sequences (Eμ and 3′) within the *IgH* locus. By contrast, *FGFR3* and *MMSET* are expressed at undetectable levels in normal plasma cells. The t(4:14) gene translocation juxtaposes the endogenous promoters of *FGFR3* and *MMSET* with the strong IgH enhancers, and promotes the aberrant expression of both genes [12]

of osteolytic lesions in MM [6]. These discoveries have already reached applications in clinical practice with the recent introduction of the anti-RANKL agent denosumab for the treatment of osteoporosis and metastatic bone disease. The targeted therapy of bone disease in MM has been reviewed elsewhere [7–9].

Molecular Biology of Multiple Myeloma

MM originates from a post-germinal center B-cell that expresses immunoglobulin heavy chain (IgH) post-switch isotypes. An early transformation event in the development of MM involves an illegitimate class switch recombination in the non-functioning allele of the immunoglobulin heavy chain (IgH) locus [10, 11]. In fact, translocations involving the IgH locus (14q32) are the most frequently detected karyotypic abnormality in MM cells. As recombination errors in immunoglobulin gene rearrangement are common in all B-cell malignancies, it seems that this process, which provides remarkable diversity to the humoral immune system, also leaves this cell lineage particularly vulnerable to oncogenic transformation. Transcriptional activity of the IgH locus is heightened in B-cells and plasma cells, and therefore the translocation and juxtaposition of an oncogene to the IgH locus, which contains powerful enhancer sequences, may result in upregulation of the oncogene [12]. The t(4:14) translocation, involving the *FGFR3*, *MMSET*, and *IgH* genes, is illustrated in Fig. 1. The most common MM chromosomal translocations and the oncogenes that they dysregulate are listed in Table 1.

A single cytogenetic abnormality that is directly responsible for the malignant transformation of plasma cells has yet to be identified. According to current knowledge, MM is not a cancer driven by a single somatic mutation, such as the formation of PML-RAR and BCR-AB fusion genes in acute promyelocytic leukemia (APL) and chronic myelogenous leukemia (CML), respectively. In those two hematologic malignancies, transgenic mouse models have demonstrated that a single gene product is responsible for the malignant transformation [13, 14]. Instead, MM cells are characterized by chromosomal instability, and the individual gene products generated by

Table 1 Typical cytogenetic abnormalities in multiple myeloma

Cytogenetic abnormality	Involved loci	Involved genes	Approximate frequency
t(4;14)	(p16;q32)	*MMSET/FGFR3*	15%
t(6;14)	(p21;q32)	*Cyclin D3*	2%
t(8;14)	(q24;q32)	*MAF-A*	<1%
t(11;14)	(q13;q32)	*Cyclin D1*	15%
t(12;14)	(p13;q32)	*Cyclin D2*	<1%
t(14;16)	(q32;q23)	*c-MAF*	5%
t(14;20)	(q32;q12)	*MAF-B*	<1%
1q gain	1q21	*(Multiple)*	30%
1p loss	1p32	*(Multiple)*	20%
13q-, -13	–	*(Multiple, RB)*	50%
17p-	–	*(Multiple, p53)*	10%

oncogenic translocations may not be sufficient for the pathogenesis of the disease [15]. It is believed that occurrence of dysregulated oncogenes represents consecutive stages of MM pathogenesis. In fact, current knowledge supports a stepwise transformation model with the accumulation of genetic alterations and proliferative capacity during the tumor progression of plasma cell dyscrasias [16]. Therefore, it is not surprising that the search for an effective single therapeutic agent in MM, with targeted activity similar to that of retinoic acid in APL or imatinib mesylate in CML, has been challenging.

Chromosomal aberrations other than translocations are also common in MM and have prognostic value. Table 1 shows the most common chromosomal gains, losses and deletions, their approximate frequency, and the genes that are thought to be dysregulated as a result of the abnormality [reviewed in 17]. In particular, the detection of complete loss of chromosome 13 (monosomy 13) by metaphase cytogenetics, or deletion of the short arm of chromosome 17 (17p-) by fluorescence in situ hybridization (FISH) are considered to be two significant adverse prognostic factors. For example, median overall survival after high-dose therapy for patients with 17p- was 15 months compared to 48 months for patients without this abnormality [18]. The tumor suppressor genes retinoblastoma (RB) and p53 are located on chromosomes 13 and 17, respectively, and their deletion is believed to account for the negative clinical outcomes associated with these chromosomal abnormalities.

Targeted Therapies in Multiple Myeloma

IMiDs

Thalidomide and its derivatives represent the class of antineoplastic compounds called ImmunoModulatory Drugs (IMiDs). The efficacy of these agents in MM and other hematologic malignancies is attributed to their immunomodulatory, anti-inflammatory, and antiangiogenic properties. IMiDs target tumor cells directly by

inducing cytotoxicity and indirectly by interfering with components of the bone marrow microenvironment that promote MM progression [19]. Thalidomide, initially introduced in Germany in 1957 as a sedative, was withdrawn from the market in 1961, when it was linked to severe fetal malformations. The renewed interest in thalidomide was related to the discovery of its activity in patients with MM [20]. Thalidomide induces apoptosis of MM cells and down-regulates the expression of several cytokines involved in cell proliferation and survival, such as TNFα, IL-6, and VEGF. However, its precise mechanism of action has not been fully elucidated [21]. Although the molecular target of thalidomide has yet to be identified, a recent study found that cereblon (CRBN), a protein encoded by a candidate gene for mental retardation, bound thalidomide and mediated its teratogenicity [22]. Clinically, the use of thalidomide in relapsed/refractory MM is associated with response rates (RR) ranging from 25% to 65% [20]. The main toxicities of thalidomide include sedation, peripheral neuropathy, bradycardia, hypotension, constipation, and venous thromboembolism. Due to the increased risk of venous thromboembolism (VTE), antithrombotic prophylaxis is always recommended (with aspirin, warfarin, or low-molecular weight heparin, depending on the clinical scenario and the presence or absence of other coexistent risk factors for VTE).

The thalidomide analogues lenalidomide and pomalidomide (CC4047) are second-generation IMiDs developed to enhance the anticancer properties and reduce the adverse effects associated with thalidomide. Lenalidomide proved effective in refractory patients including those who had relapsed following thalidomide treatment [23]. Furthermore, lenalidomide generated superior response rates along with progression-free and overall survival compared to thalidomide in newly diagnosed patients [24]. Pomalidomide elicited responses in 47% of patients who had received three or more previous regimens, including lenalidomide [25]. More clinical studies investigating the activity of pomalidomide are needed, but these data suggest that pomalidomide is clinically effective in advanced MM, even when the disease is refractory to other IMiDs. Table 2 lists several important clinical trials of IMiDs in MM, chosen based on the number of patients enrolled and the use of monotherapy whenever possible. The intent is to indicate the clinical activity of these agents in MM without the confounding effectiveness of other agents.

Proteasome Inhibitors

The proteasome is a multi-subunit, cylinder-shaped protein complex that degrades ubiquitinated proteins. Plasma cells are terminally differentiated B-cells that are specialized for the mass production of immunoglobulins. The increased protein load associated with this task lowers the threshold for proteotoxic stress and increases the susceptibility of plasma cells to toxic misfolded/unfolded proteins that trigger proapoptotic signals of the unfolded protein response (UPR) and endoplasmic reticulum (ER) stress response [26]. Additionally, plasma cell differentiation is accompanied by a dramatic decrease in expression of the proteasome [27].

Table 2 Clinical trials of IMiDs in multiple myeloma

Drug	Author [Ref.]	Regimen	Description	Trial phase	N pts	Clinical outcomes	Comments
Thalidomide	Barlogie [105]	200–800 mg/day PO	Monotherapy, in relapsed/ refractory MM	II	169	RR: 30% 2-y EFS: 20% 2-y OS: 48%	Grade 3–4 toxicities: sedation (25%), constipation (16%),
Thalidomide	Mileshkin [106]	200–800 mg/day PO	Monotherapy, in relapsed/ refractory MM	II	75	RR: 28% PFS: 5.5 months OS: 14.6 months	Most common toxicities grade >2: constipation (52%), lethargy (34%)
Thalidomide	Tosi [107]	100–800 mg/day PO	Monotherapy, in relapsed/ refractory MM	II	65	RR: 28% DOR: 8 months	19 pts received IFNα after 12 weeks
Thalidomide	Rajkumar [108]	200–800 mg/day PO	Monotherapy, in smoldering/asympto-matic MM	II	31	RR: 34% 1-y PFS: 80% 2-y PFS: 63%	Thalidomide is not approved by the FDA
Thalidomide	Rajkumar [109]	200 mg/day PO	Thal-DexvsDex, in newly diagnosed MM	III	207	RR: 63%	Regimen used as induction therapy: survival was not an endpoint
Lenalidomide	Richardson [110]	30 mg/day PO, days 1–21 (cycles: 28 days)	Monotherapy, in relapsed/ refractory MM	II	222	RR: 26% PFS: 4.9 months OS: 23.2 months	Main toxicity: hemato-logic (grade 3–4 in 60% of pts)
Lenalidomide	Dimopoulos [111]	25 mg/day PO, days 1–21 (cycles: 28 days)	Len-DexvsDex, in relapsed/refractory	III	351	RR: 60% TTP: 11.3 months	Main grade 3–4 toxicities: neutropenia (29%),

Lenalidomide	Rajkumar [112]	25 mg/day PO, days 1–21 (cycles: 28 days)	Len-LD Dexvs Len-HD Dex, in newly diagnosed MM	III	445	RR: 79% withHD Dex, 68% with LD Dex 1-y OS: 87 with HD Dex, 96% with LD Dex	Len-LD Dex provided better survival and less toxicity than Len-HD Dex
Pomalidomide	Lacy [25]	2 mg/day PO, days 1–28 (cycles: 28 days)	Given with Dex, in relapsed/refractory MM	II	34	RR: 47% PFS: 4.8 months OS: 13.9 months	Main toxicity: hematologic (grade 3–4 in 53% of pts)

Dex dexamethasone, *DOR* duration of response (median), *DVT* deep venous thrombosis, *EFS* event-free survival (median, unless otherwise indicated), *HD* high dose, *LD* low dose, *Len* lenalidomide, *PFS* progression-free survival (median, unless otherwise indicated), *MM* multiple myeloma, *OS* overall survival (median, unless otherwise indicated), *PO* orally, *pts* patients, *RR* response rate, *Thal* thaliddomide, *TTP* time to progression (median), *y* year, *FDA* U.S. Food and Drug Administration

Taken together, these cellular characteristics are thought to make plasma cells particularly sensitive to inhibitors of the proteasome. In addition, the proteasome regulates the expression of proteins and cytokines that promote MM growth and angiogenesis, and inhibit apoptosis, such as NF-κB [28]. The proteasome inhibitor bortezomib was developed for the treatment of MM based on this rationale, and it has been widely recognized as a remarkable clinical success. Bortezomib received accelerated approval from the U.S. Food and Drug Administration (FDA) in 2003, after clinical efficacy was demonstrated in refractory MM [29]. Bortezomib was later approved for first-line treatment of MM. Despite the efficacy of bortezomib, MM cells invariably develop resistance to it. Carfilzomib, a second generation proteasome inhibitor, has shown efficacy against bortezomib-resistant MM cell lines and primary patient samples in vitro [30], and has also exhibited promising activity in patients [31]. The activity of carfilzomib against bortezomib-resistant MM cells may be due to its pharmacological profile, which differs from bortezomib. Both drugs inhibit the same proteasomal subunit (20S chymotrypsin-like β5 subunit), but only carfilzomib does so irreversibly. Another second-generation proteasome inhibitor, marizomib (NPI-0052), is an orally bioavailable non-peptide based compound with encouraging preclinical activity in MM [32].

Table 3 lists several important clinical trials of bortezomib in MM. We chose trials based on the number of patients enrolled and the use of monotherapy (whenever possible) in order to determine the clinical effectiveness of this agent in MM without the influence of confounding factors. However, we recognize that combination chemotherapy regimens are frequently administered both in trials and in clinical practice, with the goal of increasing response rates and survival outcomes. For example, the VRD regimen, which combines bortezomib, lenalidomide, and dexamethasone, is an exceptionally effective regimen in MM, where it has reportedly produced an unprecedented response rate of 100% in patients with newly diagnosed disease [33].

Histone Deacetylase Inhibitors

Histone deacetylase (HDAC) inhibitors are a class of molecules that epigenetically regulate gene transcription by modulating the structure of chromatin and DNA. These drugs can increase the transcription of genes previously down-regulated by histone acetylation [34]. The molecular target of HDAC inhibitors in MM cells is a subject of debate, particularly because they promote the acetylation of non-histone proteins as well. According to recent evidence, HDACs are critical targets of proteasome inhibitors [35], and clinical trials combining these two classes of agents are in progress. The most commonly used HDAC inhibitors are vorinostat, panobinostat (LBH589), and romidepsin. In a phase I trial of single-agent vorinostat, only one minor response was seen among 13 patients with relapsed/refractory MM, but maximum tolerated dose (MTD) was not reached due to early study termination by

Table 3 Relevant clinical trials of proteasome inhibitors in multiple myeloma

Drug	Author [Ref.]	Regimen	Description	Trial phase	N pts	Clinical outcomes	Comments
Bortezomib	Richardson [29]	1.3 mg/m^2 IV on days 1, 4, 8, 11 (cycles: 21 days)	Relapsed/refractory MM. Dex added if response was suboptimal	II	202	RR: 35% DOR: 12 months OS: 16 months 1-y OS: 80%	Grade 4 toxicities: 14%. Grade 3 toxicities: thrombocytopenia (28%), fatigue (12%), neuropathy (12%), neutropenia (11%)
Bortezomib	Richardson [113]	1.3 mg/m^2 IV on days 1, 4, 8, 11 every 21 d (cycles 1–8), and 1, 8, 15, 22	Bor-DexvsDex, in relapsed/refractory MM	III	669	RR: 38% TTP: 6.2 months DOR: 8 months	There was a survival advantage for pts receiving Bor, despite the fact that 44%
Bortezomib	Jagannath [114]	1.3 mg/m^2 IV on days 1, 4, 8, 11 (cycles: 21 days)	Newly diagnosed MM. Dex added if no response	II	32	RR: 88% (CR/nCR: 25%)	Most common toxicities, grade ≥2: neuropathy (31%), constipation (28%), myalgia (28%), fatigue (25%)
Bortezomib	Orlowski [115]	1.3 mg/m^2 IV on days 1, 4, 8, 11 (cycles: 21 days)	BorvsBor-PLD, in relapsed/refractory MM	III	646	Without PLD: RR: 41% TTP: 6.5 months	Grade 3–4 toxicities were seen in 80% of pts with Bor-PLD and 64% with Bor
Bortezomib	Mikhael [116]	1.3 mg/m^2 IV on days 1, 4, 8, 11 (cycles: 21 days)	Relapsed/refractory MM. Dex added if no response	IIIB	482	RR: 67%(including 16% minimal response)	Dex added in 33% of patients. Neuropathy (any grade) seen in 25% of pts

Bor bortezomib, *CR* complete response, *Dex* dexamethasone, *DOR* duration of response (median), *IV* intravenously, *MM* multiple myeloma, *nCR* near-complete response, *OS* overall survival (median, unless otherwise indicated), *PLD* pegylated liposomal doxorubicin, *pts* patients, *RR* response rate, *TTP* time to progression (median), *y* year

sponsor decision [36]. In another phase I study of 23 MM patients, vorinostat in combination with bortezomib produced a response rate of 42% (including three partial responses among nine bortezomib refractory patients) [37]. Similarly, the combination of vorinostat with bortezomib produced clinical benefit among six patients with relapsed/refractory MM after previous bortezomib treatment (partial response in one patient and stable disease in five patients) [38]. Preliminary findings with romidepsin have been disappointing: in a phase II trial, no objective responses were observed in 12 patients with relapsed/refractory MM after administration of romidepsin (13 mg/m^2) as a 4 h intravenous infusion on days 1, 8, and 15 every 28 days [39]. HDAC inhibitors that are currently in development include ACY-1215, which specifically targets the HDAC6 isoform and has demonstrated promising preclinical activity in models of MM [40].

Heat Shock Proteins Inhibitors

Heat shock proteins (Hsps) are molecular chaperones that facilitate proper folding of their "client" proteins. Plasma cells, both normal and malignant, produce and secrete massive amounts of immunoglobulins and rely heavily on Hsps to facilitate proper folding into their correct tertiary structures. Therefore, Hsp inhibition was developed as a rational therapeutic strategy for targeting MM. Indeed, inhibition of Hsp90 was shown to induce ER stress and a proapoptotic unfolded protein response in MM cells, suggesting that proteotoxicity is one mechanism by which Hsp90 inhibitors may induce MM cell death [41]. Others have shown that the anti-MM activity of Hsp90 inhibitors arises from their ability to inhibit the activity of prosurvival and proliferative signaling molecules, such as insulin-like growth factor 1 receptor, interleukin-6 receptor, Akt, and ERK [42, 43]. Tanespimycin, an inhibitor of Hsp90, is the only agent of this class of drugs evaluated in clinical trials. In a phase I trial of single-agent tanespimycin, this agent temporarily stabilized disease in 52% of heavily pretreated patients [44]. In the phase II trial, tanespimycin was combined with bortezomib, based on the preclinical data showing anti-tumor synergy between the two drugs. Objective responses were observed, although the study was closed prematurely for resource-based reasons [45].

The Nuclear Hormone Receptor Superfamily

Corticosteroids are among the most commonly used drugs in the treatment of MM, where they have been shown to induce response rates of approximately 60% in newly diagnosed patients when used as monotherapy [46]. Glucocortocoid receptors, members of the nuclear hormone receptor superfamily, are the molecular targets of prednisone and dexamethasone—two corticosteroids commonly administered to MM patients. The superfamily of nuclear hormone receptors consists of structurally related proteins, encoded by homologous genes that function as receptors

Table 4 Cancer therapy involving the nuclear hormone receptor superfamily

Receptor	Gene	Hormone/ligand used in therapy	Type of cancer
Glucocorticoid receptor	*GR*	Prednisone, dexamethasone	MM, HL, NHL
Estrogen receptor	*ER*	Tamoxifen	Breast cancer
Androgen receptor	*AR*	Testosterone (inhibited by flutamide)	Prostate cancer
Progesterone receptor	*PR*	Megestrol acetate	Breast cancer
Retinoic acid receptor	*RARα*	ATRA	APL
Retinoid X receptor	*RXR*	Bexarotene	CTCL

APL acute promyelocytic leukemia, *ATRA* all-trans retinoic acid, *CTCL* cutaneous T-cell lymphoma, *HL* Hodgkin's lymphoma, *NHL* non-Hodgkin's lymphoma

for several hormones, including glucocorticoids, estrogen, progesterone, androgens, thyroid hormone, vitamin D, retinoids, and others. Structurally, these molecules share a small lipophilic molecular core, which can pass through the lipid bilayer of the cell membrane and bind to their specific receptors in the cytoplasm. The receptors are nuclear transcription factors that, after being activated by the binding to their hormones, translocate to the nucleus and regulate transcription of target genes that control biologic processes important in development, cell proliferation, and differentiation [47]. The nuclear hormone receptors typically consist of five domains: A/B (modulating region), C [DNA-binding region, containing two zinc-fingers which specifically recognize the hormone-responsive element (HRE), a DNA enhancer sequence that is located near the promoter region of target genes], D (hinge region, important for nuclear localization), E (hormone-binding region, which also contains leucine zippers, the sites of dimerization), and F (modulating region). Following ligand binding, hormone-receptor complexes dimerize with other hormone-receptor complexes and translocate to the nucleus, where they bind to and initiate transcription of HRE expressing genes. The interaction between the receptor dimer and the HRE activates transcriptional machinery, leading to the expression of a specific set of genes. Table 4 shows examples of hormone therapy in oncology, and provides a unifying view of this group of drugs.

Although they are not traditionally recognized as targeted therapeutics, corticosteroids represent some of the most simple and direct forms of targeted cancer therapy. These therapies target proteins whose structures contain both the ligand-binding and DNA-binding sites. Therefore, a single receptor molecule is capable of directly connecting the external therapeutic agent (hormone or ligand) to specific target genes. By comparison, other classes of targeted cancer therapeutics, such as small molecular weight inhibitors or monoclonal antibodies, may be highly specific for their molecular targets, but their anti-neoplastic activity is indirect, requiring inhibition of downstream signaling intermediates. For example, receptor tyrosine kinase inhibitors may bind exclusively to a designated kinase, but their anti-tumor activity is due to inhibition of prosurvival and proliferative signaling pathways downstream of the receptor. Other drugs used in cancer therapy may act more directly by physically interacting and damaging DNA (e.g., the alkylating agent melphalan), but their activity is not targeted, as they bind to DNA non-specifically and do not distinguish between specific genetic targets.

Emerging Therapeutic Targets and Signaling Pathways in Multiple Myeloma

The PI3K/Akt/mTOR Pathway

The PI3K/Akt/mTOR signaling pathway stimulates cell growth, survival, and proliferation, and is the most commonly activated pathway in human cancers [48]. The importance of the PI3K/Akt/mTOR pathway in the growth and survival of MM cells has been extensively studied in preclinical models [49–54], and mTOR inhibition specifically has been considered one of the more exciting strategies to be pursued in trials for MM [55]. The potential benefit of this approach has been demonstrated not only in xenograft models of MM, but also clinically. A phase II study of the mTOR inhibitor temsirolimus in MM has shown a response rate of 38% and a median time to progression of about 5 months in patients with relapsed or refractory disease [56]. Other agents that target this pathway include perifosine (KRX-0401), an orally active alkylphsopholipid that inhibits Akt, which is currently being investigated in clinical trials for MM.

The IGF-1 Pathway

Insulin-like growth factor I (IGF-I) is known to promote the growth of MM cells [57, 58]. The inhibition of the IGF-1 signaling has not been completely explored in the treatment of MM. The anti-MM activity of CP-751871 (figitumumab), a monoclonal antibody (mAb) directed against IGF-1 receptor (IGF-1R), was explored in a phase I clinical trial [59]. Responses were observed in nine of 27 patients with relapsed/refractory MM who were treated with the IGF-1R antibody in combination with dexamethasone (of note, two of the patients who reached a partial remission were previously refractory to single agent dexamethasone). Other IGF-1R targeted agents that have been tested clinically include the mAb AVE1642 [60]. This phase 1 study demonstrated a favorable toxicity profile, but the authors concluded that further development was unwarranted given a lack of responses alone or in combination with bortezomib.

Hepatocyte Growth Factor

The hepatocyte growth factor (HGF) is a potent MM growth and survival cytokine, and malignant plasma cells are known to express c-Met, the receptor for HGF [61]. Critical effector pathways that are activated by c-Met include the Ras/Raf/MEK/ERK and PI3K/Akt pathways. Since the HGF/c-Met pathway has anti-apoptotic effects in MM cell lines and primary MM cells [62], inhibitors of c-Met or neutralizing

antibodies directed against HGF may have a clinical activity in MM patients. To date, PHA-665752, a small molecule c-Met tyrosine kinase inhibitor, is the only c-Met targeted agent to show preclinical activity in MM [63].

Therapeutic mAbs

The introduction of the mAb rituximab has revolutionized the clinical care of B-cell lymphomas. However, despite many years of effort, the search for a clinically effective mAb for patients with MM has been less productive thus far [64].

Rituximab is a monoclonal antibody against the B-cell specific membrane protein CD20. The rationale for this therapeutic strategy was that CD20 is expressed in 10–15% of MM plasma cells [65]. Unfortunately, the use of rituximab provided no clinical benefit in a phase II study of ten patients with MM [66], and it did not demonstrate significant clinical activity in a cohort of 14 patients selected for CD20-expressing MM [67].

Interleukin 6 (*IL-6*) is known to play an important role in growth, differentiation, and survival of normal and malignant plasma cells. The transforming potential of IL-6 is underscored by the fact that IL-6 overexpressing transgenic mice show accelerated development of malignant plasmacytomas [68]. Monoclonal antibodies against IL-6 have been developed, and they have been used with success against Castleman's disease, a rare B-cell lymphoproliferative disorder [69], but clinical experience in MM is currently lacking. Preclinical models have shown promising activity with the IL-6 neutralizing antibodies siltuximab (formerly CNTO 328) [70, 71] and mAb 1339 [72]. Another agent targeting IL-6 signaling is tocilizumab, a monoclonal antibody against the IL-6 receptor (IL-6R), which was approved in 2005 in Japan for the treatment of Castleman's disease, and by the U.S. Food and Drug Administration (FDA) in 2010 for the treatment of rheumatoid arthritis.

Elotuzumab is a humanized monoclonal antibody directed against CS1 (also known as CD319), a cell surface glycoprotein highly expressed on MM cells that plays a role in cell adhesion. Elotuzumab has shown anti-MM activity both in vitro as well as in an in vivo MM xenograft model [73].

Many monoclonal antibodies are currently being evaluated in clinical trials for MM, with different targets and strategies. Some of these antibodies are designed to target surface proteins of plasma cells (e.g., CD38, CD56) or MM growth factors (e.g., IL-6), while others have been coupled to cytotoxins or chemotherapy agents (see Table 5).

Agents Directed Against Dysregulated Translocation Products

The t(11;14) (q13;q32) chromosomal translocation is a common translocation detected in MM. The t(11;14) translocation juxtaposes the IgH locus with the

Table 5 Selected novel targeted agents in clinical development for relapsed/refractory multiple myeloma

Drug	Mechanism of action	Regimen	Phase	Protocol ID
Pomalidomide	IMiD	Pomalidomide	III	NCT01324947
Pomalidomide	IMiD	Pomalidomide	II	NCT01319422
Pomalidomide	IMiD	Pomalidomide + Dex	III	NCT01311687
Pomalidomide	IMiD	Pomalidomide + Dex	II	NCT00558896
Pomalidomide	IMiD	Pomalidomide + Dex + Clar	II	NCT01159574
Pomalidomide	IMiD	Pomalidomide + Dex + Cy	I–II	NCT01432600
Pomalidomide	IMiD	Pomalidomide + Dex + Bor	I	NCT01497093
Carfilzomib	Proteasome inhibitor	Carfilzomib	III	NCT01302392
Carfilzomib	Proteasome inhibitor	Carfilzomib + Dex	II	NCT01495559
Carfilzomib	Proteasome inhibitor	Carfilzomib + Dex + Len	III	NCT01080391
Carfilzomib	Proteasome inhibitor	Carfilzomib + Dex + Cy	II	NCT01346787
Carfilzomib	Proteasome inhibitor	Carfilzomib + Mel + Pred	I–II	NCT01279694
MLN9708	Proteasome inhibitor	MLN9708	I	NCT00963820
MLN9708	Proteasome inhibitor	MLN9708 + Dex	II	NCT01415882
MLN9708	Proteasome inhibitor	MLN9708 + Dex + Len	I–II	NCT01383928
MLN9708	Proteasome inhibitor	MLN9708 + Mel + Pred	I–II	NCT01335685
CEP-18770	Proteasome inhibitor	CEP-18770	I–II	NCT01023880
CEP-18770	Proteasome inhibitor	CEP-18770 + Dex + Len	I–II	NCT01348919
ONX 0912	Porteasome inhibitor	ONX 0912	I–II	NCT01416428
NPI-0052	Proteasome inhibitor	NPI-0052	I	NCT00461045
Perifosine	PI3K/AKT inhibitor	Perifosine + Bor + Dex	III	NCT01002248
GSK2110183	PI3K/AKT inhibitor	GSK2110183 +/– Bor	II	NCT01445587
GSK2110183	PI3K/AKT inhibitor	GSK2110183 + Dex + Bor	I	NCT01428492
Temsirolimus	mTOR inhibitor	Temsirolimus + Bor	I–II	NCT00483262
INK128	TORC1/2 inhibitor	INK128	I	NCT01118689
Vorinostat	HDAC inhibitor	Vorinostat + Len	I	NCT00729118
Vorinostat	HDAC inhibitor	Vorinostat + Bor	II	NCT00839956
Vorinostat	HDAC inhibitor	Vorinostat + Dex + Len	I–II	NCT01502085
Pabinostat	HDAC inhibitor	Panobinostat + Bor	I	NCT00891033
Pabinostat	HDAC inhibitor	Panobinostat + Bor + Dex	III	NCT01023308
Panobinostat	HDAC inhibitor	Panobinostat + Mel	I–II	NCT00743288
Romidepsin	HDAC inhibitor	Romidepsin + Bor	I–II	NCT00431990
JNJ 26481585	HDAC inhibitor	JNJ 26481585 + Dex + Bor	I	NCT01464112
ACY-1215	HDAC6 inhibitor	ACY-1215 +/– Dex-Bor	I–II	NCT01323751
Ganetespib	HSP90 inhibitor	Ganetespib +/– Dex-Bor	I	NCT01485835
KW-2478	HSP90 inhibitor	KW-2478 + Bor	I–II	NCT01063907
Daratumumab	Monoclonal Ab anti-CD38	Daratumumab	I–II	NCT00574288
MOR03087	Monoclonal Ab anti-CD38	MOR03087 +/– Dex-Len-Bor	I–II	NCT01421186
Lorvotuzumab	Monoclonal Ab anti-CD56	Lorvotuzumab	I	NCT00991562
Siltuximab	Monoclonal Ab anti-IL6	Siltuximab + Dex	II	NCT00402181

(continued)

Table 5 (continued)

Drug	Mechanism of action	Regimen	Phase	Protocol ID
Siltuximab	Monoclonal Ab anti-IL6	Siltuximab + Dex + Bor	I	NCT01309412
Elotuzumab	Monoclonal Ab anti-CS1	Elotuzumab + Dex-Len	III	NCT01335399
Elotuzumab	Monoclonal Ab anti-CS1	Elotuzumab + Dex-Bor	II	NCT01478048
Cetuximab	Monoclonal Ab anti-EGFR	Cetuximab +/− Dex	II	NCT00368121
Anti-ICAM-1	Monoclonal Ab anti-ICAM-1	Anti-ICAM-1	I	NCT01025206
LY2127399	Monoclonal Ab anti-BAFF	LY2127399 + Bor	I	NCT00689507
IPH2101	Monoclonal Ab anti-KIR	IPH2101 + Len	I–II	NCT01217203
MFGR1877S	Monoclonal Ab anti-FGFR3	MFGR1877S	I	NCT01122875
Bevacizumab	Monoclonal Ab anti-VEGF	Bevacizumab + Len + Dex	II	NCT00410605
Bevacizumab	Monoclonal Ab anti-VEGF	Bevacizumab + Bor	II	NCT00464178
Bevacizumab	Monoclonal Ab anti-VEGF	Monotherapy	II	NCT00482495
Aflibercept	VEGF Trap	NSC 724770	II	NCT00437034
hLL1-DOX	Milatuzumab-Dox conjugate	hLL1-DOX	I–II	NCT01101594
BT062	Toxin-coupled Ab	Monotherapy	I–II	NCT01001442
Dinaciclib	CDK inhibitor	Dinaciclib	II	NCT01096342
PD 0332991	CDK inhibitor	PD 0332991 + Bor + Dex	I–II	NCT00555906
P276-00	CDK inhibitor	P276-00	I–II	NCT00882063
AT7519M	CDK inhibitor	AT7519M +/− Bor	I–II	NCT01183949
ARRY-520	KSP inhibitor	ARRY-520	I–II	NCT00821249
CX-4945	CK2 protein kinase inhibitor	CX-4945	I	NCT01199718
MLN8237	Aurora A kinase inhibitor	MLN8237 + Bor	I–II	NCT01034553
Obatoclax	BCL-2 inhibitor	Obatoclax + Bor	I–II	NCT00719901
Masitinib	Tyrosine kinase inhibitor	Masitinib	III	NCT01470131
Dasatinib	Tyrosine kinase inhibitor	Dasatinib + Dex + Len	II	NCT00560391
AT9283	JAK2 inhibitor	AT9283	II	NCT01145989
MLN4924	NEDD8 A.E. Inhibitor	MLN4924	I	NCT00722488
OPB-31121	STAT3 inhibitor	OPB-31121	I	NCT00511082
Veliparib	PARP inhibitor	Veliparib + Dex-Bor	I	NCT01495351
GDC-0449	Hedgehog antagonist	GDC-0449	I	NCT01330173
BMS-833923	Hedgehog antagonist	BMS-833923 +/− Dex-Len-Bor	I	NCT00884546
Imetelstat	Telomerase antagonist	Imetelstat +/− Len	II	NCT01242930

(continued)

Table 5 (continued)

Drug	Mechanism of action	Regimen	Phase	Protocol ID
Tivantinib	c-MET inhibitor	Tivantinib	II	NCT01447914
GSK1120212	MEK inhibitor	GSK1120212 + GSK2110183	I	NCT01476137

List of actively recruiting clinical trials, obtained from the web site www.clinicaltrials.gov, accessed on 02 May 2012

Ab antibody, *BAFF* B cell activating factor, *Bor* bortezomib, *CDK* cyclin-dependent kinase, *Clar* Clarithromycin, *Cy* cyclophosphamide, *Dex* dexamethasone, *Dox* doxorubicin, *HDAC* histone deacetylase, *ICAM1* Intercellular Adhesion Molecule-1, *KSP* kinesin spindle protein, *Len* lenalidomide, *Mel* Melphalan, *MM* multiple myeloma, *Pred* prednisone

CCND1 gene (also called *BCL1*), which leads to overexpression of the *CCND1* gene product cyclin D1. Cyclin D1 associates with cyclin-dependent kinase 4 (CDK4) to form a catalytically active complex that drives progression through the G1/S phase of the cell cycle. Consequently, up-regulation of cyclin D1 as a result of the t(11:14) translocation has been implicated in the uncontrolled proliferation of MM plasma cells [74, 75]. The selective targeting of cyclin D1 in one model was accompanied by compensatory up-regulation of cyclin D2 and demonstrated only modest inhibition of MM cell proliferation in vitro [76]. By contrast, targeting CDK4 kinase activity with the small molecule inhibitor P276-00 was a more potent strategy for inhibiting growth of MM cells in vitro and tumors in vivo [77]. Flavopiridol (alvocidib), another cyclin-dependent kinase inhibitor tested in MM patients, failed to induce clinical responses in 18 patients with advanced disease in one study[78], but induced a partial response in one of two bortezomib refractory patients when used in combination with bortezomib in another trial [79]. Flavopiridol is a broad spectrum CDK inhibitor with activity against CDK1, CDK2, CDK4, CDK7, and CDK9. Targeting CDK9 is a promising strategy for the treatment of MM. CDK9 is a key regulator of transcription as it functions as a subunit of the P-TEFb (*P*ositive-*T*ranscription *E*longation *F*actor b) complex, which phosphorylates the carboxy-terminus of RNA polymerase II, a signaling event that releases the enzyme into the elongation phase of transcription [80]. In addition to flavopiridol, SNS-032 and AT7519 (inhibitors of CDK9 and other CDKs) are currently in clinical development for the treatment of MM [81, 82]. Lastly, the agent JQ1, a member of the new class of bromodomain inhibitors, has demonstrated potent preclinical anti-MM activity by disrupting P-TEFb recruitment to c-Myc target genes [83], further demonstrating that CDK9 is a promising molecular target in the treatment of MM.

The t(4;14) (p16;q32) chromosomal translocation is also common in MM, and is associated with poor prognosis. It results in the formation of a fusion IgH-MMSET (multiple myeloma SET domain) transcript in the der(4) chromosome [12]. This translocation may induce overexpression of the fibroblast growth factor receptor 3 (FGFR3) gene, which encodes for a receptor tyrosine kinase. All cases overexpress MMSET, a histone methyltransferase, but about one third of cases do not overexpress FGFR3 [84]. Moreover, FGFR3 amplification may occur even in the absence of t(4;14) [85]. These mutations produce a constitutively active receptor, which exhibits ligand-independent dimerization and autophosphorylation. NF449, a novel

compound that antagonizes FGFR3 signaling, was found to be active against MM in vitro [86]. Anti-FGFR3 agents, such as CHIR-258, a small-molecule inhibitor of multiple receptor tyrosine kinases including FGFR3 [87], and PRO-001, an FGFR3-specific mAb [88], showed activity in mouse models of MM. In view of these promising results, anti-FGFR3 agents are currently being evaluated in clinical trials for FGFR3-expressing MM (see Table 5).

Targeting MM Cancer Stem Cells

The cancer stem cell (CSC) model proposes that a small percentage of tumor cells possess the majority of the tumorigenic potential of the cancer. CSCs are believed to have limitless self-renewal capabilities and inherent resistance to conventional chemotherapy, which enables them to avoid treatment-induced cytotoxicity and repopulate the cancer after disease-free periods. The existence of an MM CSC has been proposed for decades, as MM is a disease that nearly always recurs even after complete and durable responses. However, the MM CSC and its immunophenotype has yet to be identified. Work by Matsui and colleagues has demonstrated that a sub-population of CD138- MM cells may represent clonogenic, less-differentiated precursors of malignant plasma cells with the capacity to avoid cell death in response to cytotoxic therapy [89]. These clonogenic CD138- MM cells were found to express CD19, CD20 and CD27, a phenotype that is also typical of normal memory B cells. Based on these findings it has been proposed that CD20-targeted agents (i.e. rituximab) may be an effective approach to eliminating putative MM CSCs. However, although there is anecdotal evidence of the effectiveness of rituximab in MM, clinical trials have demonstrated a lack of efficacy, even when patients were selected for CD20 expression [66, 67]. A potential explanation for this lack of efficacy is that the clinical benefit of targeting rare subpopulations of MM CSCs may only be evident after long-term follow-up with distant endpoints. Traditional response criteria in MM (i.e. analysis of serum or urine M protein, and bone marrow plasmacytosis) are more likely to reflect an early response derived from eliminating the malignant plasma cells rather than the clonogenic MM cells that give rise to them [90]. Continued work in the area of MM CSC identification is critical and could have a significant impact on the development of new MM treatment approaches that aim to specifically target clonogenic MM CSCs.

Targeting the MM Tumor Microenvironment

The role of the tumor microenvironment in the development, progression, and resistance of various tumor types to therapy is well recognized [91]. In MM, the impact of tumor microenvironmental factors such as hypoxia, angiogenesis, and interactions between MM and bone marrow stromal cells have become an important

consideration for understanding disease progression, resistance to therapy, and have been incorporated into novel drug screening approaches [92]. For instance, bone marrow angiogenesis has been implicated in MM disease progression, as it progressively increases along the spectrum of plasma cell dyscrasias, from monoclonal gammopathy of undetermined significance (MGUS) to smoldering myeloma, and advanced MM [93]. Malignant plasma cells not only secrete vascular endothelial growth factor (VEGF), a soluble protein that stimulates the growth of new blood vessels, but they can also express its receptors, VEGFR-1 and VEGFR-2 [94, 95]. After the availability and success of the anti-VEGF monoclonal antibody bevacizumab in the clinical practice against several types of solid malignancies [96], anti-angiogenic therapy was tested in MM, although the results with this strategy have been disappointing. Three studies merit mentioning. Zangari et al. used SU5416, a small molecule VEGFR-2 inhibitor, in 27 patients with advanced MM. Four patients had disease stabilization for at least 4 months, but no objective responses were observed [97]. In a phase II trial of vandetanib (formerly ZD6474), a small molecule receptor tyrosine kinase inhibitor of both VEGFR and epidermal growth factor receptor (EGFR), no responses were found among 18 patients with relapsed MM [98]. Similarly, no clinical responses were observed in another phase II trial of 21 MM patients with the use of pazopanib, a multi-targeted receptor tyrosine kinase inhibitor of VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-α/β, and c-kit [99]. The fact that not a single response was observed among a total of 66 MM patients in three different clinical trials using anti-angiogenic drugs casts doubt that this therapeutic strategy will be further explored in MM.

Other Targeted Therapies

Several targeted therapies were initially judged as promising in the treatment of MM based on preclinical evidence or their scientific rationale, yet clinical trials using these agents failed to demonstrate their utility in humans. We report the following selected experiences:

The farnesyltransferase inhibitor *tipifarnib* was administered to 43 patients with advanced MM, at a dose of 300 mg PO bid for 3 weeks every 4 weeks. The most common toxicity was fatigue (66%). Although 64% of patients had disease stabilization, no complete nor partial responses were observed [100].

Oblimersen is an antisense drug (a short sequence of RNA which hybridizes with and inactivates a specific mRNA, preventing the formation of the protein) blocking the Bcl-2 oncogene. Despite its activity in other hematologic malignancies, a phase III randomized study that included 224 patients found no clinical benefit of in MM [101].

Etanercept is a tumor necrosis factor (TNF) alpha-neutralizing agent. It is a soluble protein engineered by fusing part of the TNF-receptor with the Fc portion of an IgG antibody. Treatment with etanercept produced no response among ten patients with refractory MM [102].

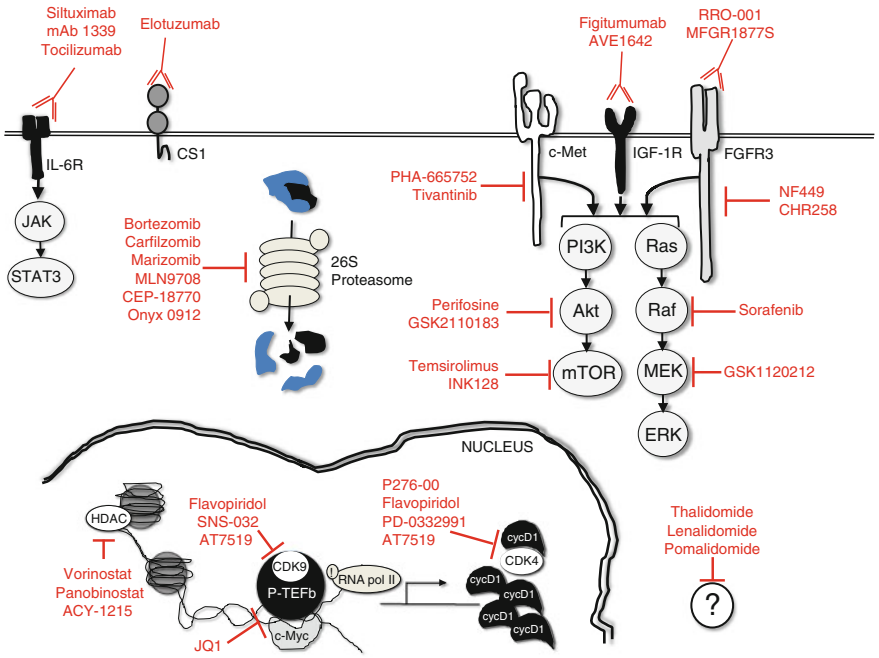

Fig. 2 Emerging therapies and therapeutic targets in MM. Molecular targets are shown along with targeted therapeutic agents (*red*) that are approved for the treatment of MM or are currently in development

Imatinib is a tyrosine kinase inhibitor that blocks the activity of c-Abl, c-Kit, and PDGF receptors. The development of imatinib has been the most successful achievement of molecular biology applied to hematological malignancies. In a phase II trial of imatinib in 28 patients with refractory/relapsed MM, no responses were observed. Of note, 52% of cases had positive c-kit staining [103].

Table 5 lists novel therapeutic agents that are currently in clinical development for the treatment of MM along with the associated clinical protocol ID. Fig. 2 illustrates the potential molecular targets in MM along with the target-specific agents that are in preclinical and clinical development.

Conclusions

In this review of MM targeted therapy we have discussed the molecular mechanisms of action and clinical efficacy of several agents, some of which (i.e. thalidomide, lenalidomide, and bortezomib) have already significantly improved the care of both newly diagnosed and relapsed/refractory patients. Furthermore, we have reviewed the many promising targeted agents for MM that are currently being developed or

are under clinical evaluation. It is clear that further advances in the treatment of MM, and the ultimate goal of a cure, cannot be achieved by intensifying conventional chemotherapy regimens, but requires the integration of novel small molecules and biologics that specifically target the pathological mechanisms underlying the progression of MM. Ultimately, gene and protein profiling and oncogenomic studies will identify specific molecules that contribute to MM pathogenesis, and will facilitate the development of new agents that block the molecular events that induce MM. Efforts in MM whole genome sequencing have already revealed potential new therapeutic targets [104]. The introduction of IMiDs and proteasome inhibitors has already revolutionized the treatment of MM. In the future, the development of new targeted agents will provide patients with more therapeutic options and further improve clinical outcomes to a point where MM may one day become a chronic and hopefully curable disease rather than an incurable one.

References

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2009. CA Cancer J Clin. 2009;59:225–49.
2. Talamo G, Farooq U, Zangari M, et al. Beyond the CRAB symptoms: a study of presenting clinical manifestations of multiple myeloma. Clin Lymphoma Myeloma Leuk. 2010;10:464–8.
3. Kumar SK, Rajkumar SV, Dispenzieri A, et al. Improved survival in multiple myeloma and the impact of novel therapies. Blood. 2008;111:2516–20.
4. Stewart AK, Richardson PG, San-Miguel JF. How I treat multiple myeloma in younger patients. Blood. 2009;114(27):5436–43.
5. Anderson KC, Alsina M, Bensinger W, et al. NCCN clinical practice guidelines in oncology: multiple myeloma. J Natl Compr Canc Netw. 2009;7:908–42.
6. Roodman GD. Pathogenesis of myeloma bone disease. Leukemia. 2009;23:435–41.
7. Roodman GD, Dougall WC. RANK ligand as a therapeutic target for bone metastases and multiple myeloma. Cancer Treat Rev. 2008;34:92–101.
8. Berenson JR. Therapeutic options in the management of myeloma bone disease. Semin Oncol. 2010;37 Suppl 1:S20–9.
9. Zangari M, Zhan F, Tricot G. Bone and paraproteinemias. Curr Opin Support Palliat Care. 2010;4:195–9.
10. Fenton JA, Pratt G, Rawstron AC, et al. Isotype class switching and the pathogenesis of multiple myeloma. Hematol Oncol. 2002;20:75–85.
11. Gonzalez D, van der Burg M, Garcia-Sanz R, et al. Immunoglobulin gene rearrangements and the pathogenesis of multiple myeloma. Blood. 2007;110:3112–21.
12. Chesi M, Nardini E, Lim RS, et al. The t(4;14) translocation in myeloma dysregulates both FGFR3 and a novel gene, MMSET, resulting in IgH/MMSET hybrid transcripts. Blood. 1998;92:3025–34.
13. He LZ, Tribioli C, Rivi R, et al. Acute leukemia with promyelocytic features in PML/RARalpha transgenic mice. Proc Natl Acad Sci USA. 1997;94:5302–7.
14. Wong S, Witte ON. Modeling Philadelphia chromosome positive leukemias. Oncogene. 2001;20:5644–59.
15. Fiancette R, Amin R, Truffinet V, et al. A myeloma translocation-like model associating CCND1 with the immunoglobulin heavy-chain locus 3′ enhancers does not promote by itself B-cell malignancies. Leuk Res. 2010;34:1043–51.
16. Rasmussen T, Theilgaard-Monch K, Hudlebusch HR, et al. Occurrence of dysregulated oncogenes in primary plasma cells representing consecutive stages of myeloma pathogenesis: indications for different disease entities. Br J Haematol. 2003;123:253–62.

17. Nahi H, Sutlu T, Jansson M, et al. Clinical impact of chromosomal aberrations in multiple myeloma. J Intern Med. 2011;269:137–47.
18. Chang H, Qi C, Yi QL, et al. p53 Gene deletion detected by fluorescence in situ hybridization is an adverse prognostic factor for patients with multiple myeloma following autologous stem cell transplantation. Blood. 2005;105:358–60.
19. Knight R. IMiDs: a novel class of immunomodulators. Semin Oncol. 2005;32:S24–30.
20. Palumbo A, Facon T, Sonneveld P, et al. Thalidomide for treatment of multiple myeloma: 10 years later. Blood. 2008;111:3968–77.
21. Paravar T, Lee DJ. Thalidomide: mechanisms of action. Int Rev Immunol. 2008;27:111–35.
22. Ito T, Ando H, Suzuki T, et al. Identification of a primary target of thalidomide teratogenicity. Science. 2010;327:1345–50.
23. Weber DM, Chen C, Niesvizky R, et al. Lenalidomide plus dexamethasone for relapsed multiple myeloma in North America. N Engl J Med. 2007;357:2133–42.
24. Gay F, Hayman SR, Lacy MQ, et al. Lenalidomide plus dexamethasone versus thalidomide plus dexamethasone in newly diagnosed multiple myeloma: a comparative analysis of 411 patients. Blood. 2010;115:1343–50.
25. Lacy MQ, Hayman SR, Gertz MA, et al. Pomalidomide (CC4047) plus low dose dexamethasone (Pom/dex) is active and well tolerated in lenalidomide refractory multiple myeloma (MM). Leukemia. 2010;24:1934–9.
26. Obeng EA, Carlson LM, Gutman DM, et al. Proteasome inhibitors induce a terminal unfolded protein response in multiple myeloma cells. Blood. 2006;107:4907–16.
27. Cenci S, Mezghrani A, Cascio P, et al. Progresively impaired proteasomal capacity during terminal plasma cell differentiation. EMBO J. 2006;25:1104–13.
28. Chauhan D, Hideshima T, Anderson KC. Targeting proteasomes as therapy in multiple myeloma. Adv Exp Med Biol. 2008;615:251–60.
29. Richardson PG, Barlogie B, Berenson J, et al. A phase 2 study of bortezomib in relapsed, refractory myeloma. N Engl J Med. 2003;348:2609–17.
30. Kuhn DJ, Chen Q, Voorhees PM, et al. Potent activity of carfilzomib, a novel, irreversible inhibitor of the ubiquitin-proteasome pathway, against preclinical models of multiple myeloma. Blood. 2007;110:3281–90.
31. O'Connor OA, Stewart AK, Vallone M, et al. A phase 1 dose escalation study of the safety and pharmacokinetics of the novel proteasome inhibitor carfilzomib (PR-171) in patients with hematologic malignancies. Clin Cancer Res. 2009;15:7085–91.
32. Singh AV, Palladino MA, Lloyd GK, et al. Pharmacodynamic and efficacy studies of the novel proteasome inhibitor NPI-0052 (marizomib) in a human plasmacytoma xenograft murine model. Br J Haematol. 2010;149:550–9.
33. Richardson PG, Weller E, Lonial S, et al. Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma. Blood. 2010;116:679–86.
34. Kim TY, Bang YJ, Robertson KD. Histone deacetylase inhibitors for cancer therapy. Epigenetics. 2006;1:14–23.
35. Kikuchi J, Wada T, Shimizu R, et al. Histone deacetylases are critical targets of bortezomib-induced cytotoxicity in multiple myeloma. Blood. 2010;116:406–17.
36. Richardson P, Mitsiades C, Colson K, et al. Phase I trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) in patients with advanced multiple myeloma. Leuk Lymphoma. 2008;49:502–7.
37. Badros A, Burger AM, Philip S, et al. Phase I study of vorinostat in combination with bortezomib for relapsed and refractory multiple myeloma. Clin Cancer Res. 2009;15:5250–7.
38. Mazumder A, Vesole DH, Jagannath S. Vorinostat plus bortezomib for the treatment of relapsed/refractory multiple myeloma: a case series illustrating utility in clinical practice. Clin Lymphoma Myeloma Leuk. 2010;10:149–51.
39. Niesvizky R, Ely S, Mark T, et al. Phase 2 trial of the histone deacetylase inhibitor romidepsin for the treatment of refractory multiple myeloma. Cancer. 2011;117:336–42.
40. Santo L, Hideshima T, Kung AL, Tseng JC, Tang D, Yang M, et al. Preclinical activity, pharmacodynamic and pharmacokinetic properties of a selective HDAC6 inhibitor, ACY-1215, in combination with bortezomib in multiple myeloma. Blood. 2012;19:2579–89. Epub ahead of print.

41. Davenport EL, Moore HE, Dunlop AS, et al. Heat shock protein inhibition is associated with activation of the unfolded protein response pathway in myeloma plasma cells. Blood. 2007;110:2641–9.
42. Mitsiades CS, Mitsiades NS, McMullan DJ, et al. Antimyeloma activity of heat shock protein-90 inhibition. Blood. 2006;107:1092–100.
43. Okawa Y, Hideshima T, Steed P, et al. SNX-2112, a selective Hsp90 inhibitor, potently inhibits tumor cell growth, angiogenesis, and osteoclastogenesis in multiple myeloma and other hematologic tumors by abrogating signaling via Akt and ERK. Blood. 2009;113:846–55.
44. Richardson PG, Chanan-Khan AA, Alsina M, et al. Tanespimycin monotherapy in relapsed multiple myeloma: results of a phase 1 dose-escalation study. Br J Haematol. 2010;150:438–45.
45. Richardson PG, Badros AZ, Jagannath S, et al. Tanespimycin with bortezomib: activity in relapsed/refractory patients with multiple myeloma. Br J Haematol. 2010;150:428–37.
46. Kumar S, Lacy MQ, Dispenzieri A, et al. Single agent dexamethasone for pre-stem cell transplant induction therapy for multiple myeloma. Bone Marrow Transplant. 2004;34:485–90.
47. Mangelsdorf DJ, Thummel C, Beato M, et al. The nuclear receptor superfamily: the second decade. Cell. 1995;83:835–9.
48. Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. Nat Rev Drug Discov. 2009;8:627–44.
49. Pene F, Claessens YE, Muller O, et al. Role of the phosphatidylinositol 3-kinase/Akt and mTOR/P70S6-kinase pathways in the proliferation and apoptosis in multiple myeloma. Oncogene. 2002;21:6587–97.
50. Stromberg T, Dimberg A, Hammarberg A, et al. Rapamycin sensitizes multiple myeloma cells to apoptosis induced by dexamethasone. Blood. 2004;103:3138–47.
51. Frost P, Moatamed F, Hoang B, et al. In vivo antitumor effects of the mTOR inhibitor CCI-779 against human multiple myeloma cells in a xenograft model. Blood. 2004;104:4181–7.
52. Yan H, Frost P, Shi Y, et al. Mechanism by which mammalian target of rapamycin inhibitors sensitize multiple myeloma cells to dexamethasone-induced apoptosis. Cancer Res. 2006;66:2305–13.
53. Francis LK, Alsayed Y, Leleu X, et al. Combination mammalian target of rapamycin inhibitor rapamycin and HSP90 inhibitor 17-allylamino-17-demethoxygeldanamycin has synergistic activity in multiple myeloma. Clin Cancer Res. 2006;12:6826–35.
54. Raje N, Kumar S, Hideshima T, et al. Combination of the mTOR inhibitor rapamycin and CC-5013 has synergistic activity in multiple myeloma. Blood. 2004;104:4188–93.
55. Harvey RD, Lonial S. PI3 kinase/AKT pathway as a therapeutic target in multiple myeloma. Future Oncol. 2007;3:639–47.
56. Farag SS, Zhang S, Jansak BS, et al. Phase II trial of temsirolimus in patients with relapsed or refractory multiple myeloma. Leuk Res. 2009;33:1475–80.
57. Georgii-Hemming P, Wiklund HJ, Ljunggren O, et al. Insulin-like growth factor I is a growth and survival factor in human multiple myeloma cell lines. Blood. 1996;88:2250–8.
58. Ge NL, Rudikoff S. Insulin-like growth factor I is a dual effector of multiple myeloma cell growth. Blood. 2000;96:2856–61.
59. Lacy MQ, Alsina M, Fonseca R, 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;26:3196–203.
60. Moreau P, Cavallo F, Leleu X, Hulin C, Amiot M, Descamps G, Facon T, Boccadoro M, Mignard D, Harousseau JL. 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.
61. Borset M, Hjorth-Hansen H, Seidel C, et al. Hepatocyte growth factor and its receptor c-met in multiple myeloma. Blood. 1996;88:3998–4004.
62. Derksen PW, de Gorter DJ, Meijer HP, et al. The hepatocyte growth factor/Met pathway controls proliferation and apoptosis in multiple myeloma. Leukemia. 2003;17:764–74.
63. Hov H, Holt RU, Rø TB, Fagerli UM, Hjorth-Hansen H, Baykov V, Christensen JG, Waage A, Sundan A, Børset M. A selective c-met inhibitor blocks an autocrine hepatocyte growth

factor growth loop in ANBL-6 cells and prevents migration and adhesion of myeloma cells. Clin Cancer Res. 2004;10(19):6686–94.
64. Maloney DG, Donovan K, Hamblin TJ. Antibody therapy for treatment of multiple myeloma. Semin Hematol. 1999;36:30–3.
65. Kapoor P, Greipp PT, Morice WG, et al. Anti-CD20 monoclonal antibody therapy in multiple myeloma. Br J Haematol. 2008;141:135–48.
66. Zojer N, Kirchbacher K, Vesely M, et al. Rituximab treatment provides no clinical benefit in patients with pretreated advanced multiple myeloma. Leuk Lymphoma. 2006;47:1103–9.
67. Moreau P, Voillat L, Benboukher L, et al. Rituximab in CD20 positive multiple myeloma. Leukemia. 2007;21:835–6.
68. Kovalchuk AL, Kim JS, Park SS, et al. IL-6 transgenic mouse model for extraosseous plasmacytoma. Proc Natl Acad Sci U S A. 2002;99:1509–14.
69. van Rhee F, Fayad L, Voorhees P, et al. Siltuximab, a novel anti-interleukin-6 monoclonal antibody, for Castleman's disease. J Clin Oncol. 2010;28:3701–8.
70. Hunsucker SA, Magarotto V, Kuhn DJ, et al. Blockade of interleukin-6 signaling with siltuximab enhances melphalan cytotoxicity in preclinical models of multiple myeloma. Br J Haematol. 2011;152:579–92.
71. Voorhees PM, Chen Q, Small GW, et al. Targeted inhibition of interleukin-6 with CNTO 328 sensitizes pre-clinical models of multiple myeloma to dexamethasone-mediated cell death. Br J Haematol. 2009;145:481–90.
72. Fulciniti M, Hideshima T, Vermot-Desroches C, et al. A high-affinity fully human anti-IL-6 mAb, 1339, for the treatment of multiple myeloma. Clin Cancer Res. 2009;15:7144–52.
73. van Rhee F, Szmania SM, Dillon M, et al. Combinatorial efficacy of anti-CS1 monoclonal antibody elotuzumab (HuLuc63) and bortezomib against multiple myeloma. Mol Cancer Ther. 2009;8:2616–24.
74. Lesage D, Troussard X, Sola B. The enigmatic role of cyclin D1 in multiple myeloma. Int J Cancer. 2005;115:171–6.
75. Marsaud V, Tchakarska G, Andrieux G, et al. Cyclin K and cyclin D1b are oncogenic in myeloma cells. Mol Cancer. 2010;9:103.
76. Tchakarska G, Le Lan-Leguen A, Roth L, et al. The targeting of the sole cyclin D1 is not adequate for mantle cell lymphoma and myeloma therapies. Haematologica. 2009;94:1781–2.
77. Raje N, Hideshima T, Mukherjee S, et al. Preclinical activity of P276-00, a novel small-molecule cyclin-dependent kinase inhibitor in the therapy of multiple myeloma. Leukemia. 2009;23:961–70.
78. Dispenzieri A, Gertz MA, Lacy MQ, et al. Flavopiridol in patients with relapsed or refractory multiple myeloma: a phase 2 trial with clinical and pharmacodynamic end-points. Haematologica. 2006;91:390–3.
79. Holkova B, Perkins EB, Ramakrishnan V, Tombes MB, Shrader E, Talreja N, Wellons MD, Hogan KT, Roodman GD, Coppola D, Kang L, Dawson J, Stuart RK, Peer C, Figg Sr WD, Kolla S, Doyle A, Wright J, Sullivan DM, Roberts JD, Grant S. Phase I trial of bortezomib (PS-341; NSC 681239) and alvocidib (flavopiridol; NSC 649890) in patients with recurrent or refractory B-cell neoplasms. Clin Cancer Res. 2011;17(10):3388–97.
80. Saunders A, Core LJ, Lis JT. Breaking barriers to transcription elongation. Nat Rev Mol Cell Biol. 2006;7:557–67.
81. Tong WG, Chen R, Plunkett W, Siegel D, Sinha R, Harvey RD, Badros AZ, Popplewell L, Coutre S, Fox JA, Mahadocon K, Chen T, Kegley P, Hoch U, Wierda WG. Phase I and pharmacologic study of SNS-032, a potent and selective Cdk2, 7, and 9 inhibitor, in patients with advanced chronic lymphocytic leukemia and multiple myeloma. J Clin Oncol. 2010;28(18):3015–22.
82. Santo L, Vallet S, Hideshima T, Cirstea D, Ikeda H, Pozzi S, Patel K, Okawa Y, Gorgun G, Perrone G, Calabrese E, Yule M, Squires M, Ladetto M, Boccadoro M, Richardson PG, Munshi NC, Anderson KC, Raje N. AT7519, a novel small molecule multi-cyclin-dependent kinase inhibitor, induces apoptosis in multiple myeloma. Oncogene. 2010;29(16):2325–36.
83. Delmore JE, Issa GC, Lemieux ME, Rahl PB, Shi J, Jacobs HM, Kastritis E, Gilpatrick T, Paranal RM, Qi J, Chesi M, Schinzel AC, McKeown MR, Heffernan TP, Vakoc CR, Bergsagel

PL, Ghobrial IM, Richardson PG, Young RA, Hahn WC, Anderson KC, Kung AL, Bradner JE, Mitsiades CS. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell. 2011;146(6):904–17.
84. Santra M, Zhan F, Tian E, et al. A subset of multiple myeloma harboring the t(4;14)(p16;q32) translocation lacks FGFR3 expression but maintains an IGH/MMSET fusion transcript. Blood. 2003;101:2374–6.
85. Quintero-Rivera F, El-Sabbagh Badr R, Rao PN. FGFR3 amplification in the absence of IGH-FGFR3 fusion t(4;14) in myeloma. Cancer Genet Cytogenet. 2009;195:92–3.
86. Krejci P, Murakami S, Prochazkova J, et al. NF449 is a novel inhibitor of fibroblast growth factor receptor 3 (FGFR3) signaling active in chondrocytes and multiple myeloma cells. J Biol Chem. 2010;285:20644–53.
87. Xin X, Abrams TJ, Hollenbach PW, et al. CHIR-258 is efficacious in a newly developed fibroblast growth factor receptor 3-expressing orthotopic multiple myeloma model in mice. Clin Cancer Res. 2006;12:4908–15.
88. Trudel S, Stewart AK, Rom E, et al. The inhibitory anti-FGFR3 antibody, PRO-001, is cytotoxic to t(4;14) multiple myeloma cells. Blood. 2006;107:4039–46.
89. Matsui W, Wang Q, Barber JP, et al. Clonogenic multiple myeloma progenitors, stem cell properties, and drug resistance. Cancer Res. 2008;68:190–7.
90. Huff CA, Matsui W. Multiple myeloma cancer stem cells. J Clin Oncol. 2008;26:2895–900.
91. Mueller MM, Fusenig NE. Friends or foes—bipolar effects of the tumour stroma in cancer. Nat Rev Cancer. 2004;4:839–49.
92. McMillin DW, Delmore J, Weisberg E, Negri JM, Geer DC, Klippel S, Mitsiades N, Schlossman RL, Munshi NC, Kung AL, Griffin JD, Richardson PG, Anderson KC, Mitsiades CS. Tumor cell-specific bioluminescence platform to identify stroma-induced changes to anticancer drug activity. Nat Med. 2010;16(4):483–9.
93. Rajkumar SV, Mesa RA, Fonseca R, et al. Bone marrow angiogenesis in 400 patients with monoclonal gammopathy of undetermined significance, multiple myeloma, and primary amyloidosis. Clin Cancer Res. 2002;8:2210–6.
94. Ria R, Roccaro AM, Merchionne F, et al. Vascular endothelial growth factor and its receptors in multiple myeloma. Leukemia. 2003;17:1961–6.
95. Kumar S, Witzig TE, Timm M, et al. Expression of VEGF and its receptors by myeloma cells. Leukemia. 2003;17:2025–31.
96. Van Meter ME, Kim ES. Bevacizumab: current updates in treatment. Curr Opin Oncol. 2010;22:586–91.
97. Zangari M, Anaissie E, Stopeck A, et al. Phase II study of SU5416, a small molecule vascular endothelial growth factor tyrosine kinase receptor inhibitor, in patients with refractory multiple myeloma. Clin Cancer Res. 2004;10:88–95.
98. Kovacs MJ, Reece DE, Marcellus D, et al. A phase II study of ZD6474 (zactima, a selective inhibitor of VEGFR and EGFR tyrosine kinase in patients with relapsed multiple myeloma–NCIC CTG IND.145. Invest New Drugs. 2006;24:529–35.
99. Prince HM, Honemann D, Spencer A, et al. Vascular endothelial growth factor inhibition is not an effective therapeutic strategy for relapsed or refractory multiple myeloma: a phase 2 study of pazopanib (GW786034). Blood. 2009;113:4819–20.
100. Alsina M, Fonseca R, Wilson EF, et al. Farnesyltransferase inhibitor tipifarnib is well tolerated, induces stabilization of disease, and inhibits farnesylation and oncogenic/tumor survival pathways in patients with advanced multiple myeloma. Blood. 2004;103:3271–7.
101. Chanan-Khan AA, Niesvizky R, Hohl RJ, et al. Phase III randomised study of dexamethasone with or without oblimersen sodium for patients with advanced multiple myeloma. Leuk Lymphoma. 2009;50:559–65.
102. Tsimberidou AM, Waddelow T, Kantarjian HM, et al. Pilot study of recombinant human soluble tumor necrosis factor (TNF) receptor (p75) fusion protein (TNFR:Fc; Enbrel) in patients with refractory multiple myeloma: increase in plasma TNF alpha levels during treatment. Leuk Res. 2003;27:375–80.

103. Dispenzieri A, Gertz MA, Lacy MQ, Geyer SM, Greipp PR, Rajkumar SV, Kimlinger T, Lust JA, Fonseca R, Allred J, Witzig TE. A phase II trial of imatinib in patients with refractory/relapsed myeloma. Leuk Lymphoma. 2006;47(1):39–42.
104. Chapman MA, Lawrence MS, Keats JJ, et al. Initial genome sequencing and analysis of multiple myeloma. Nature. 2011;471(7339):467–72.
105. Barlogie B, Desikan R, Eddlemon P, et al. Extended survival in advanced and refractory multiple myeloma after single-agent thalidomide: identification of prognostic factors in a phase 2 study of 169 patients. Blood. 2001;98:492–4.
106. Mileshkin L, Biagi JJ, Mitchell P, et al. Multicenter phase 2 trial of thalidomide in relapsed/refractory multiple myeloma: adverse prognostic impact of advanced age. Blood. 2003;102:69–77.
107. Tosi P, Zamagni E, Cellini C, et al. Salvage therapy with thalidomide in patients with advanced relapsed/refractory multiple myeloma. Haematologica. 2002;87:408–14.
108. Rajkumar SV, Gertz MA, Lacy MQ, et al. Thalidomide as initial therapy for early-stage myeloma. Leukemia. 2003;17:775–9.
109. Rajkumar SV, Blood E, Vesole D, et al. Phase III clinical trial of thalidomide plus dexamethasone compared with dexamethasone alone in newly diagnosed multiple myeloma: a clinical trial coordinated by the Eastern Cooperative Oncology Group. J Clin Oncol. 2006;24:431–6.
110. Richardson P, Jagannath S, Hussein M, et al. Safety and efficacy of single-agent lenalidomide in patients with relapsed and refractory multiple myeloma. Blood. 2009;114:772–8.
111. Dimopoulos M, Spencer A, Attal M, et al. Lenalidomide plus dexamethasone for relapsed or refractory multiple myeloma. N Engl J Med. 2007;357:2123–32.
112. Rajkumar SV, Jacobus S, Callander NS, et al. Lenalidomide plus high-dose dexamethasone versus lenalidomide plus low-dose dexamethasone as initial therapy for newly diagnosed multiple myeloma: an open-label randomized controlled trial. Lancet Oncol. 2010;11:29–37.
113. Richardson PG, Sonneveld P, Schuster MW, et al. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N Engl J Med. 2005;352:2487–98.
114. Jagannath S, Durie BG, Wolf J, et al. Bortezomib therapy alone and in combination with dexamethasone for previously untreated symptomatic multiple myeloma. Br J Haematol. 2005;129:776–83.
115. Orlowski RZ, Nagler A, Sonneveld P, et al. Randomized phase III study of pegylated liposomal doxorubicin plus bortezomib compared with bortezomib alone in relapsed or refractory multiple myeloma: combination therapy improves time to progression. J Clin Oncol. 2007;25:3892–901.
116. Mikhael JR, Belch AR, Prince HM, et al. High response rate to bortezomib with or without dexamethasone in patients with relapsed or refractory multiple myeloma: results of a global phase 3b expanded access program. Br J Haematol. 2009;144:169–75.

Current and Future Trials of Targeted Therapies in Cutaneous Melanoma

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Abstract In order to effectively treat melanoma, targeted inhibition of key mechanistic events regulating melanoma development such as cell proliferation, survival, angiogenesis and invasion or metastasis needs to be accomplished. The Mitogen Activated Protein Kinase (MAPK) pathway has been identified as a key player in melanoma development making this cascade an important therapeutic target. However, identification of the ideal pathway member to therapeutically target for maximal clinical benefit remains a challenge. In normal cells, the MAPK pathway relays extracellular signals from the cell membrane to the nucleus via a cascade of phosphorylation events, which promote cancer development. Dysregulation of the MAPK pathway occurs frequently in many human cancers including melanoma. Mutations in the B-RAF and RAS genes, genetic or epigenetic modifications are the key aberrations observed in this signaling cascade. Constitutive activation of this pathway causes oncogenic transformation of cells by promoting cell proliferation, invasion, metastasis, migration, survival and angiogenesis. This review provides an overview of (a) key members of MAPK signaling regulating melanoma

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W.S. El-Deiry (ed.), *Impact of Genetic Targets on Cancer Therapy*, Advances in Experimental Medicine and Biology 779, DOI 10.1007/978-1-4614-6176-0_10,

development; (b) key proteins which can serve as biomarkers to assess disease progression; (c) the clinical efficacy of various pharmacological agents targeting MAPK pathway; (d) current clinical trials evaluating downstream targets of the MAPK pathway; (e) issues associated with pharmacological agents such as drug resistance, induction of cancers; and finally (e) various strategies overcoming drug resistance.

Keywords AZD6244 • Drug resistance • MAPK signaling • Melanoma • PLX-4032 • ^{V600E}B-Raf

Melanoma Background

Skin cancer is the most common malignancy in the United States [1]. Although melanoma represents a small subset, it is the most deadly cutaneous neoplasm and is an increasingly common malignancy affecting a younger population than most cancers. Melanoma is diagnosed more commonly in whites than non-whites with the lifetime risk of developing invasive melanoma being 2.04% for white men and 1.45% for white women [2]. In other words, about one in 74 Americans will be diagnosed with melanoma with the median age at diagnosis of 57 years. Numerous risk factors for development of melanoma have been identified, including white skin, fair hair, light eyes, sun sensitivity, tendency to freckle, family history of melanoma, dysplastic nevi, increased numbers of typical nevi, large congenital nevi and immunosuppression. Although sun exposure is a risk factor for melanoma, cutaneous melanomas can arise frequently in areas of the body not exposed to the sun. Sun exposure in childhood and having more than one blistering sunburn in childhood are associated with an increased risk of melanoma [3].

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There are four major subtypes of invasive cutaneous melanoma including superficial spreading which accounts for approximately 70% of all melanomas, nodular melanoma which accounts for 15–30% of all melanomas, lentigomaligna and acrallentiginous. Most melanomas arise as superficial tumors confined to the epidermis and may remain for several years in a stage known as the horizontal or "radial" growth phase in which they are almost always curable by surgical excision alone. Melanomas that infiltrate into the dermis are considered to be in a "vertical" growth phase and have metastatic potential. Vertical growth phase melanoma is most strongly predicted by measuring the thickness of the tumor (i.e., Breslow depth), in millimeters, from the granular cell layer of the epidermis to the deepest malignant cell in the dermis [4]. Nodular melanomas have no identifiable radial growth or in situ phase, and enter the vertical growth phase almost from their inception. Other histologic factors that affect metastatic potential include ulceration of the tumor, mitotic rate, presence of lymphovascular invasion, microsatellites, regression, perineural invasion, and the presence of lymphocytes infiltrating the tumor.

The primary mode of treatment for localized cutaneous melanoma is surgery. Surgical margins of 5 mm are currently recommended for melanoma in situ, and margins of 1 cm are recommended for melanomas ≤1 mm in depth [5]. For tumors of intermediate thickness (1–4 mm Breslow depth), randomized prospective studies show that 2-cm margins are appropriate, although 1-cm margins have been proven effective for tumors of 1- to 2-mm thickness [6, 7]. Margins of 2 cm are recommended for cutaneous melanomas greater than 4 mm in thickness (high-risk primaries) to prevent potential local recurrence in or around the scar site.

Numerous adjuvant therapies have been investigated for the treatment of localized cutaneous melanoma following complete surgical removal. Adjuvant interferon (IFN) alfa-2b is the only adjuvant therapy approved by the US Food and Drug Administration for high-risk melanoma. However, no overall survival benefit has been demonstrated for adjuvant chemotherapy, nonspecific (passive) immunotherapy (including interferon), radiation therapy, retinoid therapy, vitamin therapy, or biologic therapy [8]. This makes evaluating for targeted therapies vitally important in treatment of melanoma.

Overview of the MAPK Signaling Pathway

The classical MAPK pathway consists of RAS, RAF, MEK1/2 and ERK1/2, which sequentially relay proliferative signals generated at cell surface receptors through cytoplasmic signaling into the nucleus (Fig. 1) [9–13]. In normal cells, the signaling cascade is stimulated by the binding of mitogens, hormones, or neurotransmitters to receptor tyrosine kinases, which upon dimerization triggers the activation of oncogenic RAS to increase cellular RAS-GTP levels [10, 14]. Activated RAS then triggers the formation of the "MAPK complex" with downstream RAF, MEK1/2, ERK1/2 and several scaffolding proteins initiating the MAPK cascade. The activated RAS activates RAF, which in turn causes the dissociation of ERK1/2 from the MAPK complex.

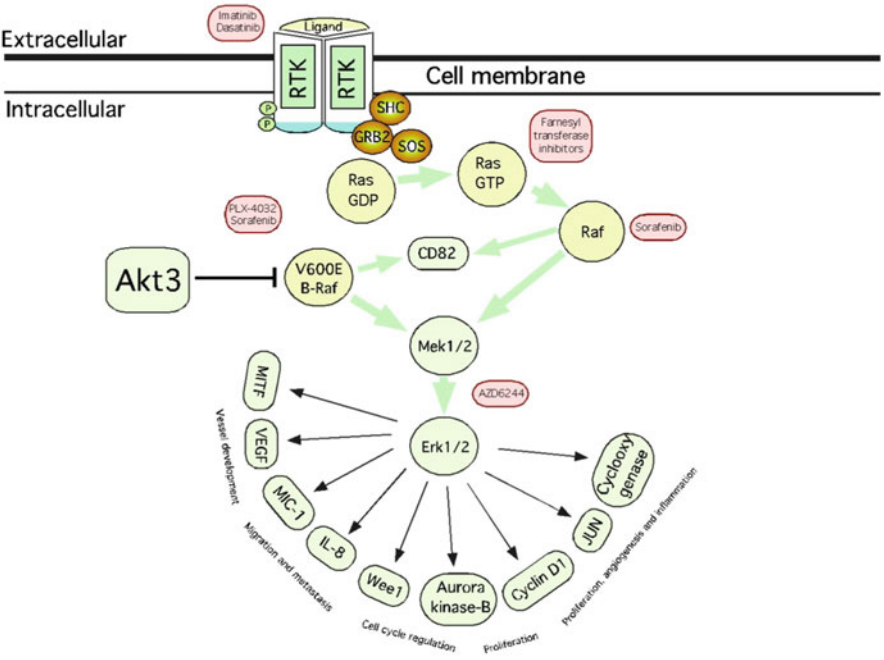

Fig. 1 MAPK signaling cascade: diagram depicts initial ligand binding to receptor tyrosine kinases (RTK), leading to activation of RAS, then RAF, then MEK 1/2, then Erk 1/2 followed by several downstream targets. In pink are therapies directed at these targets, which are discussed in this review

Activation of MAPK pathway regulates the expression of several genes involved in cell proliferation, differentiation, angiogenesis and survival by phosphorylating nuclear transcription factors such as ETS, ELK-1, MYC or indirectly by targeting intracellular signaling molecules [11, 13, 15]. For instance, activated MAPK pathway induces angiogenesis by increasing the levels of VEGF and MIC-1. MAPK pathway also effects the post-translational phosphorylation of apoptotic regulatory molecules like BAD, BIM, MCL-1, caspase 9 and BCL-2, thereby regulating cellular apoptosis [12, 15]. In melanoma, active mutant ^{V600E}B-Raf induces the expression of proliferation marker Cyclin D1.

Targeting RAS in Melanoma

The RAS family of small G-proteins consists of K-RAS, H-RAS, and N-RAS, which are involved in triggering MAPK signaling by activating downstream proteins such as RAF and PI3K [11, 14, 16]. Structurally in the catalytic domain of the RAS family proteins, the first 80 amino acids are identical and the next 85 amino acids differ only by 5%. In mammalians, all these three RAS genes are universally

expressed, even though the expression pattern for each gene is quantitatively different depending on the organ. RAS proteins function as molecular switches regulating cell proliferation and survival [9, 10, 17] and are activated by upstream activation of cell surface receptors, mutation and loss of the RAS-GAP NF-1 [11, 14, 18].

In one third of all human cancers, including melanoma, oncogenic mutations in RAS family members have been reported [11, 14, 18]. Although oncogenic mutations have been frequently reported in codons 12, 13 and 61 of RAS, substitution of leucine for glutamine at residue 61 is the most common aberration observed in N-RAS present in melanomas [18, 19]. Mutant RAS lacks GTPase activity and remains active leading to uncontrolled cell proliferation and a transformed phenotype [18]. Furthermore, expression of RAS can suppress the tumor-suppressors p16INK4A, p53, or p14ARF [20, 21]. Introduction of activated RAS into melanocytes leads to melanoma tumor formation in mice [22, 23] and knockdown of H-RAS expression using siRNAs can cause melanoma regression in an inducible melanoma tumor model [24].

Given the involvement of RAS in tumor growth and control of cell proliferation, it was felt to be a potential drug target for several years.

Clinical Efficacy of Drugs Targeting RAS

Given the fact that the activation of RAS requires farnesylation of the carboxy-terminal cysteine residues by farnesyltransferase (FT), it has been proposed that targeting FT using farnesyltransferase inhibitors (FTI) or farnesyl cysteine mimetics such as farnesylthiosalicylic acid (FTS) derivatives might prevent growth of melanomas [25, 26]. Unfortunately, efforts to pharmacologically inhibit RAS or its regulatory components for cancer therapy have so far met with minimal success.

A potent FT inhibitor, SCH66336, was noted in preclinical studies to induce G1-phase cell cycle arrest and retinoblastoma protein inactivation to kill melanoma cells [26]. Additionally, a combination of farnesylthiosalicylic acid and SCH66336 markedly enhanced cisplatin-mediated apoptosis demonstrating the chemosensitizing activity of FTIs in melanoma [26, 27]. Lonafarnib, another farnesyltransferase inhibitor, was tested in regards to regulation of proliferation, survival and invasive potential of melanoma cells in monolayer or organotypic culture systems either alone or in combination with chemotherapeutic agents (temozolomide/cisplatin, or MAPK inhibitors sorafenib/U0126/PD98059, or AKT inhibitorsLY294002/wortmannin/rapamycin). In these studies, lonafarnib was neither able to inhibit the growth of metastatic melanoma cells nor sensitize them to the chemotherapeutic agents tested [28]. However, lonafarnib did significantly augment the growth inhibitory effects of the multi-kinase inhibitor sorafenib in eight different cultured metastatic melanoma cell lines [28]. Furthermore, lonafarnib combined with sorafenib was able to trigger apoptosis and prohibit the invasive potential of melanoma cells [28]. Despite FTIs promise in preclinical studies, in a Phase-II study of 14 metastatic melanoma patients, oral administration of FT inhibitor R115777 (300 mg orally twice a day for 21 days) was toxic and lacked therapeutic efficacy [29–32].

In addition to FTIs, direct RAS inhibitors, such as BMS-214662 and L-778123, which are potent non-peptide direct inhibitors of H-RAS and K-RAS respectively, have been evaluated for the treatment in melanoma patients [33–37]. In review of a phase I study of patients with solid tumors receiving oral BMS-214662 (given once or twice daily for 2 weeks in a 3-week cycle), the patients experienced dose-limiting toxicity of nausea and diarrhea with additional toxicities of vomiting, abdominal cramping, anorexia, fatigue and fever. Additionally, of the 23 patients treated, all but 1 had progressive disease [38]. L-778123 has also been evaluated clinically as a 5 day continuous infusion either alone or in combination with radiation and paclitaxel for treatment of NSCLC, as well as head and neck carcinomas. Despite a good clinical response, studies were discontinued due to evidence of cardiac toxicity, manifested as a prolongation of the QTc interval [39–42]. Unfortunately, in addition to limiting toxicity, both compounds have been ineffective in melanoma since these tumors harbor N-RAS and not H-RAS or K-RAS mutations targeted by these agents.

Despite the promise of agents directed towards RAS in preclinical studies, they have failed in clinical trials since FTs farnesylate many proteins other than just RAS, other mechanisms activate RAS proteins promoting development of resistance and deregulation of the pathway by other oncogenes [25]. Thus, therapeutically targeting RAS in melanoma is relatively ineffective suggesting that other points in the MAPK pathway might be more promising to target.

Targeting RAF in Melanoma

The RAF family consists of A-RAF, B-RAF, and C-RAF (or RAF-1), and are downstream effectors of RAS (14, 43). All three mammalian RAF isoforms share three conserved regions (CR1, CR2, CR3) and areas of variable sequences. The CR1 (131 amino acids length) contains a RAS binding domain and a cysteine-rich domain [11, 43]. The CR2 (16 amino acids length) domain contains threonine and serine residues which play a role in regulating the activity of B-RAF upon phosphorylation. The CR3 (293 amino acids length) contains a kinase domain and key phosphorylation sites that regulate enzymatic activity [11]. A complex process that involves a series of events including membrane translocation; protein dimerization; phosphorylation likely by SRC-family tyrosine kinases; dissociation from RAF kinase inhibitory proteins; and, association with scaffolding proteins is required for the activation of normal non-mutated RAF proteins [11, 44, 45].

Greater than 60% of advanced melanomas express constitutively active mutant B-RAF, which is the most mutated gene in the MAPK signaling cascade [14, 46, 47]. These mutations are acquired, somatic, post-zygotic events and are not inherited in families [13, 46]. Mutated ^{V600E}B-RAF does not require RAS-mediated membrane translocation to exhibit enzymatic activity and is 10.7-fold more active than wild type protein [47]. It also confers resistance to negative feedback regulation by S579A mutation of B-RAF and Sprouty proteins [11]. Even though there are over 65 different mutations that occur in more than 30 B-RAF codons, a single-base

missense T to A substitution(at nucleotide 1,799) is prevalent in 90% of melanoma tumors, causing a change of valine to glutamic acid at codon 600 (V600E) in exon 15 [13, 14, 47, 48]. The glutamic acid then acts as a phosphomimetic between the Thr^{598} and Ser^{601} phosphorylation sites which causes a conformational change in protein structure and activation of the protein [14, 49, 50].

^{V600E}B-RAF leads to hyperactivation of the MAPK pathway, which in turn triggers survival pathways and cell division to promote tumor development by inducing proliferation [11, 50–52]. However, only moderate levels of MAPK pathway activation are required for increased in vitro colony formation, elevation of ERK1/2 activities and transformation and immortalization of mouse melanocytes [10, 13, 51, 53]. Recent studies have shown that ^{V600E}B-RAF regulates expression of IL-8, a pro-inflammatory chemokine and autocrine factor, to promote angiogenesis and tumor growth [54]. Additionally, mutant B-RAF can control metastatic development by promoting IL-8 mediated anchoring of melanoma cells to the vascular endothelium to aid extravasation as well as triggering invasive cellular behavior in the development of lung metastases [54, 55]. ^{V600E}B-RAF also induces formation of new blood vessels by promoting macrophage inhibitory cytokine-1 (MIC-1) secretion and vascular endothelial growth factors (VEGF) [56, 57].

^{V600E}B-RAF can also activate the MAPK pathway to levels that inhibit cellular growth and induce senescence in a wide variety of normal and early melanocytic lesion cells [58–60]. However, mutant ^{V600E}B-RAF has been shown to initially stimulate melanocyte proliferation, indicating that it contributes to development of nevi and melanogenesis [48, 50, 58]. This is followed by senescence and subsequent growth inhibition as indicated by proliferative arrest due to increases in β-Gal and $p16^{Ink4a}$ [48, 50, 58]. Increased cyclin-dependent kinase inhibitors, such as $p21^{Cip1}$, $p16^{Ink4a}$, and $p27^{Kip1}$, leads to sensescence induction and acts as a putative defense mechanism to overcome oncogene activation in normal cells [59–61]. A recent study in transformed melanocytes has also shown that senescence and apoptosis induction triggered by ^{V600E}B-RAF can be mediated by insulin growth factor binding protein-7 secretion [62]. Furthermore, additional genetic changes such as loss of p53, $p16^{INK4a}$, PTEN or elevation in AKT3 activity is required for melanoma development to occur in nevi containing ^{V600E}B-RAF and for the quiescent melanocytic cells to overcome the ^{V600E}B-RAF induced senescence in order to reenter the cell cycle [58, 63, 64]. In one study, zebrafish expressing ^{V600E}B-RAF protein developed fish-nevi; however, only when expressed in p53-deficient zebrafish did rapid progression of melanocytic lesions develop into invasive melanomas, resembling those occurring in human tumors [65]. This provided direct evidence that linked melanoma development to an interaction between the ^{V600E}B-RAF and p53 pathways [66]. ^{V600E}B-RAF has also been shown to occur with $p16^{INK4A}$ loss in ~60% of melanomas [63]. A recent study showed that absence of activated B-RAF and $p16^{INK4a}$ expression were independent predictors of melanoma tumor chemosensitivity in a group of patients who underwent isolated limb infusion with cytotoxic drugs actinomycin-D and melphalan for metastatic melanoma [67]. In regards to PTEN, genetically altered mice harboring conditional melanocytes expressing ^{V600E}B-RAF, developed benign melanocytic hyperplasia but failed to develop melanoma. Only when PTEN was lost did melanoma develop, which metastasized to

lymph nodes and lungs [64]. AKT3 has been shown to release cells from ^{V600E}B-RAF-mediated senescence via phosphorylating ^{V600E}B-RAF on S364 and/or S428 in order to reduce its activity to levels that promote rather than inhibit melanoma development from melanocytes [58]. Occurrence of B-RAF mutation is likely an early event, with the alteration of the PTEN/AKT pathway occurring later in tumor progression [68]. Therefore, a successful targeted therapy will likely require targeting both pathways simultaneously.

Clinical Efficacy of Therapies Targeting RAF

Given the importance of B-RAF mutations in melanoma, small molecule inhibitors targeting mutated ^{V600E}B-RAF kinase have shown efficacy in the clinic. Initially, the RAF inhibitor Sorafenib was studied following both oral or intraperitoneal administration. Sorafenib (BAY 43-9006) reduced growth of subcutaneous melanoma tumors by inhibiting cell proliferation and vascular development [57, 69]. However, clinical trials using sorafenib as a monotherapy in advanced melanoma have failed to demonstrate significant anti-tumor activity. Only 19% of patients exhibited stable disease with a progression free survival of 16–37 weeks, while 62% showed progressive disease with progression free survival of about 11 weeks [70]. No relationship between B-RAF mutational status and disease stability was observed raising concerns regarding the clinical utility of targeting B-RAF to treat melanoma [70]. It is felt that failure of Sorafenib clinically is likely due to its inhibition of other kinases (FGFR1, c-Kit, p38 MAPK) or angiogenic factors (VEGFR1, VEGFR2, VEGFR3, and PDGF), rather than solely due to inhibition of RAF [69, 71–73].

Given the concerns raised regarding Sorafenib, other mutant B-RAF kinase inhibitors have been developed. Of these, PLX4032 (Vemurafenib) directly targets ^{V600E}B-Raf. It was first discovered using a scaffold-based drug design approach [74], along with another promising mutant B-RAF kinase inhibitor PLX4720. Initial xenograft studies with PLX4032 revealed dose dependent inhibition of tumor growth in those with B-RAF mutation and no effect on tumors containing wild type protein. Both of these B-RAF inhibitors were chosen for further study over similar compounds because of their consistent pharmokinetics in rodents and PLX4032 was ultimately chosen for clinical trials over PLX4720 because of more favorable outcomes in beagle dogs and cynomolgus monkeys [75]. During Phase I clinical trials, the maximum tolerated dose of reformulated PLX4032 as a micro-precipitated bulk powder was discovered to be 960 mg po bid and an extension of this trial was performed with 32 patients with B-RAF mutant melanomas as detected via PCR analysis. Of those treated, 24 achieved partial remissions, and three achieved complete remission. Respondents had near complete inhibition of ERK signaling, which may be needed for significant tumor response as those patients with tumor regressions showed a greater than 80% inhibition in cytoplasmic ERK phosphorylation. The median progression free survival in this Phase I extension cohort has not been reached, but is estimated to be about 7 months [76].

A randomized Phase III trial comparing vemurafenib (PLX4032) and dacarbazine, a commonly used chemotherapeutic agent in melanoma, was recently published. In this trial, a total of 675 metastatic melanoma patients with the ^{V600E}BRAF mutation that had not been previously treated were randomly assigned to vemurafenib (960 mg po bid) or dacarbazine (1,000 mg per square meter of body surface area IV q 3 weeks). In this trial, overall survival with a 95% confidence interval was 84% in the vemurafenib group and 64% in the dacarbazine group. Vemurafenib was associated with a relative reduction in the risk of death of 63% and 74% in the risk of either death or disease progression compared with dacarbazine ($p<0.001$) [77].

Additionally, GSK2118436 is another BRAF inhibitor that has been studied and recently is starting Phase III trials. During the ESMO 2010 meeting in Milan, Phase I data was presented and revealed that treatment shrunk the overall size of brain metastases by 20–100% (3 mm or larger before treatment) in nine out of ten treated patients, which was noted to be remarkable as typical treatment responses are 10–15% [78]. Additionally, treatment with GSK2118436 in these trials revealed an impressive 60% response for melanomas outside of the brain. At this point, PLX4032 is likely to reach the market first since results from its Phase III trial as noted above has shown significantly extended survival in metastatic melanoma.

Toxicities and Development of Resistance of Drugs Targeting ^{V600E}B-RAF

Major concerns related to B-RAF inhibitors include development of resistance and to a lesser extent its side effects. Minor side effects included rash, joint pain and fever [76]. Additionally, approximately 23% of patients developed cutaneous squamous cell carcinoma during the first few months of treatment [79]. In the recently published Phase III trial of vemurafenib (PLX4032), initial observations reported side effects that included arthralgia, rash, fatigue, alopecia, photosensitivity, nausea, diarrhea, keratocanthoma or squamous cell carcinoma with 38% of patients requiring a dose modification to lessen these issues [77].

A more serious concern related to patients treated with Vemurafenib has been disease recurrence as early as 3–4 months in those who initially responded to the drug [79]. To characterize the underlying mechanisms leading to development of drug resistance, Nazarian et al. examined three cell lines with the ^{V600E}B-RAF that were very sensitive to growth inhibition from PLX4032. These cell lines were subjected to chronic PLX4032 exposure to develop resistant sublines [80]. Analysis of these cell lines revealed that the ^{V600E}B-RAF did not develop secondary mutations promoting the development of resistance. Resistance developed by the formation of RAF dimers either via RAS activation or increased RAF expression, since binding of these inhibitors to RAF dimers leads to transactivation of the nonbound member of the dimer, bypassing the inhibitory effect [81]. Melanomas with the ^{V600E}B-RAF, do not have Ras levels high enough to promote dimerization of RAF. However, in in vitro cell lines that developed resistance, N-Ras mutations occurred, leading to

increased RAS activation and thereby RAF dimerization and development of resistance [80]. Another mechanism leading to resistance was by overexpression of mitogen-activated protein kinase 8 (MAP3K8), which encodes the protein kinase COT that activates ERK through phosphorylation in a RAF-independent manner, leading to resistance to RAF inhibition [82]. In ^{V600E}B-RAF cells, expression of MAP3K8 mRNA levels and its associated COT protein were undetectable. However, treatment with the RAF inhibitor PLX4720 increased COT protein levels in a dose dependent manner. Clinically, two thirds of biopsied samples from ^{V600E}B-RAF melanoma patients treated with PLX4032 showed increased MAP3K8 mRNA expression by quantitative PCR analysis. Resistance can also develop due to overexpression of platelet-derived growth factor receptor (PDGFR), activating a receptor tyrosine kinase dependent survival pathway and through parallel signaling pathways triggering downstream effectors of cell transformation [80, 81]. Clinically, the overexpression of PDGFR was observed in 4/11 patient derived samples from resistant tumors. B-RAF inhibitors, such as PLX4720, also appear to cause hyperactivation of the MEK-ERK 1/2 pathway in mutant N-Ras melanoma cells which can cause pathway hyperactivation leading to apoptotic resistance [83].

Targeting MEK in Melanoma

MEK-1 and MEK-2 are dual-specificity tyrosine/threonine protein kinases that lie downstream of B-RAF and are found to be active in ~30% of all human cancers with activated MAPK signaling [14]. The only known substrate of MEK-1 and MEK-2 kinases is ERK [14]. Therefore, MEK-1/2 is a popular therapeutic target in the MAPK signaling cascade [84]. It has been shown that tumors that harbor ^{V600E}B-RAF are sensitive to MEK inhibition but not those that harbor mutant RAS [85]. Therefore, when selecting MEK inhibitors for melanoma therapy, B-RAF mutational status is a critical factor needing consideration [85].

Clinical Efficacy of Therapies Targeting MEK

A wide range of different cancer cell lines possessing either K-RAS, N-RAS or B-RAF mutations are sensitive to AZD6244 at <1 μmol/L which is a selective, potent, allosteric inhibitor of MEK [86]. Initial in vitro studies by Davies et al. noted that the majority of cell lines that are sensitive to AZD6244 possess a mutation in the RAF or RAS genes, while none of the resistant lines possessed a B-Raf mutation. Thus, most cell lines containing mutant B-RAF are dependent on MEK activity and therefore sensitive to MEK inhibition. In contrast, presence of K-RAS mutation makes cells less sensitive to MEK inhibition, which might be due to RAS initiating signaling through other signaling pathways implicated in cancer development [86].

Efficacy of AZD6244 was tested in nude mice containing xenografts from cells with B-Raf and K-Ras mutations that were highly sensitive to AZD6244

(Colo-205, Calu-6, and SW620) [86]. Dosing with 25 mg/kg bid resulted in 94% inhibition of Calu-6 tumor growth, 73% inhibition of SW620 tumor growth and stasis of Colo-205 tumors if started when tumors were about 0.2 cm^3 or partial regression if dosing started when tumors were larger at about 0.55 cm^3. Phosphorylated-ERK levels were measured to determine the level of inhibition in each of these xenografts. In Calu-6 xenografts, an acute dose of 25 mg/kg sufficiently inhibited p-ERK by >90% after 1 h as measured by immunohistochemistry or western blotting. Moreover, inhibition of ERK phosphorylation was significant but to a lesser degree in Colo-205 and SW620 xenografts. In the two most sensitive xenografts, Colo-205 and Calu-6, a single dose of AZD6244 was sufficient to trigger apoptosis. A combination of AZD6244 with either irinotecan or docetaxel has also been shown to significantly inhibit xenografted tumor development in this study [86].

Phase I clinical trials with AZD6244 were published in 2008 with 57 patients enrolled [87]. The maximum tolerated dose in this trial was 100 mg po bid. Pharmacokinetics revealed a median half life of approximately 8 h, supporting twice daily dosing, and pharmacodynamic studies demonstrated dose dependent inhibition of ERK phosphorylation with up to 100% inhibition occurring 1 h after treatment with the first dose. Additionally, Ki-67, a marker of cell proliferation, decreased compared to pretreatment levels in tumor biopsies, but not as consistently as pERK levels did. The most common side effect was a rash followed by gastrointestinal related toxicities including nausea and diarrhea. Stable disease lasted for five or more months in nine of 57 patients enrolled, stable disease at end of cycle 2 (each cycle is 28 days) for 19 of the patients and one patient with uveal melanoma and renal cell carcinoma with stable disease for 22 cycles and another with medullary thyroid cancer that had stable disease for 19 cycles.

A non-ATP competitive MEK 1/2 inhibitor with a unique structure and mechanism of action is R05068760. Daouti et al. published an in vivo characterization of the pharmacokinetics, pharmacodynamic and efficacy of RO5068760 in multiple xenograft tumor models [88]. The estimated EC_{50} in plasma was 1.36 umol/L (880 ng/ml) in the LOX melanoma models and a plasma drug concentration of 0.65 or 5.23 umol/L was needed for tumor growth inhibition (>90%) in ^{V600E}B-Raf or K-ras mutant tumor models.

Development of Resistance to Drugs Targeting MEK 1/2

Certain melanoma cells are resistant to MEK1/2 inhibitors [89]. While mechanisms leading to MEK1/2 inhibitor resistance remains uncertain, a recent study sequenced tumors obtained from relapsed patients following treatment with the allosteric MEK inhibitor AZD6244 and resistant clones generated from a MEK1 random mutagenesis screen [90]. Mutations were identified disrupting the allosteric drug binding pocket or alpha-helix C, which led to an ~100-fold increase in resistance to MEK inhibition [90]. Mutations in MEK1, Q65P and P124L have also been identified in

patients treated with the MEK inhibitor AZD6244. These mutations affected MEK1 codons located within or adjacent to the N-terminal negative regulatory helix A and conferred resistance.

Cells from patients that initially showed transient disease stabilization after being treated with AZD6244 followed by relapse on this drug have been subsequently treated with PLX4720 (a BRAF inhibitor described above) [90]. AZD6244-resistant melanoma cells exhibited resistance to PLX4720 with a GI50 value of >10 μM compared to 5–10 nM in treatment-naïve cells. Mechanistically, the cause was P124L and P124S MEK mutations, which conferred two- to threefold more resistance compared to wild-type MEK1. Meanwhile, robust resistance of >50-fold to PLX4720 compared to the MEK (DD) allele was conferred by the Q56P mutation. Clinically relevant MEK1 resistance mutations may confer cross-resistance to B-RAF inhibition as evidenced by pMEK levels following PLX4720 treatment that showed comparable reduction across all MEK1 resistance alleles [90].

Preventing MEK mediated resistance will likely require targeting multiple points in the MAPK pathway. Exposing melanoma cells containing mutant B-RAF simultaneously to PLX4720 (a ^{V600E}B-RAF inhibitor) and AZD6244 (a MEK inhibitor) prevented emergence of resistant clones, which indicates the potential of targeting multiple points in this signaling cascade to prevent development of resistance and to kill melanoma cells [90]. Therefore, combined inhibition of MEK and RAF might bypass acquired resistance to targeted therapeutics directed against the MAP kinase pathway.

Targeting ERK in Melanoma

ERK is the only known downstream substrate for MEK 1/2 [14]. Elevated ERK activity is frequently observed in human tumors as well as proliferating metastatic melanoma cell lines and is a good indicator of tumor progression [91, 92]. Growth factors in melanomas can activate ERK either by the "classical" pathway (utilizing receptor tyrosine kinases such as the c-KIT ligand SCF), or through a pathway that is coupled to G-protein receptors (such as the α-MSH activated melanocortin receptors) [9]. In melanocytes, ERK activity can also be stimulated by mitogens such as bFGF and endothelin-1 [93]. However, the degree of contribution of each pathway to the overall stimulation of ERK in melanomas remains to be determined. Additionally, sustained activation of ERK in melanoma cells has been shown to confer resistance to various therapeutic agents. Although elevated ERK activity has been shown to promote cell proliferation; under certain circumstances, the activation of ERK can inhibit cell cycle by up-regulating p53 and p16INK4a expression [13, 58, 61, 63].

Further evidence of ERK expression in melanomas was performed using immunohistochemical studies with antibodies to ERK 1/2 and phosphorylated ERK (p-ERK). In these cases, ERK was noted to be expressed in varying degrees in formalin fixed sections from 42 primary melanomas, 38 metastases, and 20 nevi (14 of the primary melanomas were in the radial and 28 in the vertical growth phase),

either in the cytoplasm and/or nucleus. Only low levels of ERK1/2 were detected in melanocytes and no pERK was detected in normal skin [94]. In patients with metastatic melanoma, higher levels of pERK were noted in subcutaneous metastases compared to lymph node metastases or compound nevi. Since N-RAS and B-RAF mutations are more frequent in cutaneous or soft tissue melanoma metastases, this could partially account for the differences in pERK levels in these cases. Additionally, there was a non-significant relationship between the depths of melanoma and pERK levels [94]. Higher percentage of p-ERK-positive cells have been reported in nodular melanoma compared with benign nevi and superficial spreading melanoma. Thus ERK activation is directly related to the stage of disease with higher activity occurring in more advanced melanomas [94].

Currently, the MEK1/2 inhibitors as described above are employed as inhibitors of its downstream effector ERK1/2, as MEK1/2 is known to activate ERK1/2 selectively [95] (Table 1).

Targeting Other Downstream Targets

Targeting Aurora Kinases in Melanoma

The aurora kinase family consists of aurora kinase A (AURKA), aurora kinase B (AURKB), and aurora kinase C (AURKC), which are involved in mitotic spindle assembly regulating centrosome duplication and separation, microtubule-kinetochore attachment, spindle-checkpoint, and cytokinesis [96–98]. The family members range in size from 309 to 403 amino acids with AURKA sharing 53% homology with AURKB and 73% homology with AURKC [99–101]. AURKA is involved in mitotic spindle formation and centrosome maturation that are required for chromosome segregation [102]. AURKB is a chromosomal passenger protein regulating early mitotic stage transition of prophase to metaphase [103, 104]. Inhibition halts a crucial spindle checkpoint causing premature exit from mitosis disrupting chromosome segregation and cytokinesis. AURKC is localized to the centrosome and involved in spermatogenesis.

In humans, although three isoforms of Aurora kinases, Aurora-A, -B and -C, were identified, only Aurora-A and -B are expressed at detectable levels in all somatic cells, therefore, have been characterized in greater detail for their involvement in cellular pathways relevant to the development of cancer [105]. Elevated expression of AURKs has been reported in cancers of skin, breast, colon, prostate and ovaries [106]. In addition, genetic variants of AURKs have been found in various clinical biopsies excised from patients suffering from non-melanoma skin cancer, and cancers of breast, prostate and ovaries [107, 108]. For example, a genetic variant of AURKA, STK15 T + 91A, which resulting in the amino acid substitution F31I, has been associated with increased aneuploidy in colon tumors and cell transformation in vitro [109]. Furthermore, meta-analysis of 9,549 cases of breast,

Table 1 Classes of therapies and associated resistance patterns in targeted therapies most extensively studied to date

Target	Drug	Specificity of the drug	Development of Resistance	Overcoming of Resistance
Raf Inhibitors	PLX-4032 (vemurafenib)	100-fold selective to mutant ^{V600E}B-Raf over wild-type B-Raf.	Insensitivity to drug secondary to promotion of RAF dimer formation via: *-Overexpression of RAF1 -RAS activation secondary to RAS mutation*	Requires further study Add an inhibitor to RAS (studies have failed to find an inhibitor to RAS)
	Sorafenib	Inhibits both wild type and mutant B-Raf activities		
		Inhibits both wild type and mutant B-Raf activities		
	XL281	100-fold selective to mutant ^{V600E}B-Raf over wild-type B-Raf	Overexpression of MAP3K8 (COT)	Add an inhibitor of MAP3K8
	GSK2118436		Overexpression of PDGFRβ	Add an inhibitor of PDGFRβ
			Hyperactivation of MEK-ERK 1/2 signaling	Add a MEK 1/2 inhibitor
MEK 1/2 inhibitors	AZD6244 R05068760	Inhibits MEK phosphorylation of ERK	Unclear at this time. Further clinical trials are needed	
Aurora Kinase Inhibitors	SNS-314 VX680 MLN8054	Inhibits phosphorylation of serine 10 of aurora kinases	Unclear at this time. Further clinical trials are needed	

colon, ovarian, prostate, lung, esophageal and non-melanoma skin cancers showed an increased risk in T + 91A homozygotes of breast and colorectal cancers. In addition, genomic analysis of cutaneous melanomas showed frequent gains at chromosome 20q that includes Aurora A gene.

Clinical Efficacy of Therapies Directed at Aurora Kinase

Since elevated levels of these kinases have been detected in several cancers, the aurora family of serine/threonine kinases are another target of therapies [110–112]. In vivo studies of 12 cancer cell lines, including melanoma, was performed in female mice that had subcutaneous implantation of tumor cells with the aurora kinase inhibitor, SNS-314 [110]. These assays revealed decreased phosphorylation of histone H3 on serine 10, a marker of activity of aurora kinases, and significant tumor growth inhibition in a dose dependent manner. This trial concluded that SNS-314 is a potent small molecule inhibitor of Aurora kinases and may be a novel therapeutic agent for human cancers, including melanoma [110].

Additionally, a recent study evaluating the effect of inhibiting Aurora kinase-A and Aurora kinase-B activities using isoform specific pharmacological agents VE-465 and ZM447439, respectively, demonstrated that Aurora kinase-A targeting is more effective than Aurora kinase-B inhibition for the induction of melanoma cell death [113]. A phase I trial examining the safety, pharmacokinetics and pharmacodynamics of an oral Aurora kinase-A inhibitor, MLN8054 has been performed in patients with advanced solid tumors. The data showed induction of two dose limiting toxicities when MLN8054 was given QID at a dose of 80 mg [114, 115]. Aurora kinase-A inhibition was evidenced by pharmacodynamic analysis of skin and tumor mitotic indices, mitototic cell chromosome alignment and spindle bipolarity. Recently a more potent second generation Aurora kinase-A inhibitor MLN8237 was synthesized and is currently in early phase clinical trials [116].

Targeting Macrophage Inhibitory Cytokine-1 in Melanoma

MIC-1, also known as PTGF-β, PLAB, GDF15, PDF, and NAG-1, is a member of the transforming growth factor-beta super-family proteins implicated in melanoma development [56, 117]. Expression of MIC-1 is upregulated in 66% of metastatic melanoma cell lines (35/53) and 100% metastatic patient biopsies (16/16) compared to normal melanocyte controls [56]. Another recent study also showed elevated MIC-1 expression in 67% advanced melanomas [118]. In addition, five- to sixfold increase in secreted MIC-1 protein was observed in the serum of these patients indicating that MIC-1 can serve as a prognostic marker for identifying melanoma patients [56, 118]. Prior studies also showed elevated expression of MIC-1 in a wide variety of tumors including carcinomas of prostate, large bowel and breast. Expression of MIC-1 is regulated by MAP kinase and PI3 kinase pathways in melanoma [56, 118].

For example, pharmacological agents U0126 and PD098059 inhibiting MEK1/2 activity decreased expression of MIC-1 [56]. Similarly, cells treated with PI3 kinase inhibitor LY294002 also modestly reduced expression. MITF, a key member of MAP kinase pathway regulating the expression of various cell cycle and cell proliferation proteins, has been shown to control MIC-1 protein levels [56]. Factors stimulating MITF activity such as stem cell factor or exposure to PMA elevated expression of MIC-1. A prior study using short-hairpin RNAs demonstrated that MIC-1 inhibition decreased xenografted melanoma tumors development compared to cells transfected with control sh-RNAs [56]. Similarly, another recent study also demonstrated that targeting Mic-1 using siRNAs reduces the xenografted melanoma tumors growth [118]. Similar to VEGF, MIC-1 also stimulated the vessels development, thereby augmented tumor growth.

Although MIC-1 expression has been shown to be upregulated in advanced melanomas, the precise role of MIC-1 in tumor biology is unclear. For example, it is not known whether MIC-1 expression is leading to metastasis development or metastatic tumors are releasing MIC-1 into serum to perform some yet unknown role. Furthermore, the role of MIC-1 in different stages of melanoma development needs to be studied in detail as recent studies demonstrated that the MIC-1 function varies with the stage and extent of the tumor producing it. At present time, there are no apparent trials evaluating direct MIC-1 inhibitors in melanoma.

Targeting Interleukin-8 (IL-8) in Melanoma

IL-8 is an important autocrine multifunctional cytokine implicated in melanoma cell proliferation, angiogenesis, migration and metastasis development [119]. IL-8 is also an indicator of tumor aggressiveness as elevated expression of this cytokine is observed in melanoma tumor biopsies [120]. Furthermore, introduction of IL-8 into non-metastatic melanoma cells lines or cells that are negative for IL-8 expression induced expression and activity of MMP-2, which increased invasion and angiogenesis thereby transforming them in to highly tumorigenic, metastatic cell types [121]. IL-8 expression can be induced by phosphoglucoseisomerase/autocrine motility factor (AMF) in autocrine manner thereby promoting melanoma cell migration [122]. Targeting IL-8 using siRNAs reduced IL-8 secretion from melanoma cells, which resulted in the down regulation of β2 integrin on neutrophils thereby inhibiting metastasis development [54, 123]. Tumor-derived cytokines IL-6 and IL-8 can act as attractants for circulating tumor cells (CTCs) thereby promoting a process called “tumor self-seeding” [124]. Tumor self-seeding is a process in which tumor cells colonize their tumors of origin. Tumor self-seeding is primarily responsible for local recurrence occurring after complete tumor excision.

Signaling pathways regulating IL-8 expression and secretion in melanoma cells involve MAP kinase pathway signaling [54, 123]. A recent study demonstrated that AMF induced IL-8 production was mediated by ERK1/2 in melanoma cells [122]. Therefore, targeting members of MAP kinase signaling could potentially decrease

IL-8 levels thereby inhibiting melanoma tumor and metastasis development. For example, knockdown of mutant (V600E) B-Raf inhibited the constitutive secretion of IL-8 thereby decreasing melanoma cell extravasation and subsequent metastasis development [54, 123]. Pharmacological agents targeting members of MAP kinase pathway also reduced the production of IL-8 in melanoma cells [125, 126]. For example, inhibition of B-RAF using derivatives of diarylimidazoles decreased colony formation in soft agar, reduced proliferation and retarded melanoma tumor growth in animal models. Targeting B-Raf reduced IL-8 in the plasma of animals, suggesting that it could serve as a marker for clinical assessment of B-Raf inhibition [125]. MEK inhibitor PD0325901 has been shown to reduce IL-8 and VEGF levels thereby decreasing melanoma cell proliferation and angiogenesis [126].

Other key regulators of IL-8 production in melanoma cells include STAT3 and PAR-1. For example, whereas introduction of constitutively active STAT3 into WM35 melanoma cells enhanced IL-8 production, targeted inhibition of STAT3 in 1205 Lu cells reduced IL-8 levels [127]. Similarly, systemic delivery of PAR-1 siRNA incorporated into neutral liposomes [1, 2-dioleoyl-sn-glycero-3-phosphatid ylcholine (DOPC)] decreased VEGF and IL-8 production thereby reducing melanoma growth and metastasis in animals [128].

While IL-8 can serve as a biomarker of B-Raf inhibition, some pharmacological agents targeting MAP kinase signaling can induce the production of IL-8. For example, dacarbazine, an FDA approved agent for melanoma, activates the extracellular signal-regulated kinase pathway, and increases expression and secretion of IL-8 and VEGF [129, 130]. In addition, some selenium containing chemotherapeutic agents such as PBISe targeting Akt induce MAP kinase pathway activity [13, 131]. However, it is unknown whether increased MAP kinase activity mediated by PBISe promotes IL-8 secretion. Therefore, clinical trials should consider using IL-8 neutralizing antibodies such as ABX-IL8 while evaluating these agents [132]. In fact, the use of fully human antibodies against IL-8 have been studied thus far in vitro [133]. In this study, fully human IL-8 antibodies reduced the invasion of metastatic melanoma cells. They also appeared to sensitize tested cell lines when treated with dacarbazine and also decreased cell viability in metastatic melanoma cell lines.

Targeting WEE1 in Melanoma

WEE1, another downstream member in the MAPK signaling, is a key protein kinase involved in maintaining G(2)-cell-cycle checkpoint arrest for pre-mitotic DNA repair [134, 135]. WEE1 phosphorylates Tyr-15 of CDC2 thereby inhibiting its activity, which results in G2/M arrest [136]. Elevated expression of WEE1 is observed in glioblastoma and breast cancer [134, 136]. Furthermore, studies have also shown that targeting WEE1 using siRNA or pharmacological agents inhibited cancer cell survival and reduced the development of xenografted tumors demonstrating the therapeutic potential of targeting this key kinase for cancer therapy [137, 138]. In addition, targeting WEE1 in combination with either radiotherapy or treatment

with cytostatic agents enhanced the therapeutic potential. Pharmacological inhibition of WEE1 using MK-1775 selectively sensitized p53 deficient cancer cells to DNA damaging agents such as gemcitabine, cisplatin and carboplatin [137]. PD0166285, a known WEE1 inhibitor, radio-sensitized cells to radiation-induced cell death in a p53 dependent manner [139]. Cells lacking p53 showed higher sensitivity to WEE1 inhibition compared to those harboring p53. Mechanistically, targeting WEE1 induces a mitotic catastrophe due to premature entry into mitosis with unrepaired lethal damaged DNA [139]. Since cancer cells, unlike normal cells that require G1-arrest, largely depend on G2-M arrest for damaged DNA repair, targeting WEE1 in combination with DNA damaging agents is a promising therapy for cancers. While the role of WEE1 is well studied in several other cancer types, a few studies have reported the therapeutic potential of targeting WEE1 in mouse melanoma cells. Targeting WEE1 using PD0166285 reduced cell proliferation by decreasing Cyclin-D levels [140]. Since melanomas are known to contain functionally active p53 protein, it is interesting to determine whether targeted inhibition of WEE1 alone is effective for retarding melanoma development. At present time, there are no apparent trials evaluating direct WEE1 inhibitors in melanoma.

Targeting VEGF in Melanoma

VEGF is another key target in melanomas regulating angiogenesis, which is required for invasive tumor growth and metastasis [141–143]. Immunohistochemical studies have shown that 20–77% of pr human primary melanomas express VEGF (Potti, A. Anticancer Res. 2003. pp 4023–2026). Targeted inhibition of VEGF may be a valuable approach to cancer therapy. Studies have shown that targeting B-Raf inhibits VEGF expression in melanomas [57]. siRNA mediated inhibition of B-Raf reduced endogenous as well as secreted VEGF levels, which in turn decreased blood vessel development thereby retarding xenografted melanoma tumors growth [118]. Furthermore, prior studies have demonstrated that inhibition of Raf, either by the use of MEK inhibitor (PD98059) or by siRNA specific to B-Raf, significantly lowered VEGF-A expression [144]. Treating melanoma cells with sorafenib also decreased MAPK activity and reduced blood vessel density through the inhibition of VEGF [57]. Proof of principle studies using siRNAs targeting VEGF retarded melanoma tumor development indicating VEGF could be a therapeutic target for inhibiting melanoma [57]. Further circulating VEGF (cVEGF) has been correlated with disease progression in melanomas, indicating VEGF could be a biomarker for disease diagnosis as well as a marker for measuring the therapeutic efficacy of various treatment interventions. However, a recent study showed that cVEGF may not be a good indicator of assessing the disease severity and treatment efficacy as the true VEGF levels in cancer patients appears to be very low, except in renal cell carcinoma [145]. High levels of VEGF measured in clinical specimens could be due to the artificial release from activated platelets. Activated platelets in cancers have been found to secrete increased VEGF, primarily during the blood harvest procedure [145]. Therefore care must be taken while considering VEGF as a biomarker for disease prognosis.

Sorafenib, which originally was developed as a BRAF inhibitor, also selectively inhibits VEGFR-2 and -3, was initially studied but did not show evidence to improve standard of care [146]. Axitinib, a potent oral inhibitor of VEGF Receptor Tyrosine Kinase 1, 2, and 3 is currently in development by Pfizer Inc for the potential treatment of a variety of solid tumors. Thus far, preclinical and clinical data is available for axitinib [147]. Phase II studies in many tumor types including malignant melanoma and renal, pancreatic, thyroid, breast, lung and colorectal carcinomas showed that axitinib is well-tolerated [147]. However, in metastatic melanoma, recent Phase II trials revealed an unimpressive overall response rate of 15.6% and median survival of 6.8 months [146]. Additionally, due to frequent side effects including fatigue, hypertension, diarrhea, hand-foot syndrome and proteinurea, its clinical development has been hampered [147, 148]. Recent ongoing phase III studies in pancreatic and metastatic renal cell carcinoma will ultimately define the therapeutic role of this targeted agent for the treatment of melanoma and other malignancies [147]. Thus far, the failures of these selective tyrosine kinase VEGF inhibitors, along with others including sunitinib, dovitinib and vatalanib are believed to be multifactorial secondary to the chemoresistant nature of metastatic melanoma, the cystostatic rather than cytotoxic nature of tyrosine kinase inhibitors, and that these studies have been primarily for inhibition ability in established metastatic tumors [146].

More recently, monoclonal antibodies directed against VEGF ligand have been reported with bevacizumab (Avastin). A recent phase II trial for first line therapy for malignant melanoma tested carboplatin and paclitaxel chemotherapy with and without bevacizumab and reported that progression free survival had improved to 22% and overall survival improved to 21% in patients treated with bevacizumab [146]. The primary progression free survival endpoint was not met but the gain in overall survival has led to a planned subsequent definitive trial [146]. Larger Phase II trials are needed to further delineate the use of VEGF monoclonal antibodies.

Additionally, afibercept, a fusion protein that incorporates portions of human VEGFR-1 and VEGFR-2 with human IgG1 has been studied. Acting as a soluble decoy VEGF receptor, preclinical studies showed a favorable profile over other VEGF inhibitors [149]. In an interim analysis of a current Phase II study, one of 21 patients with treatment-naïve metastatic melanoma received complete remission [146].

Targeting Cyclin D-1 and B-RAF in Melanoma

D-type cyclins, which are regulated themselves by B-Raf, regulate G1 cell cycle progression by enhancing the expression and activities of cyclin-dependent kinases [150]. In normal cells, levels of cyclins and cyclin-dependent kinase (Cdk) inhibitors are tightly controlled. However, in melanomas, this normal balance is frequently dysregulated. In one study, immunohistochemical analysis of cyclin D1 showed elevated expression in early melanomas [151]. Cyclin D1 positivity increased during tumor progression, but was observed in lower levels in metastases. Survival analysis in this study failed to detect any linkage to shorter or longer survival among patients expressing either cyclin D1 c-Kit, or p-ERK [151]. Additionally, this study

found that cyclin D1 expression lacked prognostic potential as low levels of cyclin D1 occurred in metastatic melanomas [151]. In contradiction to this report, another found that cyclin D1 expression may be related to malignant phenotype and might be associated with high proliferation rates in metastatic melanomas. Analysis of formalin-fixed paraffin-embedded material from 21 common melanocytic nevi, 42 dysplastic nevi, and 17 primary cutaneous metastatic melanomas showed elevated Cyclin D1 expression in advanced compared to earlier stage lesions [152].

Currently, a Phase II Study to evaluate the safety and efficacy of P276-00, a cyclin D1 inhibitor, has finished recruiting and is currently ongoing with results pending at this time. Previously, P276-00 was studied in vitro and in vivo [153]. In this study, P276-00 was tested for its antiproliferative potential in a panel of 16 cisplatin-resistant and cisplastin sensitive cell lines and noted to have a ~30-fold higher effect than cisplatin. Furthermore, 22 human xenografts in a clonogenic assay showed tumor sensitivity to P276-00 was ~26-fold more potent than cisplatin and also effective against cisplatin resistant lines in melanomas, CNS, renal and prostate cancer. In flow cytometry testing, an asynchronous population of human prostate cancer and human promyelocytic leukemia cells showed arrest of prostate cancer cells in G2-M with no significant apoptosis and significant apoptosis in faster growing promyelocytic leukemia cells. P276-00 in synchronized human non-small cell lung cancer showed arrest of cells in G1 followed by apoptosis if exposed for 48 h. Further testing was performed with P276-00 in vivo with murine tumor and human xenograft models showing significant growth inhibition in murine colon cancer when administered i.p. at 50 mg/kg for 20 treatments and in murine lung cancer models when administered i.p. at 60 mg/kg every alternate day for seven treatments. In human xenograft models, P276-00 showed significant inhibition in human colon carcinoma HCT-116 xenografts at a dose of 35 mg/kg i.p for 10 days and human non-small cell lung carcinoma H-460 xenograft at a dose of 50 mg/kg daily or 30 mg/kg twice daily i.p. for 20 treatments [153]. Cyclin-D1 remains an interesting potential target for therapies in melanoma and results from the noted Phase II trial are pending.

Targeting Members of Other Pathways

Agents currently used in treatment of melanoma, such as Dacarbazine or the derivative temozolomide, are only effective in 15–20% of patients [154, 155], partly secondary to deregulation of many pathways in melanoma cells that promote highly metastatic phenotypes and resistance to chemotherapeutics [13]. As such, most clinicians and researchers in the melanoma field believe that multiple signaling cascades will need to be targeted simultaneously to effectively inhibit melanoma development. Therefore targeting of the members of MAPK cascade or other oncogenic proteins from different signaling pathways combined with these therapies will be required to achieve better clinical efficacy [156].

As alluded to in the previous sections, preclinical studies have shown that targeting PI3K and MAPK signaling pathways using siRNA or pharmacological agents can

sensitize cells to chemotherapeutic agents and synergistically inhibit melanoma development [58, 157]. For instance, co-targeting RAF and mTOR using sorafenib and rapamycin, respectively, more effectively inhibited melanoma cell proliferation, inhibited melanoma cell invasion and induced cell death [158]. Likewise, treatment of melanoma cells with cisplatin or temozolomide in combination with LY294002 or rapamycin effectively reduced melanoma cell growth and survival [158]. Similarly, simultaneous inhibition of CDK 4 kinases and MEK using pharmacological inhibitors PD98059 and 219476, respectively, significantly increased apoptosis compared to single agents alone [159]. Another independent study combined MAPK and PI3K signaling pathway inhibition to show that the anti-proliferative and pro-apoptotic effects of inhibitors alone were disappointing compared to using a panel of pharmacological inhibitors (BAY 43-9006, PD98059, U0126, wortmannin, LY294002) which significantly inhibited growth and enhanced apoptosis in monolayer culture [160].

Targeting oncogenes while expressing tumor suppressors is another alternative approach for inhibiting melanoma development. For instance, massive apoptosis in melanomas was observed when ^{V600E}B-RAF was targeted using siRNA while expressing the tumor suppressor INK4A cDNA compared to either of these events alone [63]. Building on this approach, targeting multiple members of a single pathway or members of different pathways is an approach to more effectively treat melanomas that will continue to evolve in the next decade. However, the combination would need to be selected based on the genetic pathway activated and available approaches to target them.

Impact of Tumor Microenvironment and Cancer Stem Cells—The Future of Melanoma Targeted Therapy?

Melanomas, like many other cancer types, depend on interactions with microenvironment for tumor growth as well as metastasis formation [161]. Therefore, tissue microenvironment does play a critical role in cell survival and growth and likely contributes to cell transformation and tumor development [162]. Cellular interactions with the stroma and with other cells provide key signals that control cellular arrest or division, survival or death, and entrance or exit from a quiescent state [161]. For example, tumor cell adhesion to blood vessel endothelial cells (EC) followed by trans-endothelial migration is critical event responsible for the metastasis development [163–165].

Recent studies have shown the involvement of polymorphonuclear neutrophils (PMNs) for facilitating melanoma cell adhesion to the endothelium as well as subsequent extravasation under flow conditions [166, 167]. Experiments demonstrating the involvement of neutrophils in the development of melanoma metastasis showed enhanced metastatic tumors when neutrophils were injected immediately after melanoma cells injection [123]. Mechanistically, entrapped melanoma cells produced interleukin-8 thereby attracting neutrophils. In addition, IL-8 also increased the beta [2] integrin expression by 75–100% leading to the anchoring of melanoma cells to

endothelial cells via interaction with ICAM-1 on melanoma cells. Targeted inhibition of IL-8 secretion from melanoma cells decreased Beta-2-integrin on neutrophils by 50%, which in turn reduced neutrophil-mediated extravasation, and resulting in 50% fewer melanomas in lungs. Several studies have shown direct regulation of IL-8 expression and ^{V600E}B-Raf activity in melanomas. Therefore targeted inhibition of V600EB-Raf might be inhibiting metastasis development through reducing the IL-8 mediated melanoma cells-neutrophil interactions, further demonstrating the involvement of extracellular matrix in the melanoma metastasis formation [123, 168].

Melanoma cells actively interact with the tumor microenvironment, through molecular signals, to promote tumor formation [161]. For example, collagen, a key extracellular matrix component regulate the development of melanomas [169]. Melanoma cells containing tumor suppressor KLF6 when grown in collagen rich media failed to develop tumors [170]. However, when similar cells were grown in polyHEMA coated plates or plastic plates they grew with the proliferation rates similar to KLF6 null cells, indicating the involvement of tumor microenvironment in the tumor development. Mechanistically, KLF6 inhibited pErk1/2 as well as cyclin D1 levels thereby reduced melanoma cell proliferation in a collagen rich environment. Therefore, loss of KLF6 promotes melanoma tumor development by upregulating MAPK pathway [170].

Several studies have reported elevated COX-2 levels in human melanomas [171–173]. In addition, studies have also shown that COX-2 expression is regulated by MAPK pathway, and inhibiting ^{V600E}B-Raf in melanomas effectively reduces COX-2 expression without altering COX-1 levels [174]. Elevated COX-2 triggers cell proliferation, invasion and metastatic abilities of melanoma cells thereby promote metastasis formation in distant organs [175–177]. For example, a recent study showed that inhibition of COX-2 decreases systemic and bone metastasis of melanomas [176]. Furthermore, inhibition of COX-2 using celecoxib reduced melanoma bone metastasis incidence as well as tumor volume in mice models. Since COX-2 inhibition retarded melanoma metastasis and tumor formation, several derivatives of COX-2 have been prepared and tested for efficacy for inhibiting melanoma.

Furthermore, the concept of cancer stem cells (CSCs) has been first established for human myeloid leukemia in the 1960s [178]. Recent studies found CSCs in many other solid tumors including cancers of breast, brain and skin [179]. A study isolated morphologically heterogeneous populations of cells, as demonstrated by the coexistence of multiple genetic sub-clones, in melanomas and showed their involvement in tumor recurrence as well as drug resistance [180–182]. A recent study also showed that melanoma stem cells are also responsible for tumor initiation, development, growth as well as metastasis [183]. However, it is presently unclear which role a sufficiently characterized population of melanoma stem cells plays in cancer promotion and progression [181].

Melanoma stem cells have been isolated from about 20% of the metastatic melanomas cultured in growth medium suitable for human embryonic stem cells and their properties studied [184]. It has been observed that multipotent melanoma stem cells possess self-renewal ability and persisted after serial cloning in vitro and transplantation

in vivo. In vivo studies also showed the enhanced tumorigenic potential of melanoma stem cells compared to melanoma cells, suggesting targeting this sub-population might help to eliminate melanomas more effectively. Furthermore, dedifferentiated melanoma cells have been found to be more resistant to various treatments compared to melanoma cells and formed tumors more quickly [185].

Strategies to Overcome Drug Delivery Issues Using Nanotechnology Based Therapeutic Agents

A continued barrier in the availability of effective treatment options and drugs in melanoma that target the MAPK pathway, despite our knowledge of this pathway to date, continues to be the lack of clinically effective pharmacological agents and delivery vehicles to get the drug into the melanoma cells [186]. Nanotechnology, which is capable of encapsulating one or more therapeutic agents as a single drug in order to evaluate its efficacy in clinical trials, may be part of the possible solution to this problem [186–188]. Additionally, many nanotechnologies are shown to improve circulation time, enhanced drug uptake into tumors, avoid the reticulo-endothelial system, and minimize toxicity [186]. There are currently a wide variety of nanotechnology delivery systems that have been developed for treating tumor including silicon and gold nanoshells, polymeric nanoparticles, carbon-based nanostructures, dendrimers, and liposomes [189].

Currently in various stages of development are liposomes that contain chemotherapeutic agents, antisense-ODNs, siRNA, DNA, or radioactive particles that could target the MAPK pathway [186, 188]. For instance, liposomes loaded with siRNAs targeting ^{V600E}B-RAF and AKT3 synergistically inhibited melanoma tumor growth in mice [157, 186]. Similarly, sorafenib in combination with ceramide-containing liposomes synergistically inhibited melanoma development in animals [190]. Additionally, a Phase-I study has shown that liposomal cisplatin can enhance drug delivery up to 200 fold in tumors [191]. Another study showed the use of other nanoparticle technology, such as the unique hexadentate-polyD,L-lactic acid-co-glycolic acid polymer chemically conjugated to PD98059 (MEK1 inhibitor), which induced apoptosis in vitro, retarded tumor growth in vivo and inhibited melanoma cell proliferation [187]. Furthermore, the antitumor efficacy of cisplatins have also been enhanced by use of nanoparticles [187]. Thus, nanoparticle delivery systems provide one technology to load multiple drugs, which could be genetic or pharmacological, into a single vehicle and to target to the melanoma cells.

Another potential approach that is currently being evaluated is the use of RNAi technology to target the MAPK pathways. siRNA can specifically inhibit target genes in the MAPK pathway; however rapid degradation in animals has been a major obstacle [187, 192, 193]. Liposomes can protect RNAi from being “detected” by RNAses, and if coupled to specific antibodies or ligands can deliver the particles specifically into melanoma cells. Approximately 1,200 different classes of “lipidoids”, which are lipid-like barriers, were noted to be about 100 times more efficient at

delivering small interfering RNA than the earlier reported lipid-based barriers in a recent report from the Massachusetts Institute of Technology and Alnylam Pharmaceuticals Inc. [194]. Clinical efficacy of this approach for targeting the MAP kinase pathways remains to be demonstrated [195].

Conclusion

In order to effectively treat melanomas, targeted inhibition of key mechanistic events regulating melanoma development such as cell proliferation, survival, angiogenesis and invasion or metastasis is required to prevent the tumor growth. A targeted approach, particularly targeting the MAPK pathway, will likely be a component of any therapeutic regimen for cutaneous melanomas. As this review demonstrates, targeting B-RAF or MEK may be the best approach for clinical efficacy and combining inhibition of key members of this signaling cascade and its downstream targets that regulate melanoma growth may be required to prevent the progression of this disease and development of resistance. Furthermore, understanding the molecular mechanisms that lead to the development of resistance to chemotherapeutic agents, as well as strategies to overcome resistance is needed. The use of nanotechnology might prove to be a potential avenue to overcome some of these issues by providing a single platform in which multiple genetic or pharmacological agents can be loaded to synergistically inhibit melanoma development and overcome the occurrence of resistance. The challenge remains in identifying the optimal targets in addition to discovery of drugs that have negligible toxicity-related side effects and are bioavailable.

Grant Support NIH CA-127892-01A and The Foreman Foundation for Melanoma Research (GPR).

References

1. Rager EL, Bridgeford EP, Ollila DW. Cutaneous melanoma: update on prevention, screening, diagnosis, and treatment. Am Fam Physician. 2005;72(2):269–76.
2. Edwards BK, Howe HL, Ries LA, Thun MJ, Rosenberg HM, Yancik R, et al. Annual report to the nation on the status of cancer, 1973–1999, featuring implications of age and aging on U.S. cancer burden. Cancer. 2002;94(10):2766–92.
3. Whiteman DC, Whiteman CA, Green AC. Childhood sun exposure as a risk factor for melanoma: a systematic review of epidemiologic studies. Cancer Causes Control. 2001;12(1):69–82.
4. Breslow A. Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. Ann Surg. 1970;172(5):902–8.
5. NIH Consensus conference. Diagnosis and treatment of early melanoma. JAMA. 9;268(10):1314–9.
6. Balch CM, Urist MM, Karakousis CP, Smith TJ, Temple WJ, Drzewiecki K, et al. Efficacy of 2-cm surgical margins for intermediate-thickness melanomas (1 to 4 mm). Results of a multi-institutional randomized surgical trial. Ann Surg. 1993;218(3):262–7. discussion 7–9.

7. Veronesi U, Cascinelli N, Adamus J, Balch C, Bandiera D, Barchuk A, et al. Thin stage I primary cutaneous malignant melanoma. Comparison of excision with margins of 1 or 3 cm. N Engl J Med. 1988;318(18):1159–62.
8. Veronesi U, Adamus J, Aubert C, Bajetta E, Beretta G, Bonadonna G, et al. A randomized trial of adjuvant chemotherapy and immunotherapy in cutaneous melanoma. N Engl J Med. 1982;307(15):913–6.
9. Panka DJ, Atkins MB, Mier JW. Targeting the mitogen-activated protein kinase pathway in the treatment of malignant melanoma. Clin Cancer Res. 2006;12(7 Pt 2):2371s–5.
10. Lopez-Bergami P, Fitchman B, Ronai Z. Understanding signaling cascades in melanoma. Photochem Photobiol. 2008;84(2):289–306.
11. Mercer KE, Pritchard CA. Raf proteins and cancer: B-Raf is identified as a mutational target. Biochim Biophys Acta. 2003;1653(1):25–40.
12. Russo AE, Torrisi E, Bevelacqua Y, Perrotta R, Libra M, McCubrey JA, et al. Melanoma: molecular pathogenesis and emerging target therapies (review). Int J Oncol. 2009;34(6):1481–9.
13. Madhunapantula SV, Desai D, Sharma A, Huh S, Amin S, Robertson GP. PBIse, a novel selenium containing drug for the treatment of malignant melanoma. Mol Cancer Ther. 2008;7(5):1297–308.
14. Dhomen N, Marais R. BRAF signaling and targeted therapies in melanoma. Hematol Oncol Clin North Am. 2009;23(3):529–45. ix.
15. Gollob JA, Wilhelm S, Carter C, Kelley SL. Role of Raf kinase in cancer: therapeutic potential of targeting the Raf/MEK/ERK signal transduction pathway. Semin Oncol. 2006; 33(4):392–406.
16. Fensterle J. A trip through the signaling pathways of melanoma. J Dtsch Dermatol Ges. 2006;4(3):205–17.
17. Meier F, Schittek B, Busch S, Garbe C, Smalley K, Satyamoorthy K, et al. The RAS/RAF/MEK/ERK and PI3K/AKT signaling pathways present molecular targets for the effective treatment of advanced melanoma. Front Biosci. 2005;10:2986–3001.
18. Marquette A, Bagot M, Bensussan A, Dumaz N. Recent discoveries in the genetics of melanoma and their therapeutic implications. Arch Immunol Ther Exp (Warsz). 2007;55(6):363–72.
19. Tsao H, Zhang X, Fowlkes K, Haluska FG. Relative reciprocity of NRAS and PTEN/MMAC1 alterations in cutaneous melanoma cell lines. Cancer Res. 2000;60(7):1800–4.
20. Dahl C, Guldberg P. The genome and epigenome of malignant melanoma. APMIS. 2007;115(10):1161–76.
21. Chin L, Pomerantz J, Polsky D, Jacobson M, Cohen C, Cordon-Cardo C, et al. Cooperative effects of INK4a and ras in melanoma susceptibility in vivo. Genes Dev. 1997;11(21):2822–34.
22. Mishra PJ, Ha L, Rieker J, Sviderskaya EV, Bennett DC, Oberst MD, et al. Dissection of RAS downstream pathways in melanomagenesis: a role for Ral in transformation. Oncogene. 2010;29(16):2449–56.
23. Ackermann J, Frutschi M, Kaloulis K, McKee T, Trumpp A, Beermann F. Metastasizing melanoma formation caused by expression of activated N-RasQ61K on an INK4a-deficient background. Cancer Res. 2005;65(10):4005–11.
24. Chin L, Tam A, Pomerantz J, Wong M, Holash J, Bardeesy N, et al. Essential role for oncogenic Ras in tumour maintenance. Nature. 1999;400(6743):468–72.
25. Prendergast GC, Rane N. Farnesyltransferase inhibitors: mechanism and applications. Expert Opin Investig Drugs. 2001;10(12):2105–16.
26. Smalley KS, Eisen TG. Farnesyl transferase inhibitor SCH66336 is cytostatic, pro-apoptotic and enhances chemosensitivity to cisplatin in melanoma cells. Int J Cancer. 2003; 105(2):165–75.
27. Halaschek-Wiener J, Kloog Y, Wacheck V, Jansen B. Farnesyl thiosalicylic acid chemosensitizes human melanoma in vivo. J Invest Dermatol. 2003;120(1):109–15.
28. Meier F, Niessner H, Flaherty K, Schadendorf D, Sinnberg T, Schittek B, Garbe C. Effect of the farnesyl transferase inhibitor lonafarnib on sensitivity of melanoma cells to multikinase inhibitor sorafenib and opn Rheb farnesylation and mTOR signaling. J Clin Oncol. [Abstract]. 2009;27(15S (Supplement abstract 9077).

29. End DW, Smets G, Todd AV, Applegate TL, Fuery CJ, Angibaud P, et al. Characterization of the antitumor effects of the selective farnesyl protein transferase inhibitor R115777 in vivo and in vitro. Cancer Res. 2001;61(1):131–7.
30. Sosman JA, Puzanov I. Molecular targets in melanoma from angiogenesis to apoptosis. Clin Cancer Res. 2006;12(7 Pt 2):2376s–83.
31. McCubrey JA, Steelman LS, Abrams SL, Chappell WH, Russo S, Ove R, et al. Emerging Raf inhibitors. Expert Opin Emerg Drugs. 2009;14(4):633–48.
32. Gajewsky T, Niedezwiecki D, Johnson J, Linette G, Bucher C, Blakovich M, et al. Phase-II study of farnesyltransferase inhibitor R115777 in advanced melanoma: CALGB500104. J Clin Oncol. [Abstract]. 2006;24(18S, June 20 Supplement, Abstract #8014).
33. Dellavalle RP, Nicholas MK, Schilling LM. Melanoma chemoprevention: a role for statins or fibrates? Am J Ther. 2003;10(3):203–10.
34. Kelland LR. Farnesyl transferase inhibitors in the treatment of breast cancer. Expert Opin Emerg Drugs. 2003;12(3):413–21.
35. Rose WC, Lee FY, Fairchild CR, Lynch M, Monticello T, Kramer RA, et al. Preclinical anti-tumor activity of BMS-214662, a highly apoptotic and novel farnesyltransferase inhibitor. Cancer Res. 2001;61(20):7507–17.
36. Rose WC, Long BH, Fairchild CR, Lee FY, Kadow JF. Preclinical pharmacology of BMS-275183, an orally active taxane. Clin Cancer Res. 2001;7(7):2016–21.
37. Dinsmore CJ, Bogusky MJ, Culberson JC, Bergman JM, Homnick CF, Zartman CB, et al. Conformational restriction of flexible ligands guided by the transferred noe experiment: potent macrocyclic inhibitors of farnesyltransferase. J Am Chem Soc. 2001;123(9):2107–8.
38. Camacho LH SS, Pezzulli S, et al. Dose escalation study of oral farnesyl transferase inhibitor (FTI) BMS-214662 in patients with solid tumors. Proc Annu Meet Am Soc Clin Oncol. 2001;20:311 [Abstract].
39. Rubin EAJ, Morrison BW, et al. Phase I trial of the farnesyl protein transferase inhibitor L-778123 on 14- or 28-day dosing schedule. Proc Annu Meet Am Soc Clin Oncol. 2000;19:689 [Abstract].
40. Sharma SBC, Spriggs D, et al. A Phase I trial and PK study of farnesyl transferase inhibitor L-778123 administered as a seven day continuous infusion in combination with paclitaxel. Proc Annu Meet Am Soc Clin Oncol. 2000;19:719 [Abstract].
41. Hahn SMKK, Morrison BW, et al. Phase I trial of farnesyl transferase inhibitor L-778123 in combination with radio-therapy. Proc Annu Meet Am Soc Clin Oncol. 2000;19:906 [Abstract].
42. Adjei AA. Blocking oncogenic Ras signaling for cancer therapy. J Natl Cancer Inst. 2001;93(14):1062–74.
43. Brummer T, Martin P, Herzog S, Misawa Y, Daly RJ, Reth M. Functional analysis of the regulatory requirements of B-Raf and the B-Raf(V600E) oncoprotein. Oncogene. 2006;25(47):6262–76.
44. Rajakulendran T, Sahmi M, Lefrancois M, Sicheri F, Therrien M. A dimerization-dependent mechanism drives RAF catalytic activation. Nature. 2009;461(7263):542–5.
45. Wellbrock C, Karasarides M, Marais R. The RAF proteins take centre stage. Nat Rev Mol Cell Biol. 2004;5(11):875–85.
46. Michaloglou C, Vredeveld LC, Mooi WJ, Peeper DS. BRAF(E600) in benign and malignant human tumours. Oncogene. 2008;27(7):877–95.
47. 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.
48. Kumar R, Angelini S, Czene K, Sauroja I, Hahka-Kemppinen M, Pyrhonen S, et al. BRAF mutations in metastatic melanoma: a possible association with clinical outcome. Clin Cancer Res. 2003;9(9):3362–8.
49. Garnett MJ, Marais R. Guilty as charged: B-RAF is a human oncogene. Cancer Cell. 2004;6(4):313–9.
50. Wan PT, Garnett MJ, Roe SM, Lee S, Niculescu-Duvaz D, Good VM, et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell. 2004;116(6):855–67.
51. Wellbrock C, Ogilvie L, Hedley D, Karasarides M, Martin J, Niculescu-Duvaz D, et al. V599EB-RAF is an oncogene in melanocytes. Cancer Res. 2004;64(7):2338–42.

52. Tuveson DA, Weber BL, Herlyn M. BRAF as a potential therapeutic target in melanoma and other malignancies. Cancer Cell. 2003;4(2):95–8.
53. Hoeflich KP, Gray DC, Eby MT, Tien JY, Wong L, Bower J, et al. Oncogenic BRAF is required for tumor growth and maintenance in melanoma models. Cancer Res. 2006;66(2):999–1006.
54. Liang S, Sharma A, Peng HH, Robertson G, Dong C. Targeting mutant (V600E) B-Raf in melanoma interrupts immunoediting of leukocyte functions and melanoma extravasation. Cancer Res. 2007;67(12):5814–20.
55. Sharma A, Tran MA, Liang S, Sharma AK, Amin S, Smith CD, et al. Targeting mitogen-activated protein kinase/extracellular signal-regulated kinase kinase in the mutant (V600E) B-Raf signaling cascade effectively inhibits melanoma lung metastases. Cancer Res. 2006;66(16):8200–9.
56. Boyle GM, Pedley J, Martyn AC, Banducci KJ, Strutton GM, Brown DA, et al. Macrophage inhibitory cytokine-1 is overexpressed in malignant melanoma and is associated with tumorigenicity. J Invest Dermatol. 2009;129(2):383–91.
57. Sharma A, Trivedi NR, Zimmerman MA, Tuveson DA, Smith CD, Robertson GP. Mutant V599EB-Raf regulates growth and vascular development of malignant melanoma tumors. Cancer Res. 2005;65(6):2412–21.
58. Cheung M, Sharma A, Madhunapantula SV, Robertson GP. Akt3 and mutant V600E B-Raf cooperate to promote early melanoma development. Cancer Res. 2008;68(9):3429–39.
59. Wajapeyee N, Serra RW, Zhu X, Mahalingam M, Green MR. Oncogenic BRAF induces senescence and apoptosis through pathways mediated by the secreted protein IGFBP7. Cell. 2008;132(3):363–74.
60. Dhomen N, Reis-Filho JS, da Rocha Dias S, Hayward R, Savage K, Delmas V, et al. Oncogenic Braf induces melanocyte senescence and melanoma in mice. Cancer Cell. 2009;15(4):294–303.
61. Michaloglou C, Vredeveld LC, Soengas MS, Denoyelle C, Kuilman T, van der Horst CM, et al. BRAFE600-associated senescence-like cell cycle arrest of human naevi. Nature. 2005;436(7051):720–4.
62. Nguyen LP, Emley A, Wajapeyee N, Green MR, Mahalingam M. BRAF V600E mutation and the tumour suppressor IGFBP7 in atypical genital naevi. Br J Dermatol. 2009;162(3):677–80.
63. Zhao Y, Zhang Y, Yang Z, Li A, Dong J. Simultaneous knockdown of BRAF and expression of INK4A in melanoma cells leads to potent growth inhibition and apoptosis. Biochem Biophys Res Commun. 2008;370(3):509–13.
64. Dankort D, Curley DP, Cartlidge RA, Nelson B, Karnezis AN, Damsky Jr WE, et al. Braf(V600E) cooperates with Pten loss to induce metastatic melanoma. Nat Genet. 2009;41(5):544–52.
65. Patton EE, Widlund HR, Kutok JL, Kopani KR, Amatruda JF, Murphey RD, et al. BRAF mutations are sufficient to promote nevi formation and cooperate with p53 in the genesis of melanoma. Curr Biol. 2005;15(3):249–54.
66. Yu H, McDaid R, Lee J, Possik P, Li L, Kumar SM, et al. The role of BRAF mutation and p53 inactivation during transformation of a subpopulation of primary human melanocytes. Am J Pathol. 2009;174(6):2367–77.
67. Gallagher SJ, Thompson JF, Indsto J, Scurr LL, Lett M, Gao BF, et al. p16INK4a expression and absence of activated B-RAF are independent predictors of chemosensitivity in melanoma tumors. Neoplasia. 2008;10(11):1231–9.
68. Goel VK, Lazar AJ, Warneke CL, Redston MS, Haluska FG. Examination of mutations in BRAF, NRAS, and PTEN in primary cutaneous melanoma. J Invest Dermatol. 2006;126(1):154–60.
69. Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res. 2004;64(19):7099–109.
70. Eisen T, Ahmad T, Flaherty KT, Gore M, Kaye S, Marais R, et al. Sorafenib in advanced melanoma: a phase II randomised discontinuation trial analysis. Br J Cancer. 2006;95(5):581–6.
71. Takimoto CH, Awada A. Safety and anti-tumor activity of sorafenib (nexavar) in combination with other anti-cancer agents: a review of clinical trials. Cancer Chemother Pharmacol. 2008;61(4):535–48.
72. Karasarides M, Chiloeches A, Hayward R, Niculescu-Duvaz D, Scanlon I, Friedlos F, et al. B-RAF is a therapeutic target in melanoma. Oncogene. 2004;23(37):6292–8.

73. Madhunapantula SV, Robertson GP. Is B-Raf a good therapeutic target for melanoma and other malignancies? Cancer Res. 2008;68(1):5–8.
74. Tsai J, Lee JT, Wang W, Zhang J, Cho H, Mamo S, et al. Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity. Proc Natl Acad Sci USA. 2008;105(8):3041–6.
75. Bollag G, Hirth P, Tsai J, Zhang J, Ibrahim PN, Cho H, et al. Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. Nature. 2010;467(7315):596–9.
76. 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.
77. 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.
78. Hong S, Han SB. Overcoming metastatic melanoma with BRAF inhibitors. Arch Pharm Res. 2011;34(5):699–701.
79. Agarwala SS, di Pietro A, Flaherty KT, Garbe C, Grob JJ, Kashani-Sabet M, et al. Meeting report from the third global workshop on melanoma. Pigment Cell Melanoma Res. 2010;23(5):e1–7.
80. Nazarian R, Shi H, Wang Q, Kong X, Koya RC, Lee H, et al. Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. Nature. 2010;468(7326):973–7.
81. Solit DB, Rosen N. Resistance to BRAF inhibition in melanomas. N Engl J Med. 2011;364(8):772–4.
82. Johannessen CM, Boehm JS, Kim SY, Thomas SR, Wardwell L, Johnson LA, et al. COT drives resistance to RAF inhibition through MAP kinase pathway reactivation. Nature. 2010;468(7326):968–72. Dec 16 2010.
83. Kaplan FM, Shao Y, Mayberry MM, Aplin AE. Hyperactivation of MEK-ERK1/2 signaling and resistance to apoptosis induced by the oncogenic B-RAF inhibitor, PLX4720, in mutant N-RAS melanoma cells. Oncogene. 2011;30(3):366–71.
84. Brower V. BRAF inhibitors: research accelerates in wake of positive findings. J Natl Cancer Inst. 2010;102(4):214–5.
85. Solit DB, Garraway LA, Pratilas CA, Sawai A, Getz G, Basso A, et al. BRAF mutation predicts sensitivity to MEK inhibition. Nature. 2006;439(7074):358–62.
86. Davies BR, Logie A, McKay JS, Martin P, Steele S, Jenkins R, et al. AZD6244 (ARRY-142886), a potent inhibitor of mitogen-activated protein kinase/extracellular signal-regulated kinase kinase 1/2 kinases: mechanism of action in vivo, pharmacokinetic/pharmacodynamic relationship, and potential for combination in preclinical models. Mol Cancer Ther. 2007;6(8):2209–19.
87. Adjei AA, Cohen RB, Franklin W, Morris C, Wilson D, Molina JR, et al. Phase I pharmacokinetic and pharmacodynamic study of the oral, small-molecule mitogen-activated protein kinase kinase 1/2 inhibitor AZD6244 (ARRY-142886) in patients with advanced cancers. J Clin Oncol. 2008;26(13):2139–46.
88. Daouti S, Higgins B, Kolinsky K, Packman K, Wang H, Rizzo C, et al. Preclinical in vivo evaluation of efficacy, pharmacokinetics, and pharmacodynamics of a novel MEK1/2 kinase inhibitor RO5068760 in multiple tumor models. Mol Cancer Ther. 2010;9(1):134–44.
89. Mandic A, Viktorsson K, Heiden T, Hansson J, Shoshan MC. The MEK1 inhibitor PD98059 sensitizes C8161 melanoma cells to cisplatin-induced apoptosis. Melanoma Res. 2001;11(1):11–9.
90. Emery CM, Vijayendran KG, Zipser MC, Sawyer AM, Niu L, Kim JJ, et al. MEK1 mutations confer resistance to MEK and B-RAF inhibition. Proc Natl Acad Sci USA. 2009;106(48):20411–6.
91. Lopez-Bergami P, Huang C, Goydos JS, Yip D, Bar-Eli M, Herlyn M, et al. Rewired ERK-JNK signaling pathways in melanoma. Cancer Cell. 2007;11(5):447–60.
92. Mirmohammadsadegh A, Mota R, Gustrau A, Hassan M, Nambiar S, Marini A, et al. ERK1/2 is highly phosphorylated in melanoma metastases and protects melanoma cells from cisplatin-mediated apoptosis. J Invest Dermatol. 2007;127(9):2207–15.

93. Pardo OE, Arcaro A, Salerno G, Tetley TD, Valovka T, Gout I, et al. Novel cross talk between MEK and S6K2 in FGF-2 induced proliferation of SCLC cells. Oncogene. 2001;20(52):7658–67.
94. Zhuang L, Lee CS, Scolyer RA, McCarthy SW, Palmer AA, Zhang XD, et al. Activation of the extracellular signal regulated kinase (ERK) pathway in human melanoma. J Clin Pathol. 2005;58(11):1163–9.
95. Kohno MJP. Pharmacological inhibitors of the ERK signaling pathway: applications as anti-cancer drugs. Prog Cell Cycle Res. 2003;5(Chapter 22):219–24.
96. Boss DS, Beijnen JH, Schellens JH. Clinical experience with aurora kinase inhibitors: a review. Oncologist. 2009;14(8):780–93.
97. Carmena M, Ruchaud S, Earnshaw WC. Making the Auroras glow: regulation of Aurora A and B kinase function by interacting proteins. Curr Opin Cell Biol. 2009;21(6):796–805.
98. Libertini S, Abagnale A, Passaro C, Botta G, Portella G. Aurora A and B kinases—targets of novel anticancer drugs. Recent Pat Anticancer Drug Discov. 2010;5(3):219–41.
99. Kollareddy M, Dzubak P, Zheleva D, Hajduch M. Aurora kinases: structure, functions and their association with cancer. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2008;152(1):27–33.
100. Macarulla T, Ramos FJ, Tabernero J. Aurora kinase family: a new target for anticancer drug. Recent Pat Anticancer Drug Discov. 2008;3(2):114–22.
101. Vader G, Lens SM. The Aurora kinase family in cell division and cancer. Biochim Biophys Acta. 2008;1786(1):60–72.
102. Barr AR, Gergely F. Aurora-A: the maker and breaker of spindle poles. J Cell Sci. 2007;120(Pt 17):2987–96.
103. Cimini D. Detection and correction of merotelic kinetochore orientation by Aurora B and its partners. Cell Cycle. 2007;6(13):1558–64.
104. Yeung SC, Gully C, Lee MH. Aurora-B kinase inhibitors for cancer chemotherapy. Mini Rev Med Chem. 2008;8(14):1514–25.
105. Dar AA, Goff LW, Majid S, Berlin J, El-Rifai W. Aurora kinase inhibitors—rising stars in cancer therapeutics? Mol Cancer Ther. 2010;9(2):268–78.
106. Saeki T, Ouchi M, Ouchi T. Physiological and oncogenic Aurora-A pathway. Int J Biol Sci. 2009;5(7):758–62.
107. Ju H, Cho H, Kim YS, Kim WH, Ihm C, Noh SM, et al. Functional polymorphism 57Val>Ile of aurora kinase A associated with increased risk of gastric cancer progression. Cancer Lett. 2006;242(2):273–9.
108. Tchatchou S, Wirtenberger M, Hemminki K, Sutter C, Meindl A, Wappenschmidt B, et al. Aurora kinases A and B and familial breast cancer risk. Cancer Lett. 2007;247(2):266–72.
109. Ewart-Toland A, Dai Q, Gao YT, Nagase H, Dunlop MG, Farrington SM, et al. Aurora-A/STK15 T+91A is a general low penetrance cancer susceptibility gene: a meta-analysis of multiple cancer types. Carcinogenesis. 2005;26(8):1368–73.
110. Arbitrario JP, Belmont BJ, Evanchik MJ, Flanagan WM, Fucini RV, Hansen SK, et al. SNS-314, a pan-Aurora kinase inhibitor, shows potent anti-tumor activity and dosing flexibility in vivo. Cancer Chemother Pharmacol. 2010;65(4):707–17.
111. Katayama H, Sen S. Aurora kinase inhibitors as anticancer molecules. Biochim Biophys Acta. 2010;1799(10–12):829–39.
112. Lens SM, Voest EE, Medema RH. Shared and separate functions of polo-like kinases and aurora kinases in cancer. Nat Rev Cancer. 2010;10(12):825–41.
113. Pirker C, Lotsch D, Spiegl-Kreinecker S, Jantscher F, Sutterluty H, Micksche M, et al. Response of experimental malignant melanoma models to the pan-Aurora kinase inhibitor VE-465. Exp Dermatol. 2010;19(12):1040–7.
114. Dees EC, Infante JR, Cohen RB, O'Neil BH, Jones S, von Mehren M, et al. Phase 1 study of MLN8054, a selective inhibitor of Aurora A kinase in patients with advanced solid tumors. Cancer Chemother Pharmacol. 2010;67(4):945–54.
115. Macarulla T, Cervantes A, Elez E, Rodriguez-Braun E, Baselga J, Rosello S, et al. Phase I study of the selective Aurora A kinase inhibitor MLN8054 in patients with advanced solid tumors: safety, pharmacokinetics, and pharmacodynamics. Mol Cancer Ther. 2010;9(10):2844–52.

116. Carol H, Boehm I, Reynolds CP, Kang MH, Maris JM, Morton CL, et al. Efficacy and pharmacokinetic/pharmacodynamic evaluation of the Aurora kinase A inhibitor MLN8237 against preclinical models of pediatric cancer. Cancer Chemother Pharmacol. 2011;68(5):1291–304.
117. Yamashita T, Yoneta A, Hida T. Macrophage inhibitory cytokine-1: a new player in melanoma development. J Invest Dermatol. 2009;129(2):262–4.
118. Huh SJ, Chung CY, Sharma A, Robertson GP. Macrophage inhibitory cytokine-1 regulates melanoma vascular development. Am J Pathol. 2010;176(6):2948–57.
119. Singh RK, Varney ML. IL-8 expression in malignant melanoma: implications in growth and metastasis. Histol Histopathol. 2000;15(3):843–9.
120. Zhang H, Fu T, McGettigan S, Kumar S, Liu S, Speicher D, et al. IL8 And cathepsin B as melanoma serum biomarkers. Int J Mol Sci. 2011;12(3):1505–18.
121. Jovanovic M, Stefanoska I, Radojcic L, Vicovac L. Interleukin-8 (CXCL8) stimulates trophoblast cell migration and invasion by increasing levels of matrix metalloproteinase (MMP)2 and MMP9 and integrins alpha5 and beta1. Reproduction. 2010;139(4):789–98.
122. Araki K, Shimura T, Yajima T, Tsutsumi S, Suzuki H, Okada K, et al. Phosphoglucose isomerase/autocrine motility factor promotes melanoma cell migration through ERK activation dependent on autocrine production of interleukin-8. J Biol Chem. 2009;284(47): 32305–11.
123. Huh SJ, Liang S, Sharma A, Dong C, Robertson GP. Transiently entrapped circulating tumor cells interact with neutrophils to facilitate lung metastasis development. Cancer Res. 2010;70(14):6071–82.
124. Kim MY, Oskarsson T, Acharyya S, Nguyen DX, Zhang XH, Norton L, et al. Tumor self-seeding by circulating cancer cells. Cell. 2009;139(7):1315–26.
125. Crawford S, Belajic D, Wei J, Riley JP, Dunford PJ, Bembenek S, et al. A novel B-RAF inhibitor blocks interleukin-8 (IL-8) synthesis in human melanoma xenografts, revealing IL-8 as a potential pharmacodynamic biomarker. Mol Cancer Ther. 2008;7(3):492–9.
126. Ciuffreda L, Del Bufalo D, Desideri M, Di Sanza C, Stoppacciaro A, Ricciardi MR, et al. Growth-inhibitory and antiangiogenic activity of the MEK inhibitor PD0325901 in malignant melanoma with or without BRAF mutations. Neoplasia. 2009;11(8):720–31.
127. Oka M, Sakaguchi M, Okada T, Nagai H, Ozaki M, Yoshioka T, et al. Signal transducer and activator of transcription 3 upregulates interleukin-8 expression at the level of transcription in human melanoma cells. Exp Dermatol. 2010;19(8):e50–5.
128. Villares GJ, Zigler M, Wang H, Melnikova VO, Wu H, Friedman R, et al. Targeting melanoma growth and metastasis with systemic delivery of liposome-incorporated protease-activated receptor-1 small interfering RNA. Cancer Res. 2008;68(21):9078–86.
129. Lev DC, Ruiz M, Mills L, McGary EC, Price JE, Bar-Eli M. Dacarbazine causes transcriptional up-regulation of interleukin 8 and vascular endothelial growth factor in melanoma cells: a possible escape mechanism from chemotherapy. Mol Cancer Ther. 2003;2(8):753–63.
130. Lev DC, Onn A, Melinkova VO, Miller C, Stone V, Ruiz M, et al. Exposure of melanoma cells to dacarbazine results in enhanced tumor growth and metastasis in vivo. J Clin Oncol. 2004;22(11):2092–100.
131. Chung CY, Madhunapantula SV, Desai D, Amin S, Robertson GP. Melanoma prevention using topical PBISe. Cancer Prev Res (Phila Pa). 2011;4(6):935–48.
132. Huang S, Mills L, Mian B, Tellez C, McCarty M, Yang XD, et al. Fully humanized neutralizing antibodies to interleukin-8 (ABX-IL8) inhibit angiogenesis, tumor growth, and metastasis of human melanoma. Am J Pathol. 2002;161(1):125–34.
133. Zigler M, Villares GJ, Lev DC, Melnikova VO, Bar-Eli M. Tumor immunotherapy in melanoma: strategies for overcoming mechanisms of resistance and escape. Am J Clin Dermatol. 2008;9(5):307–11.
134. Murrow LM, Garimella SV, Jones TL, Caplen NJ, Lipkowitz S. Identification of WEE1 as a potential molecular target in cancer cells by RNAi screening of the human tyrosine kinome. Breast Cancer Res Treat. 2010;122(2):347–57.
135. Walter SA, Guadagno SN, Ferrell Jr JE. Activation of Wee1 by p42 MAPK in vitro and in cycling xenopus egg extracts. Mol Biol Cell. 2000;11(3):887–96.

136. De Witt Hamer PC, Mir SE, Noske D, Van Noorden CJ, Wurdinger T. WEE1 kinase targeting combined with DNA-damaging cancer therapy catalyzes mitotic catastrophe. Clin Cancer Res. 2011;17:4200–7.
137. Hirai H, Arai T, Okada M, Nishibata T, Kobayashi M, Sakai N, et al. MK-1775, a small molecule Wee1 inhibitor, enhances anti-tumor efficacy of various DNA-damaging agents, including 5-fluorouracil. Cancer Biol Ther. 2010;9(7):514–22.
138. Smaill JB, Lee HH, Palmer BD, Thompson AM, Squire CJ, Baker EN, et al. Synthesis and structure-activityrelationshipsofsoluble8-substituted4-(2-chlorophenyl)-9-hydroxypyrrolo[3,4-c]carbazole-1,3(2H,6H)-diones as inhibitors of the Wee1 and Chk1 checkpoint kinases. Bioorg Med Chem Lett. 2008;18(3):929–33.
139. Wang Y, Li J, Booher RN, Kraker A, Lawrence T, Leopold WR, et al. Radiosensitization of p53 mutant cells by PD0166285, a novel G(2) checkpoint abrogator. Cancer Res. 2001;61(22):8211–7.
140. Hashimoto O, Shinkawa M, Torimura T, Nakamura T, Selvendiran K, Sakamoto M, et al. Cell cycle regulation by the Wee1 inhibitor PD0166285, pyrido [2,3-d] pyrimidine, in the B16 mouse melanoma cell line. BMC Cancer. 2006;6:292.
141. Park K, Lee MY, Kim KS, Hahn SK. Target specific tumor treatment by VEGF siRNA complexed with reducible polyethyleneimine-hyaluronic acid conjugate. Biomaterials. 2010;31(19):5258–65.
142. Molhoek KR, Griesemann H, Shu J, Gershenwald JE, Brautigan DL, Slingluff Jr CL. Human melanoma cytolysis by combined inhibition of mammalian target of rapamycin and vascular endothelial growth factor/vascular endothelial growth factor receptor-2. Cancer Res. 2008;68(11):4392–7.
143. Yang L, Chen G, Mohanty S, Scott G, Fazal F, Rahman A, et al. GPR56 regulates VEGF production and angiogenesis during melanoma progression. Cancer Res. 2011;71(16):5558–68.
144. Akula SM, Ford PW, Whitman AG, Hamden KE, Bryan BA, Cook PP, et al. B-Raf-dependent expression of vascular endothelial growth factor-A in Kaposi sarcoma-associated herpesvirus-infected human B cells. Blood. 2005;105(11):4516–22.
145. Niers TM, Richel DJ, Meijers JC, Schlingemann RO. Vascular endothelial growth factor in the circulation in cancer patients may not be a relevant biomarker. PLoS One. 2011;6(5):e19873.
146. Corrie PG, Basu B, Zaki KA. Targeting angiogenesis in melanoma: prospects for the future. Ther Adv Med Oncol. 2010;2(6):367–80.
147. Kelly RJ, Rixe O. Axitinib (AG-013736). Recent Results Cancer Res. 2010;184:33–44.
148. Choueiri TK. Axitinib, a novel anti-angiogenic drug with promising activity in various solid tumors. Curr Opin Investig Drugs. 2008;9(6):658–71.
149. Holash J, Davis S, Papadopoulos N, Croll SD, Ho L, Russell M, et al. VEGF-Trap: a VEGF blocker with potent antitumor effects. Proc Natl Acad Sci USA. 2002;99(17):11393–8.
150. Hu R, Aplin AE. AlphaB-crystallin is mutant B-RAF regulated and contributes to cyclin D1 turnover in melanocytic cells. Pigment Cell Melanoma Res. 2010;23:201–9.
151. Oba J, Nakahara T, Abe T, Hagihara A, Moroi Y, Furue M. Expression of c-Kit, p-ERK and cyclin D1 in malignant melanoma: an immunohistochemical study and analysis of prognostic value. J Dermatol Sci. 2011;62(2):116–23.
152. Lebe B, Pabuccuoglu U, Ozer E. The significance of Ki-67 proliferative index and cyclin D1 expression of dysplastic nevi in the biologic spectrum of melanocytic lesions. Appl Immunohistochem Mol Morphol. 2007;15(2):160–4.
153. Joshi KS, Rathos MJ, Mahajan P, Wagh V, Shenoy S, Bhatia D, et al. P276-00, a novel cyclin-dependent inhibitor induces G1-G2 arrest, shows antitumor activity on cisplatin-resistant cells and significant in vivo efficacy in tumor models. Mol Cancer Ther. 2007;6(3):926–34.
154. Mandara M, Nortilli R, Sava T, Cetto GL. Chemotherapy for metastatic melanoma. Expert Rev Anticancer Ther. 2006;6(1):121–30.
155. Huncharek M, Caubet JF, 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.

156. Smalley KS, Haass NK, Brafford PA, Lioni M, Flaherty KT, Herlyn M. Multiple signaling pathways must be targeted to overcome drug resistance in cell lines derived from melanoma metastases. Mol Cancer Ther. 2006;5(5):1136–44.
157. Tran MA, Gowda R, Sharma A, Park EJ, Adair J, Kester M, et al. Targeting V600EB-Raf and Akt3 using nanoliposomal-small interfering RNA inhibits cutaneous melanocytic lesion development. Cancer Res. 2008;68(18):7638–49.
158. Meier FE, Lasithiotakis K, Schittek B, Sinnberg T, Garbe C. Temozolomide combined with the PI3K inhibitor LY294002 or the mTOR inhibitor rapamycin inhibits melanoma cell growth, survival and invasion. J Clin Oncol. [Abstract]. 2007;25(18S (June 20 Suppl) Abstract 8559).
159. Li J, Xu M, Yang Z, Li A, Dong J. Simultaneous inhibition of MEK and CDK4 leads to potent apoptosis in human melanoma cells. Cancer Invest. 2009;28(4):350–6.
160. Meier F, Busch S, Lasithiotakis K, Kulms D, Garbe C, Maczey E, et al. Combined targeting of MAPK and AKT signalling pathways is a promising strategy for melanoma treatment. Br J Dermatol. 2007;156(6):1204–13.
161. Villanueva J, Herlyn M. Melanoma and the tumor microenvironment. Curr Oncol Rep. 2008;10(5):439–46.
162. Flach EH, Rebecca VW, Herlyn M, Smalley KS, Anderson AR. Fibroblasts contribute to melanoma tumor growth and drug resistance. Mol Pharm. 2011;8(6):2039–49.
163. Dong C, Slattery MJ, Liang S, Peng HH. Melanoma cell extravasation under flow conditions is modulated by leukocytes and endogenously produced interleukin 8. Mol Cell Biomech. 2005;2(3):145–59.
164. Liang S, Hoskins M, Dong C. Tumor cell extravasation mediated by leukocyte adhesion is shear rate dependent on IL-8 signaling. Mol Cell Biomech. 2010;7(2):77–91.
165. Ozdemir T, Zhang P, Fu C, Dong C. Fibrin serves as a divalent ligand that regulates neutrophil-mediated melanoma cells adhesion to endothelium under shear conditions. Am J Physiol Cell Physiol. 2012;302(8):C1189–201.
166. Zhang P, Ozdemir T, Chung CY, Robertson GP, Dong C. Sequential binding of alphaVbeta3 and ICAM-1 determines fibrin-mediated melanoma capture and stable adhesion to CD11b/CD18 on neutrophils. J Immunol. 2011;186(1):242–54.
167. Dong C, Robertson GP. Immunoediting of leukocyte functions within the tumor microenvironment promotes cancer metastasis development. Biorheology. 2009;46(4):265–79.
168. Liang S, Seto EY, Remais JV, Zhong B, Yang C, Hubbard A, et al. Environmental effects on parasitic disease transmission exemplified by schistosomiasis in western China. Proc Natl Acad Sci USA. 2007;104(17):7110–5.
169. van Kempen LC, Rijntjes J, Mamor-Cornelissen I, Vincent-Naulleau S, Gerritsen MJ, Ruiter DJ, et al. Type I collagen expression contributes to angiogenesis and the development of deeply invasive cutaneous melanoma. Int J Cancer. 2008;122(5):1019–29.
170. Huh SJ, Chen YL, Friedman SL, Liao J, Huang HJ, Cavenee WK, et al. KLF6 gene and early melanoma development in a collagen I-rich extracellular environment. J Natl Cancer Inst. 2010;102(15):1131–47.
171. Denkert C, Kobel M, Berger S, Siegert A, Leclere A, Trefzer U, et al. Expression of cyclooxygenase 2 in human malignant melanoma. Cancer Res. 2001;61(1):303–8.
172. Goulet AC, Einsphar JG, Alberts DS, Beas A, Burk C, Bhattacharyya A, et al. Analysis of cyclooxygenase 2 (COX-2) expression during malignant melanoma progression. Cancer Biol Ther. 2003;2(6):713–8.
173. Becker MR, Siegelin MD, Rompel R, Enk AH, Gaiser T. COX-2 expression in malignant melanoma: a novel prognostic marker? Melanoma Res. 2009;19(1):8–16.
174. Flockhart RJ, Armstrong JL, Reynolds NJ, Lovat PE. NFAT signalling is a novel target of oncogenic BRAF in metastatic melanoma. Br J Cancer. 2009;101(8):1448–55.
175. Kuzbicki L, Sarnecka A, Chwirot BW. Expression of cyclooxygenase-2 in benign naevi and during human cutaneous melanoma progression. Melanoma Res. 2006;16(1):29–36.
176. Valcarcel M, Mendoza L, Hernandez JJ, Carrascal T, Salado C, Crende O, et al. Vascular endothelial growth factor regulates melanoma cell adhesion and growth in the bone marrow microenvironment via tumor cyclooxygenase-2. J Transl Med. 2011;9:142.

177. Wilson KS. Cyclooxygenase-2 inhibition and regression of metastatic melanoma. Melanoma Res. 2006;16(5):465.
178. Goldberg GM, Rubenstone AI, Saphir O. A study of malignant lymphomas and leukemias. III. Stem cell, blast cell, and monocytic leukemias (with reference to their lymphogenous or myelogenous origin). Cancer. 1961;14:21–9.
179. Soltanian S, Matin MM. Cancer stem cells and cancer therapy. Tumour Biol. 2011;32(3): 425–40.
180. Schatton T, Frank MH. Cancer stem cells and human malignant melanoma. Pigment Cell Melanoma Res. 2008;21(1):39–55.
181. Menaa F, Houben R, Eyrich M, Broecker EB, Becker JC, Wischhusen J. Stem cells, melanoma and cancer stem cells: the good, the bad and the evil? G Ital Dermatol Venereol. 2009;144(3):287–96.
182. Mimeault M, Hauke R, Mehta PP, Batra SK. Recent advances in cancer stem/progenitor cell research: therapeutic implications for overcoming resistance to the most aggressive cancers. J Cell Mol Med. 2007;11(5):981–1011.
183. Zabierowski SE, Fukunaga-Kalabis M, Li L, Herlyn M. Dermis-derived stem cells: a source of epidermal melanocytes and melanoma? Pigment Cell Melanoma Res. 2011;24(3):422–9.
184. Fang D, Nguyen TK, Leishear K, Finko R, Kulp AN, Hotz S, et al. A tumorigenic subpopulation with stem cell properties in melanomas. Cancer Res. 2005;65(20):9328–37.
185. Kumar SM, Zhang G, Bastian BC, Arcasoy MO, Karande P, Pushparajan A, et al. Erythropoietin receptor contributes to melanoma cell survival in vivo. Oncogene. 2012;31(13):1649–60.
186. Tran MA, Watts RJ, Robertson GP. Use of liposomes as drug delivery vehicles for treatment of melanoma. Pigment Cell Melanoma Res. 2009;22(4):388–99.
187. Basu S, Harfouche R, Soni S, Chimote G, Mashelkar RA, Sengupta S. Nanoparticle-mediated targeting of MAPK signaling predisposes tumor to chemotherapy. Proc Natl Acad Sci USA. 2009;106(19):7957–61.
188. Fink W, Zimpfer-Rechner C, Thoelke A, Figl R, Kaatz M, Ugurel S, et al. Clinical phase II study of pegylated liposomal doxorubicin as second-line treatment in disseminated melanoma. Onkologie. 2004;27(6):540–4.
189. Hughes GA. Nanostructure-mediated drug delivery. Nanomedicine. 2005;1(1):22–30.
190. Tran MA, Smith CD, Kester M, Robertson GP. Combining nanoliposomal ceramide with sorafenib synergistically inhibits melanoma and breast cancer cell survival to decrease tumor development. Clin Cancer Res. 2008;14(11):3571–81.
191. Hwang TL, Lee WR, Hua SC, Fang JY. Cisplatin encapsulated in phosphatidylethanolamine liposomes enhances the in vitro cytotoxicity and in vivo intratumor drug accumulation against melanomas. J Dermatol Sci. 2007;46(1):11–20.
192. Bawa R. Nanoparticle-based therapeutics in humans: a survey. Nanotechnol Law Bus. 2008;5(2):135–55.
193. Yih TC, Al-Fandi M. Engineered nanoparticles as precise drug delivery systems. J Cell Biochem. 2006;97(6):1184–90.
194. Fulmer T. Curiously strong lipidoids. Science-Business exchange, “Cover story: targets and mechanisms” featured in nature. Nature. 2010;3(2):1–2.
195. Davis ME, Zuckerman JE, Choi CH, Seligson D, Tolcher A, Alabi CA, et al. Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. Nature. 2010;464(7291):1067–70.

Current Approaches to Epigenetic Therapy for the Treatment of Mantle Cell Lymphoma

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Abstract Epigenetics is the study of heritable changes in phenotype or gene expression caused by mechanisms other than changes in the underlying DNA sequence. Such changes can include DNA methylation or histone modifications which both serve to silence gene expression. This review describes a new development in pharmacology, epigenetic therapy, which attempts to correct these epigenetic changes for the treatment of mantle cell lymphoma (MCL) and other B cell malignancies for which no consensus on standard therapy exists. One class of drugs utilized are the histone deacetylase inhibitors, (HDACi) which result in the accumulation of acetylated histones. Hyperacetylation of histones and nonhistone proteins are postulated to mediate the anticancer effects of these drugs. Another class of epigenetic agents are hypomethylating agents, that can cause both DNA and histone hypomethylation. Epigenetic drugs may be useful in the treatment of cancer where hypermethylation of tumor suppressor genes is known to lead to silencing of these genes. The purine analog cladribine has been shown to have hypomethylating properties and has activity as a single agent or in combination with other therapies for mantle cell lymphoma. Epigenetic therapy with the DNA hypomethylating agent 5-aza-2-deoxycytidine can also cause restoration of cell surface expression of the CD20 protein and increase rituximab sensitivity in vitro. Combinations of epigenetic agents may act synergistically to further potentiate the efficacy of monoclonal antibodies like rituximab and ofatumumab and improve the treatment outcome in MCL.

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W.S. El-Deiry (ed.), *Impact of Genetic Targets on Cancer Therapy*, Advances in Experimental Medicine and Biology 779, DOI 10.1007/978-1-4614-6176-0_11,

Keywords Mantle cell lymphoma • Epigenetics • HDAC inhibitor • Cladribine • DNA methylation • Hypomethylating agent • Rituximab • 5-aza-2-deoxycytidine • CD20 • Oncogenesis • Locus control region • Cyclin D1 • B-cell lymphoma • CHOP • R-CHOP • R-Hyper-CVAD • Bortezomib • Lenalidomide • mTOR inhibitors • Bendamustine • Immunotherapy • MDS • AML • Vorinostat • Panobinostat • Romidepsin • Thrombo-embolic event • PET/CT

Background

Epigenetic regulation refers to transcriptional variability of the genome without any changes in the DNA sequence [1, 2]. Chromatin consists of the entire DNA of a chromosome and its associated proteins, called histones, comprised of four core parts: H2A, H2B, H3 and H4. Together, these proteins assemble and associate with the DNA to form the nucleosome [3]. Chromatin remodeling refers to structural modifications of the nucleosome and includes enzymatically mediated post-translational modification of the histone proteins [4].

Epigenetic changes lead to silencing of transcription by directly interfering with the binding of transcription factors (TF) as well as recruitment of other enzymes, such as histone deacetylases (HDACs) and proteins to the promoter sites that repress transcription [5, 6]. In general, the cancer genome is characterized by global hypomethylation with site-specific hypermethylation in promoter regions of tumor suppressor genes and, thus, epigenetics plays a pivotal role in oncogenesis [4, 7] (Fig. 1).

Mechanism of Gene Silencing by Histone Modification and DNA Methylation

Epigenetic changes in chromatin structure involving DNA methylation and/or histone modifications have been implicated in deregulated oncogene expression in lymphoid malignancies but remain poorly characterized [8, 9]. Epigenetic modifications of eukaryotic gene promoters such as DNA hypomethylation and histone H3 and H4 acetylation correlate with activation of transcription. Activation of genes may occur by local promoter hypomethylation and hyperacetylation and epigenetic changes involving remote regulatory elements such as enhancers or locus control regions (LCRs). Treatment of silenced genes with DNA methyltransferase inhibitors and/or inhibitors of histone deacetylase has been shown to transcriptionally activate silent genes [10].

More recently it has been demonstrated that epigenetic mechanisms are in part responsible for resistance to rituximab through the a decrease in CD20 expression on the cell surface of B cell lymphoma cells after treatment with rituximab. Epigenetic therapy with 5-aza-2-deoxycytidine can cause restoration of both cell surface expression of the CD20 protein and rituximab sensitivity in vitro [11].

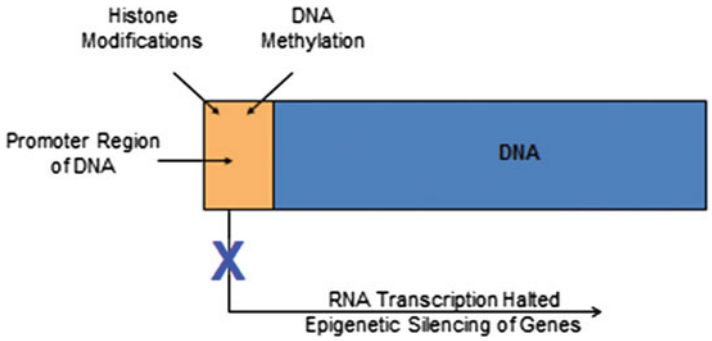

Fig. 1 Schematic diagram of the mechanisms of epigenetic silencing of genes

Mantle cell lymphoma (MCL) is now recognized as a distinct genetic, clinical and pathological subtype of B-cell NHL (6–8%) usually characterized by the expression of cyclin D1 and the presence of the t (11; 14) in >90% of patients. The pathologic diagnosis is confirmed by the characteristic absence of CD23 and CD10 expression, with expression of CD20, CD5 and/or cyclin D1. The ratio of males to females affected is about 4:1. At diagnosis, typical patients are in their 60s. Clinical features may include a history of fever, night sweat and loss of weight or appetite or the classic "B symptoms" described in NHL.

Impact of the Tumor Microenvironment and Cancer Stem Cells

The contribution of the tumor microenvironment in MCL has been explored. Dave et al. [12] provided evidence that the tumor microenvironment is important in prognostication of one type of NHL, namely follicular lymphoma. The genes in the immune-response signatures established by Dave et al. can be used as markers to identify subpopulations of immune cells that may promote or antagonize the proliferation or survival of the malignant clone.

Yang et al. [13] showed that the intensity of CD19 expression on NHL lymphoma cells is visibly decreased in about a third of all B-cell lymphomas with low-level CD19. This decrease was significantly more common in follicular lymphomas than other lymphoma types, regardless of grade, occurring in 79% of studied cases. Punitive stem cells in MCL have been identified with a CD19 negative phenotype.

Emerging therapy targeting the microenvironment and/or cancer stem cells include: It appears that this network of stromal cells creates a microenvironment that initially attracts cancer cells, allowing their early survival, and subsequently contributes to tumor growth. Recently, a newly described family of proteins has taken center stage in the dialogue between stromal and neoplastic B lymphocytes and plasma cells: the hedgehog proteins. Certainly anti-angiogenesis therapeutics fit within this as a sub-section.

Treatment of Mantle Cell Lymphoma

All patients with MCL require do not require immediate treatment [14]. There is currently no standard therapy for newly diagnosed or relapsed disease [15]. The Ki-67 index, a measure of tumor proliferative activity, may provide some insight into which patients will be more likely to progress.

A retrospective review of 249 patients with advanced-stage MCL enrolled in prospective clinical trials of CHOP or R-CHOP found significantly longer median overall survival times in patients with tumors demonstrating a Ki-67 index less than 10% when compared with those with higher Ki-67 indices [16].

For some B-cell Non-Hodgkin's lymphoma (NHL), an accepted treatment regimen is rituximab–cyclophosphamide doxorubicin vincristine prednisone (R-CHOP). Howard et al. from Dana-Farber evaluated the efficacy of rituximab and CHOP induction therapy in patients with newly diagnosed MCL. They found a 96% response rate with 48% of patients achieving a complete response (CR) and the remaining 48% achieving a partial response (PR). However, 28 of the 40 patients who received treatment relapsed or developed progressive disease with a median progression free survival (PFS) of 16.6 months [17].

Lenz et al. reported a similar 92% overall response rate (ORR) with a somewhat better PFS of 28 months using R-CHOP [18]. The MD Anderson group evaluated the response, failure-free survival (FFS), overall survival rates and toxicity of rituximab plus an intense chemotherapy regimen in patients with previously untreated aggressive MCL. This was a prospective phase II trial of rituximab plus fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone alternating every 21 days with rituximab plus high-dose methotrexate-cytarabine for a total of 6–8 cycles [19].

Of 97 assessable patients, 97% responded, and 87% achieved a CR or unconfirmed CR. With a median follow-up time of 40 months, the 3-year FFS and overall survival rates were 64% and 82%, respectively, without a plateau in the curves. For the subgroup of patients < or = 65 years of age, the 3-year FFS rate was 73%. Five patients died from acute toxicity. Four patients developed treatment-related myelodysplasia/acute myelogenous leukemia, and three patients died while in remission from MCL. A total of eight treatment-related deaths (8%) occurred [19].

Rituximab plus hyper-CVAD alternating with rituximab plus high-dose methotrexate and cytarabine is effective in untreated aggressive MCL. Toxicity was significant but expected. Because of the shorter FFS concurrent with significant toxicity in patients more than 65 years of age, this regimen is not recommended as standard therapy for this age subgroup [19]. However, in a multicenter cooperative group study, 58% of patients achieved a complete response (CR) or complete response uncertain (CRu) [20].

Although there is no clear consensus about the best treatment for individual MCL patients, a common chemotherapeutic treatment approach that has been used is R-CHOP or R-Hyper CVAD. Since both R - CHOP and R- hyper CVAD regimens appear to have issues with efficacy and toxicity; alternative regimens have been investigated in both the frontline and relapsed settings.

Progress has been made in the diagnosis of MCL, in the understanding of the relevant molecular pathways and pathogenesis of the disease, and in the development of new treatment options, including chemoimmunotherapy and targeted agents.

The panel of novel drugs approved or being tested offers new opportunities in the management of MCL from combination in the frontline setting (e.g. bortezomib-R-chemo) to post-induction strategies such as consolidation (e.g. radioimmunotherapy, bortezomib) or maintenance therapy (e.g. rituximab, lenalidomide). Other novel options including cytotoxics (bendamustine, cladribine), new biological/small molecules such as proteasome inhibitors, mTOR inhibitors, CDK inhibitors (flavopiridol); IMiDs (thalidomide, lenalidomide); HDAC inhibitors, Bcl-2 inhibitors and second or third generation monoclonal antibodies or immunotoxins are all been or being studied for the treatment of MCL [21].

Our main focus is on the use of a combination of epigenetic and immunotherapy for the treatment of MCL.

Hypomethylating Agents

Azacitadine and decitabine are hypomethylating agents indicated for the treatment of patients with myelodysplastic syndrome subtypes and chronic myelomonocytic leukemia (CMMoL). This agent has a well-established activity in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) [22, 23].

Blum et al. targeted aberrant DNA hypermethylation in chronic lymphocytic leukaemia (CLL) and non- Hodgkin lymphoma (NHL) with decitabine, thinking that this may reverse epigenetic silencing in B-cell malignancies. Two phase I trials were conducted to determine the minimum effective pharmacological dose of decitabine in patients with relapsed/refractory CLL (n = 16) and NHL (n = 4). Out of 20 patients eight had stable disease. In 17 patients, there were no significant changes in genome-wide methylation or in target gene re-expression [24].

The efficacy of this class of hypomethylating agents in treating MCL has yet to be tested. We could not find any completed or ongoing trials for its use in MCL.

Cladribine

Cladribine or 2 chlorodeoxyadenosine (2-CdA) is a purine analog with activity in human B cell malignancies. Cladribine is FDA approved for the treatment of hairy cell leukemia (HCL), an indolent B cell malignancy. Cladribine has also been shown to have hypomethylating properties in addition to its cytotoxic properties [25].

Rummel et al. demonstrated that the combination of reduced-dose 2-CdA and mitoxantrone is a highly active regimen in the treatment of low-grade lymphomas, and in particular of MCL. Fifty six of the 62 patients responded to 2-CdA resulting in an overall response rate of 90% with a CR rate of 44% and a median duration of remission of 25 months [26].

Robak et al. used rituximab combined with cladribine (RC) or with cladribine and cyclophosphamide (RCC) in heavily pretreated patients with indolent lymphoproliferative disorders and mantle cell lymphoma. Thirty-three patients with B-CLL, 12 patients with LG-NHL and 9 patients with MCL entered the study. Thirty-three patients (61%) had recurrent disease after prior therapy, and 21 patients (39%) had refractory disease. Thirty-one patients were treated on the RC regimen, and 23 patients were treated on the RCC regimen. Six patients (11%) achieved a complete response, and 33 patients (60%) achieved a partial response. The median failure-free survival (FFS) of responders was 10.5 months [27].

Another study by Inwards et al. to access the role 2-CdA alone or in combination with rituximab was investigated in 80 patients with MCL. In previously untreated patients, 2-CdA monotherapy resulted in an ORR of 81% with a 42% CR rate. The median PFS for these patients was 13.6 months, and 81% of patients remained alive at 2 years. The addition of rituximab to therapy resulted in an ORR of 66% for previously untreated patients with a 52% CR rate. The PFS was 12.1 months and 78% of patients remained alive at 2 years [28].

Spurgeon et al. performed a retrospective chart review of 31 patients with MCL (median age, 67) treated with rituximab and cladribine (RC) combination to access the role of RC as an initial treatment of MCL. The majority of responding patients also received rituximab maintenance. The overall response rate was 87%, with 61% of patients achieving a CR/CRu. The estimated median follow-up was 32.5 months, median PFS was 37.5 months, and median OS was 85.2 months. One of 19 (5.3%) subjects in CR/CRu relapsed (median follow-up of 23 months). CR/CRu was associated with improved survival ($p<0.0001$), while a high mantle cell international prognostic index (MIPI) was associated with worse survival ($p=0.05$). There was one toxic death (neutropenic pseudomonal sepsis) related to treatment. RC is an effective therapy for previously untreated MCL, and these results validate the use of RC for the initial treatment of MCL [29].

These results are in keeping with our observations that cladribine's activity is significantly greater in previously untreated patients. Cladribine inhibits histone methylation in vitro and DNA methylation in vivo and in vitro, [22], perhaps explaining cladribine's activity in lymphoma, in contrast to azacitidine and decitadine which only inhibit DNA methylation. DNA methylation and histone methylation have been shown to synergize in gene silencing. In addition, a subset of silenced genes in cancer cells are silenced independently of DNA methylation and can only be activated by inhibiting histone methylation [30, 31].

Vorinostat

Suberoylanilide hydroxamic acid (vorinostat) is a histone deacetylase (HDAC) inhibitor, derived from hydroxamic acid, which inhibits class I and II HDAC enzymes that regulate transcriptional and post-transcriptional processes by removing acetyl groups from histone proteins. Vorinostat has received regulatory approval by the FDA for the treatment of relapsed refractory cutaneous T-cell lymphoma

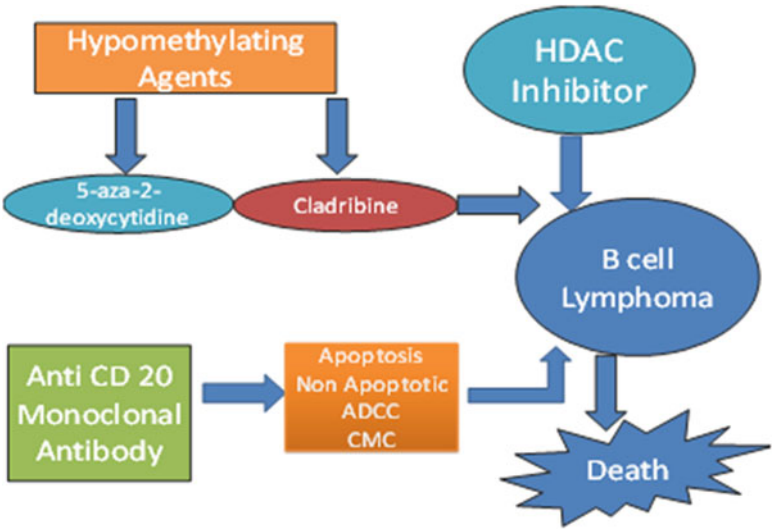

Fig. 2 Proposed syngergistic actions of epigenetic agents in combination with antiCD20 monoclonal antibodies

(CTCL) with progressive, persistent, or recurrent disease on or following two systemic therapies [32]. It is been currently evaluated in various phase I/II trials for MCL and other non-Hodgkin's lymphoma (NHL).

Kirschbaum et al. [33] performed a phase II (NCT00253630) study using oral vorinostat, a histone and protein deacetylase inhibitor, to examine its efficacy and tolerability in patients with relapsed/refractory indolent lymphoma. Patients with relapsed/refractory follicular lymphoma (FL), marginal zone lymphoma (MZL), or mantle cell lymphoma (MCL), with 4 prior therapies were eligible. Oral vorinostat was administered at a dose of 200 mg twice daily on days 1 through 14 of a 21-day cycle until progression or unacceptable toxicity. ORR was 29% five CRs and five PRs. For 17 patients with FL, ORR was 47% (four CRs, four PRs). There were two of nine responders with MZL (one CR, one PR), and no formal responders among the nine patients with MCL, although one patient maintained stable disease for 26 months. The drug was well-tolerated over long periods of treatment, with the most common grade 3 adverse events being thrombocytopenia, anemia, leucopenia, and fatigue.

Other HDAC inhibitors like panobinostat [34, 35], and romidepsin [36] are being tested alone or in combinations with other agents (Fig. 2).

Vorinostat, Cladribine and Rituximab

Spurgeon et al. has presented a phase I/II (NCT00764517) study at ASCO 2011 using combination of vorinostat (SAHA), cladribine (2-CdA), and rituximab (SCR) in previously untreated mantle cell lymphoma to explore epigenetic modifications.

Patients were given Vorinostat on days 1–14 (200 mg, 300 mg, or 400 mg) in combination with 2-CdA 5 mg/m2 IV on days 1–5, and rituximab 375 mg/m2 IV on days 3, 10, 17, and 24 with cycle 1 and then on Day 3 with subsequent cycles. Cycles are repeated every 28 days for up to 6 cycles. After vorinostat MTD determination, phase II will have enrolled an additional 40 patients including patients with newly diagnosed mantle cell lymphoma or newly diagnosed CLL [37].

Patients are monitored for response rate, overall survival (OS) and progression-free survival (PFS). Forty five patients (Phase I/II) have been enrolled and 39 patients have been treated. The ORR in evaluable relapsed patients is 32% (7/22). Of the 21 previously untreated advanced MCL patients (blastic MCL 4)all have completed ≥2 cycles) and ORR is 100% (21/21) with 69%(11/15) complete responses (CR) as assessed by metabolic imaging (PET/CT) and fluorescent in situ hybridization/flow cytometry after Cycle 2. Toxicities have primarily included reversible myelosuppression and thrombocytopenia. One grade 4 thrombo-embolic event (probably related) and one grade 5 pulmonary hemorrhage in a patient with relapsed pulmonary lymphoma were seen. The study demonstrates SCR activity in lymphoid malignancies and appears particularly promising in previously untreated mantle cell lymphoma. Myelosuppression is the primary toxicity of this regimen especially in previously treated patients. In vivo epigenetic changes have been appreciated and correlative studies are ongoing. This trial (NCT00764517) continues to accrue patients at this time with almost 50 patients enrolled at this time [37]. An update of this trial to be presented at ASH 2012 demonstrated ORR 100% and CR rate 70–80% in untreated MCL patients.

Toxicities were primarily hematologic and reversible [37]. Studies have confirmed epigenetic changes after cladribine treatment in MCL patients, including DNA methylation changes in vivo, inhibition of histone methylation in vitro and changes in gene expression in vitro and in vivo.

Conclusion

Since both R-CHOP and R- hyper CVAD regimens appear to have issues with efficacy and toxicity, alternative regimens have been investigated in both the front-line and relapsed settings for mantle cell lymphoma and other indolent non Hodgkin's lymphoma, especially for those above 65 years old. Combined epigenetic and immunotherapy with cladribine, vorinostat, and rituximab appears to be a highly active and nontoxic therapy for newly diagnosed mantle cell lymphoma.

Our hypothesis is two different epigenetic agents like hypomethylating agents and histone deacetylase (HDAC) inhibitors act synergistically to reverse resistance to and increase efficacy of monoclonal anti CD20 antibodies in B cell lymphomas.

A phase I/II trial (NCT00764517) is evaluating response rate and progression free survival based on the above hypothesis. Large randomized studies are needed to compare patients with chemotherapy naïve or relapsed mantle cell lymphoma or other indolent non Hodgkin lymphomas with other treatments such as bendamustine/rituximab.

References

1. Bird A. DNA methylation patterns and epigenetic memory. Genes Dev. 2002;16(1):6–21.
2. Bhalla KN. Epigenetic and chromatin modifiers as targeted therapy of hematologic malignancies. J Clin Oncol. 2005;23(17):3971–93.
3. Esteller M. Molecular origins of cancer: epigenetics in cancer. New Engl J Med. 2008;358(11): 1148–59.
4. Jenuwein T, Allis CD. Translating the histone code. Science. 2001;293(5532):1074–80.
5. Rountree MR, Bachman KE, Baylin SB. DNMT1 binds HDAC2 and a new co-repressor, DMAP1, form a complex at replication foci. Nat Genet. 2000;25(3):269–77.
6. Robertson KD, Ait-Si-Ali S, Yokochi T, Wade PA, Jones PL, Wolffe AP. DNMT1 forms a complex with Rb, E2F1 and HDAC1 and represses transcription from E2F-responsive promoters. Nat Genet. 2000;25(3):338–42.
7. Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. Carcinogenesis. 2010;31(1):27–36.
8. Lichtenstein M, Keini G, Cedar H, Bergman Y. B-cell-specific demethylation—a novel role for the intronic kappa-chain enhancer sequence. Cell. 1994;76(5):913–23.
9. Madisen L, Krumm A, Hebbes TR, Groudine M. The immunoglobulin heavy chain locus control region increases histone acetylation along linked c-myc genes. Mol Cell Biol. 1998;18(11):6281–92.
10. Herman JG, Baylin SB. Mechanisms of disease: gene silencing in cancer in association with promoter hypermethylation. New Engl J Med. 2003;349(21):2042–54.
11. Hiraga J, Tomita A, Sugimoto T, Shimada K, Ito M, Nakamura S, et al. Down-regulation of CD20 expression in B-cell lymphoma cells after treatment with rituximab-containing combination chemotherapies: its prevalence and clinical significance. Blood. 2009;113(20):4885–93.
12. Dave SS, Wright G, Tan B, Rosenwald A, Gascoyne RD, Chan WC, et al. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. New Engl J Med. 2004;351(21):2159–69.
13. Wei Y, Agrawal N, Patel J, Edinger A, Osei E, Thut D, et al. Diminished expression of CD19 in B-cell lymphomas. Cytom Part B-Clin Cy. 2005;63B(1):28–35.
14. Martin P, Chadburn A, Christos P, Weil K, Furman RR, Ruan J, et al. Outcome of deferred initial therapy in mantle-cell lymphoma. J Clin Oncol. 2009;27(8):1209–13.
15. Yoong Y, Kurtin PJ, Allmer C, Geyer S, Habermann TM, Nagorney M, et al. Efficacy of splenectomy for patients with mantle cell non-Hodgkin's lymphoma. Leukemia Lymphoma. 2001; 42(6):1235–41.
16. Determann O, Hoster E, Ott G, Bernd HW, Loddenkemper C, Hansmann ML, et al. Ki-67 predicts outcome in advanced-stage mantle cell lymphoma patients treated with anti-CD20 immunochemotherapy: results from randomized trials of the European MCL Network and the German Low Grade lymphoma Study Group. Blood. 2008;111(4):2385–7.
17. Howard OM, Gribben JG, Neuberg DS, Grossbard M, Poor C, Janicek MJ, et al. Rituximab and CHOP induction therapy for newly diagnosed mantle-cell lymphoma: molecular complete responses are not predictive of progression-free survival. J Clin Oncol. 2002;20(5):1288–94.
18. Lenz G, Dreyling M, Hoster E, Wormann B, Duhrsen U, Metzner B, et al. Immunochemotherapy with rituximab and cyclophosphamide, doxorubicin, vincristine, and prednisone significantly improves response and time to treatment failure, but not long-term outcome in patients with previously untreated mantle cell lymphoma: results of a prospective randomized trial of the German Low Grade Lymphoma Study Group (GLSG). J Clin Oncol. 2005;23(9):1984–92.
19. Romaguera JE, Fayad L, Rodriguez MA, Broglio KR, Hagemeister FB, Pro B, et al. High rate of durable remissions after treatment of newly diagnosed aggressive mantle-cell lymphoma with rituximab plus hyper-CVAD alternating with rituximab plus high-dose methotrexate and cytarabine. J Clin Oncol. 2005;23(28):7013–23.
20. Epner EM, Unger J, Miller T, Rimza L, Spier C, Leblanc M, et al. A multi center trial of hyperCVAD plus rituxan in patients with newly diagnosed mantle cell lymphoma. Blood. 2007;110(11):121a.
21. Goy A, Kahl B. Mantle cell lymphoma: the promise of new treatment options. Crit Rev Oncol Hemat. 2011;80(1):69–86.

22. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Finelli C, Giagounidis A, et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. Lancet Oncol. 2009;10(3):223–32.
23. Paulson K, Kumar R, Ahsanuddin A, Seftel MD. Azacytidine as a novel agent in the treatment of acute lymphoblastic leukemia. Leukemia Lymphoma. 2011;52(1):134–6.
24. Blum KA, Liu ZF, Lucas DM, Chen P, Xie ZL, Baiocchi R, et al. Phase I trial of low dose decitabine targeting DNA hypermethylation in patients with chronic lymphocytic leukaemia and Non-Hodgkin lymphoma: dose-limiting myelosuppression without evidence of DNA hypomethylation. Brit J Haematol. 2010;150(2):189–95.
25. Spurgeon S, Yu M, Phillips JD, Epner EM. Cladribine: not just another purine analogue? Expert Opin Inv Drug. 2009;18(8):1169–81.
26. Rummel MJ, Chow KU, Karakas T, Jaager E, Mezger J, Von Grunhagen U, et al. Reduced-dose cladribine (2-CdA) plus mitoxantrone is effective in the treatment of mantle-cell and low-grade Non-Hodgkin's lymphoma. Eur J Cancer. 2002;38(13):1739–46.
27. Robak T, Smolewski P, Cebula B, Szmigielska-Kaplon A, Chojnowski K, Blonski JZ. Rituximab combined with cladribine or with cladribine and cyclophosphamide in heavily pre-treated patients with indolent lymphoproliferative disorders and mantle cell lymphoma. Cancer. 2006;107(7):1542–50.
28. Inwards DJ, Fishkin PAS, Hillman DW, Brown DW, Ansell SM, Kurtin PJ, et al. Long-term results of the treatment of patients with mantle cell lymphoma with cladribine (2-CDA) alone (95-80-53) or 2-CDA and rituximab (N0189) in the North Central Cancer Treatment Group. Cancer. 2008;113(1):108–16.
29. Spurgeon SE, Pindyck T, Okada C, Chen YY, Chen ZQ, Mater E, et al. Cladribine plus rituximab is an effective therapy for newly diagnosed mantle cell lymphoma. Leukemia Lymphoma. 2011;52(8):1488–94.
30. Cedar H, Bergman Y. Linking DNA methylation and histone modification: patterns and paradigms. Nat Rev Genet. 2009;10(5):295–304.
31. Kondo Y, Shen L, Cheng AS, Ahmed S, Boumber Y, Charo C, et al. Gene silencing in cancer by histone H3 lysine 27 trimethylation independent of promoter DNA methylation. Nat Genet. 2008;40(6):741–50.
32. Duvic M, Talpur R, Ni X, Zhang CL, Hazarika P, Kelly C, et al. Phase 2 trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma, (CTCL). Blood. 2007;109(1):31–9.
33. Kirschbaum M, Frankel P, Popplewell L, Zain J, Delioukina M, Pullarkat V, et al. Phase II study of vorinostat for treatment of relapsed or refractory indolent non-Hodgkin's lymphoma and mantle cell lymphoma. J Clin Oncol. 2011;29(9):1198–203.
34. A phase II study of the Histone Deacetylase (HDAC) inhibitor LBH589 (Panobinostat) in patients with relapsed or refractory Non-Hodgkin lymphoma (NCT01261247). www.clinicaltrials.gov.
35. A phase I/II study of the Histone Deacetylase (HDAC) Inhibitor LBH589 (Panobinostat) in combination with mTOR Inhibitor RAD001 (Everolimus) in patients with relapsed multiple myeloma or lymphoma (NCT00918333). www.clinicaltrials.gov.
36. A Phase II study of depsipeptide, a histone deacetylase inhibitor, in relapsed or refractory mantle cell or diffuse large cell non-Hodgkin's lymphoma (NCT00077194). www.clinicaltrials.gov.
37. Spurgeon S, Chen AI, Okada C, Parekh S, Leshchenko VV, Palmbach G, et al. A Phase I/II Study of vorinostat (SAHA), cladribine (2-CdA), and rituximab shows significant activity in previously untreated mantle cell lymphoma. Blood. 2011;118(21):203.

Impact of Genetic Targets on Primary Brain Tumor Therapy: What's Ready for Prime Time?

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Abstract Primary brain tumors constitute a substantial public health problem with 66,290 cases diagnosed in the US in 2012, and 13,700 deaths recorded. With discovery of genetic factors associated with specific brain tumor subtypes, the goal of therapy is changing from treating a class of tumors to developing individualized therapies catering to the molecular composition of the actual tumor. For oligodendrogliomas, the loss of 1p/19q due to an unbalanced translocation improves both survival and the response to therapy, and is thus both a prognostic and a predictive marker. Several additional genetic alterations such as EGFR amplification, MGMT methylation, PDGFR activation, and 9p and 10q loss, have improved our understanding of the characteristics of these tumors and may help guide therapy in the future. For astrocytic tumors, MGMT is associated with a better prognosis and an improved response to temozolomide, and for all glial tumors, mutations in the IDH1 gene are possibly the most potent of good prognostic markers. Three of these markers – 1p/19q deletions, MGMT methylation status, and mutations in the IDH1 gene – are so potent that a new brain tumor subtype, the "triple negative" glioma (1p/19q intact, MGMT unmethylated, IDH1 non-mutated) has entered common parlance. Newer markers, such as CD 133, require additional investigation to determine their prognostic and predictive utility. In medulloblastomas, markers of WNT activation, MYCC/MCYN amplification, and TrkC expression levels are reliable prognostic indicators, but do not yet drive specific treatment selection. Many other

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W.S. El-Deiry (ed.), *Impact of Genetic Targets on Cancer Therapy*, Advances in Experimental Medicine and Biology 779, DOI 10.1007/978-1-4614-6176-0_12,

proposed markers, such as 17q gain, TP53 mutations, and hMOF protein expression show promise, but are not yet ready for prime time. In this chapter, we focus on the markers that have shown convincing prognostic, predictive, and diagnostic value, and discuss potential markers that are being currently being intensively investigated. We also discuss serum profiling of tumors in an effort to discover additional potential markers.

Keywords Glioma • Glioblastoma • Oligodendroglioma • Allelic loss • Tumor suppressor genes • TP53 • Irradiation • PCV therapy • Temozolomide • MGMT • EGFR • CDKN2A • PDGF • 9p loss • 10q loss • Autocrine • PI3K • Akt • Astrocytoma • CD133 • Nestin • IDH • Medulloblastoma • Wnt • MycN • DKK2 • WIF1 • CCDC46 • Beta-catenin • 17q • FISH • hMOF • TrkC • ELISA • Meningioma • S100B • NPY • YKL-40 • MMP9 • Osteopontin • APRIL • TNF inhibitors

Introduction

Approximately 64,530 new primary brain and other central nervous system tumors will be diagnosed in the United States in 2011 (24,070 malignant and 40,470 non-malignant tumors)an overall rate of 19.4 per 100,000 person-years (7.3 malignant and 12.1 non-malignant tumors) [1, 2], resulting in 13,140 deaths in 2010. Primary brain tumors arise from neuro-epithelial tissue and are classified histologically based on their respective cells of origin. With increasing knowledge of the genomics of the different primary brain tumor types, customization of cancer therapy based on tumor-specific molecular profiles is becoming increasingly common. In this review, we will highlight the major types of primary CNS tumors and the bedside relevance of their associated genetic alterations to tumor diagnosis and therapy.

Oligodendrogliomas

Introduction

Oligodendrogliomas are glial tumors composed of cells that histologically resemble mature oligodendrocytes. Oligodendrogliomas constitute about 5% of all intracranial tumors, arise predominantly in the cerebral hemispheres [3, 4], and are most commonly diagnosed in fourth and fifth decades of life [5]. The frequency of anaplastic oligodendrogliomas varies between 3.5 and 50% in the literature [6, 7]. Mean survival after diagnosis is 10 years for low grade and 5 years for high grade oligodendrogliomas [6, 8]. In 1988, Cairncross and Macdonald first reported the unexpected chemosensitivity of “malignant” oligodendrogliomas to PCV (procarbazine, lomustine, and vincristine) chemotherapy, sparking an interest in the neuro-oncology community to further investigate these tumors [9–11].

Loss of 1p/19q

Several studies in the early 1990s demonstrated allelic loss of 1p (the short arm of chromosome 1) and 19q (long arm of chromosome 19) in up to 83% of low grade oligodendrogliomas and up to 63% of anaplastic oligodendrogliomas [12–14]. This deletion comprises the entire chromosome arms of 1p and 19q which suggests that it is due to an unbalanced translocation. The high sequence homology of the centromeric regions of chromosome 1 and 19 supports translocation at the centromere as a mechanism for this deletion [15–18].

A clear association exists between co-deletion of 1p and 19q and a classic histological appearance with a perinuclear halo around cells and a chicken-wire vascular pattern [19–23], explaining the observation of McDonald and colleagues in 131 patients with anaplastic oligodendrogliomas, that only patients with a "classic" histology showed a survival benefit when treated with chemotherapy, compared to those with an "atypical" histology [24–26].

The improved prognosis and better response to treatment of these tumors is unlikely to be explained by a single gene. The mechanism of gene alteration due to 1p/19q co-deletion is largely unknown. Tumor suppressor genes on 1p and/or 19q may be important in the development of oligodendroglioma [13, 15]. Several responsible genes have been proposed on 1p, including CDKN22C, TP73, RAD54 [12] and on 19q, including PEG3, p190RhoGap, ZNF34 [26–28], but none have been clearly identified as key players in tumor development.

While loss of 1p/19q is common in oligodendrogliomas, loss of 1p alone or 19q alone are seen frequently in astrocytomas. These deletions are typically small, and do not convey the same prognostic implication as the complete 1p/19q deletions seen in oligodendrogliomas [14]. Similarly, partial 1p deletions occur in glioblastoma, not associated with 19q loss, are not associated with a better prognosis seen in loss of 1p/19q [21].

In mixed oligoastrocytomas with predominant oligodendroglial morphology, the percentage of tumors showing 1p and 19q loss falls to 39%, emphasizing that the presence of even a small astrocytic component significantly reduces the chance of finding 1p and 19q loss [29].

Several studies have shown that oligodendrogliomas with certain molecular subtypes can have a predilection for particular locations in the brain. 1p/19q co-deleted tumors present more commonly in the frontal, occipital or parietal lobes while oligodendrogliomas without deletions present more commonly in the temporal lobes, insula, and diencephalon [30]. Oligoastrocytomas with TP53 mutations were seen mostly in the temporal lobe when compared to oligoastrocytomas without this mutation [31].

In magnetic resonance imaging, oligodendrogliomas with 1p/19q loss have indistinct borders with mixed signal intensity on T1 and T2-weighted imaging [32]. Non-co-deleted tumors are more likely to demonstrate a ring-enhancing pattern on T1-weighted, gadolinium-enhanced images.

Two large randomized controlled phase 3 trials were started in the early 1990s and were designed to test the benefit of PCV therapy for patients with anaplastic oligodendrogliomas and anaplastic oligoastrocytomas (OAs) [7, 8]. Both trials compared radiation therapy to a combination of radiation therapy along with PCV

therapy. In the Radiation Therapy Oncology Group trial 9402, 289 patients with anaplastic oligodendrogliomas or anaplastic mixed oligoastrocytomas were randomized to treatment with radiation therapy alone or with up to four cycles of "intensified" PCV prior to the start of cranial irradiation [7]. In the European Organization for Research and Treatment of Cancer Trial 26951, 368 patients with the same tumor types were randomized to treatment with radiation therapy alone or to radiation followed by up to six cycles of standard PCV [8]. While these trials were accruing patients, it became clear that allelic loss of 1p and 19q was the most common genetic alteration in these patients with oligodendrogliomas [9]. Although the addition of PCV therapy to cranial irradiation did not demonstrate a survival benefit over RT alone, 1p/19q status clearly conveyed a survival advantage when patients were stratified by co-deletion status. Tumor specimens were available in 70% of the patients in the two trials for analysis. In RTOG 9402 trial, the median survival was not reached for co-deleted patients at the end of the trial (>7 years) while for those without co-deletion, median survival was 2.8 years. In the EORTC 26951 trial, once again the median survival was not reached for patients with co-deletion (>8 years) while median survival for those without co-deletion was 2 years. Similar improvements in progression free survival were observed in both RCTs in those with co-deletions compared to those patients without co-deletions. When the benefit of "early" PCV (administered at the time of initial diagnosis either prior to radiation—the RTOG trial—or following radiation—the EORTC trial), a prolongation of the median progression free survival by just under 1 year was seen in both trials (2.6 vs. 1.7 years, $p=0.004$ in the RTOG study; 1.9 vs. 1.1 years, $p=0.0018$ in the EORTC study). In the RTOG trial (but not in the EORTC trial), further evaluation showed that this improvement in progression free survival in PCV-treated patients was restricted to those with 1p and 19q co-deletions.

Because the large majority of patients in both the RTOG and the EORTC studies received chemotherapy at the time of tumor recurrence, and because recurrent anaplastic oligodendrogliomas and anaplastic mixed gliomas are frequently responsive to chemotherapy, the ability to show a survival advantage for the radiation plus chemotherapy groups in either of these two studies was likely subverted. Consequently, a more accurate description of the result of these two studies is that early (at the time of diagnosis) PCV chemotherapy does not seem to convey a survival advantage over late (at the time of tumor recurrence) PCV therapy, even though early chemotherapy is associated with a longer (approximately 1 year) progression-free survival. Whether early or late chemotherapy represents the best treatment strategy will remain unsettled until additional prospective trials examining the relative impacts of chemotherapy and tumor progression on quality of life are conducted. Considering the substantial toxicity of PCV, the addition of PCV to cranial irradiation in non-co-deleted patients may not be advantageous. Up to 50% of patients with anaplastic oligodendrogliomas respond, at the time of tumor recurrence, to temozolomide, with median progression free survivals of up to 12 months [16–18, 33, 34]. This response is more durable and more frequent in co-deleted patients but is seen in non-co-deleted tumors as well. In light of temozolomide's more benign toxicity profile, temozolomide may be a more appropriate first line and

recurrence therapy compared to PCV, including in patients without codeletions. However, only limited recommendations can be made currently since only indirect comparisons have been performed between PCV and TMZ [19]. Early treatment with cranial irradiation carries a risk of delayed neurotoxicity when survival is prolonged, such as in patients with co-deleted tumors, leading many to advocate neoadjuvant chemotherapy, delaying cranial irradiation until tumor recurrence [20]. These trials demonstrated that regardless of the type of treatment there is an overall improved response in patients with codeleted tumors whether radiation alone, chemotherapy alone, or a combination of treatments is used. Similarly, patients with recurrent codeleted oligodendrogliomas enjoy a survival advantage over non-co-deleted patients [29].

MGMT

Several groups have demonstrated that 1p/19q loss is correlated with MGMT promoter methylation, which results in lower levels of MGMT protein expression. In 2005, Mollemann et al. evaluated 52 oligodendrogliomas, both new and recurrent, and showed that hypermethylation of the MGMT promoter was seen in 88%, with both the frequency and degree of methylation correlating significantly with 1p/19q deletion status ($p < 0.01$) [35–37]. In contrast to patients with glioblastomas, MGMT hypermethylation did not show a significant correlation with response to treatment or survival [38, 39]. Therefore, the clinical importance of this genetic finding is uncertain.

EGFR, CDKN2A, PDGF, 9p and 10q Loss

While "typical" oligodendroglioma histology is associated with 1p/19q co-deletion, histologically "atypical" oligodendrogliomas exhibit molecular signatures of astrocytic tumors, including TP53 mutations, EGFR amplification, 10q loss, and PTEN mutations [29, 40, 41].

Subanalysis of data from EORTC 26951 showed that tumors with EGFR amplification on 7p and/or 10q loss more frequently exhibited a mixed oligoastrocytic phenotype. EGFR amplification was also inversely related to the presence of 1p/19q codeletion [42–45]. This provides genetic evidence for at least two distinct molecular pathways for the development of oligodendrogliomas from different precursor cells. This hypothesis is supported by the marked difference in outcome and prognosis, with a superior outcome in the presence of 1p and 19q loss, and with poor survival in anaplastic oligodendrogliomas with the loss of 10q and/or amplification of EGFR [40, 46].

Also when compared with low grade oligodendrogliomas, anaplastic oligodendrogliomas have additional chromosomal deletions, especially loss of heterozygosity for 9p, and/or deletion of CDKN2A (p16) [29]. The tumor suppressor CDKN2A is located on 9p21, encoding p16 (INK4A) and p14 (ARF). These genetic changes

occur in 33–50% of anaplastic oligodendrogliomas and are associated with more aggressive tumor progression and poorer prognosis [47].

Another commonly seen alteration is the activation of the receptor tyrosine kinase, PDGFR, with elevated coexpression of PDGF ligand and receptor, seen in over 90% of oligodendrogliomas [48]. This leads to an autocrine stimulation loop that can drive cellular proliferation. In tissue culture when PDGF is forcefully overexpressed in glia, the resulting high grade gliomas have histologic features resembling anaplastic oligodendrogliomas. Imatinib mesylate, a potent inhibitor of PDGFR, has been administered in several clinical trials to patients with recurrent malignant gliomas, but only a limited number of patients in those trials had oligodendrogliomas, so no conclusions could be drawn with regard to this agent's potential effectiveness in patients with recurrent oligodendrogliomas [49]. Further clinical trials will be necessary to determine a possible therapeutic effect of PDGFR inhibitors on oligodendrogliomas.

Phosphatidyl-inositol-3-kinase (PI3K-AKT) is a tyrosine kinase in a signal transduction pathway which is negatively regulated by PTEN. Although loss of PTEN is common in gliomas, this loss is also seen in up to 21% of oligodendrogliomas [50]. PTEN alterations are also associated with a more aggressive phenotype, with a median progression free survival of 31 months for those with 10q loss when compared to 118 months for those with intact 10q.

Summary

Oligodendrogliomas are a separate subset of gliomas with characteristic histologic appearance, genetic changes, and clinical behavior. The discovery of loss of 1p/19q as a prognostic factor for this tumor type should facilitate better stratification of therapy. Several additional genetic changes such as EGFR amplification, MGMT methylation, PDGFR activation, 9p and 10q loss have improved our understanding of the characteristics of these tumors and may help guide therapy in the future. While patients with 1p/19q loss have a prolonged survival, an improved response to radiation, and probably an improved response to chemotherapy as well, clinical trials to date do not provide definitive guidance with regard to the optimum type and timing of the various therapeutic modalities. Hopefully additional clinical trials will help elucidate these issues in the future.

Astrocytomas

Introduction

In 2007, the World Health Organization divided predominately astrocytic gliomas into four types [51–53]. Grades III (anaplastic astrocytoma) and IV (glioblastoma multiforme) tumors are more clinically aggressive than their lower grade counterparts

(grade I—pilocytic astrocytoma, and grade II—astrocytoma). The critical histological differences between grades include the presence of nuclear atypia, mitotic activity, neovasculaturity and necrosis. This system based on light microscopic appearance is helpful in predicting outcome in large groups of patients, but performs less well for individual patients, and does not distinguish different subgroups within broad histologic categories. Glioblastoma multiforme is defined histologically by the presence of microvascular proliferation and pseudopallisading around necrotic areas of tumor [54]. The modifier "multiforme" denotes a variegated lesion, and indeed these tumors are remarkably heterogeneous. The cellular patterns can include epithelial structures, multinucleated giant cells, granular cells, gemistocytes, lipidized cells, perivascular lymphocytes and oligodendroglial components [55].

EGFR, PTEN, and TP53

Glioblastomas are often further classified, based on clinical behavior, as "primary" or "de novo" and "secondary" [53]. Patients with primary GBMs (approximately 95% of cases) typically have a short clinical course preceding diagnosis (less than 3 months in 68%), are older, and more frequently demonstrate EGFR amplification, $P16^{INK4a}$ deletions, and PTEN mutations, and less frequently harbor TP53 mutations. Primary GBMs are more treatment-resistant, and patients have substantially shorter survivals (10–14 months). Patients with secondary glioblastomas typically have a protracted clinical course, with preceding diagnoses of WHO grade II and III tumors. Patients are younger, survive much longer than those with primary GBMs (5–7 years from the time of initial tumor diagnosis), and their tumors more frequently harbor TP53 mutations.

Data accumulated from The Cancer Genome Atlas (TCGA) Research Network allows the division of GBMs into four unique subtypes of tumor based on gene expression profiles [56, 57]: classical, mesenchymal, proneural, and neural. The most commonly seen genetic derangements in classical GBMs are amplification of the EGFR gene on chromosome 7, or loss of PTEN on chromosome 10. Mesenchymal GBMs are frequently associated with TP53 loss and the activation of the tumor necrosis factor (TNF) proteins. Recurrent tumors often shift their mRNA signatures to this subtype and as a consequence become more malignant [58]. Proneural GBMs tend to express genes commonly seen during neuronal development such as DCX, SOX, DLL3, TCF4 and ASCL1. Interestingly, proneural GBMs are associated with mutations similar to those seen in the evolution of secondary GBMs. One such alteration is the missense mutation in enzyme isocitrate dehydrogenase 1 (IDH1). This mutation is commonly seen in younger GBM patients who have a protracted clinical course [59]. Less information is know about mutations seen in neural GBMs. Multi-institutional projects like the TCGA will continue to shed light on the genomic patterns of GBM beyond the broad diagnostic categories that we currently employ.

In the last decade a new paradigm concerning the development of solid tumors has gathered momentum and support. Tumor initiating cells, which appear to have stem cell properties, are now thought to propel tumor growth and underlie resistance

to chemo-and radiation therapies [60–62]. Glioblastomas are composed of a heterogeneous population of motile cells, with the ability to recruit stromal, inflammatory and vascular tissue. A fraction of the cell population is believed to express stem cell characteristics or "stemness". The neurosphere assay allows isolation of cells from human glioblastomas with the ability to self renew and recapitulate the aggressive behavior and invasion pattern of the parent tissue [63, 64]. These cells also exhibit multipotency [65, 66]. It is thought that tumor-initiating cells possess neural stem cell (NSC) attributes, including key cell surface markers. These initiating cells eventually acquire a host of genetic alterations to unlock an end-stage level of high malignancy.

CD133

Cluster of differentiation 133 (CD 133 or Prominin-1), is a 120 kDa cell surface five span transmembrane glycoprotein located on cellular protrusions and is a valuable stem cell marker [66, 67]. Elevated expression of CD 133 in GBM may decrease patient survival and increase risk of recurrence [68]. In vivo studies have shown that the malignancy of CD 133 bearing cells is markedly greater than CD 133 negative cells. A few hundred CD 133 positive cells are able to form mature tumors in vivo within neural tissue, while several thousand ($>10^5$) CD 133 deficient cells are unable to recreate orthotopic tumors [69]. The functional significance of CD 133 expression is currently unknown, and CD 133 negative cells can give rise to CD 133 positive cells [70]. CD 133 may be expressed during cell stress given its association with mitochondrial function in low energy states [71].

Nestin

Nestin is a type VI intermediate filament (IF) protein. As a cytosolic structural element, its function is thought to regulate cell morphology and radial axonal growth. Although expressed heavily during embryogenesis, its presence dwindles into adulthood where its sole sanctuary appears to be the subventricular zone (SVZ) [72]. As neural stem cells lose their multipotency and differentiate, the expression of nestin decreases and expression of either glial or neuronal markers rise [73, 74]. Nestin has been found in a variety of brain tumors isolated from human samples and lends some proof to the presence of stem-like tumor initiating cells within human glioblastomas [75]. The migratory capacity of glioma cells may be related to re-expression of nestin, given its developmental role as a primordial cytoskeletal protein [76].

The origin of glioblastoma is hotly debated [77]. According to the stem cell theory, NSCs acquire an increasing burden of genetic alterations, and the resulting dysregulation leads to gliomatous change. Mature glioma cells may also acquire stem-like characteristics through de-differentiation [78]. In either case the presence of a sub-population of cells within the tumor with self-renewal capabilities can

increase the tumor's proliferative capacity. This can cause asymmetric division of cells with defective apoptotic mechanisms making these tumor-initiating cells a considerable treatment challenge.

MGMT

Methylation status of the promoter region of the O^6 methylguanine DNA methyltransferase (MGMT) gene has also been widely studied [79]. Clinicians have begun to use the characteristics of this gene to predict response to chemotherapy in many tumor types The MGMT gene is located on chromosome 10q26 and encodes a suicide DNA repair protein by the same name. The gene possesses a rich CpG island, which in normal tissue remains largely unmethylated and contains the promoter region. Hypermethylation results in silencing of this gene and thus reduction or absence MGMT protein expression. The homeostatic role of this protein is to catalyze the removal of alkyl adducts or methyl groups from the O 6 position of guanine and the O 4 position of thymine [80]. Temozolomide (TMZ) is a chemotherapy agent that introduces alkyl groups at multiple sites along the DNA chain, thus leading to apoptosis or cellular breakdown. TMZ unequivocally increases survival in patients with glioblastomas when used concurrently with cranial irradiation, and as an adjuvant treatment following radiation [81]. The MGMT gene product works to reverse the major DNA-associated damage caused by TMZ allowing the cell to escape cell death. When the MGMT promoter is silenced by hypermethylation the cell succumbs more readily to effects of TMZ. This unique property has been examined in glioblastoma, where MGMT promoter methylation is seen in up to 40% of cases [82]. A recent study of patients with newly diagnosed glioblastomas treated with TMZ showed an increase in median survival in patients with promoter methylation from 15.3 to 21.7 months, as compared to unmethylated tumors [83]. MGMT promoter methylation has also been shown to lengthen progression free survival in patients undergoing TMZ treatment [84]. The prognostic usefulness of this genetic aberration is currently being investigated for prognostication in grade II and III astrocytomas, and in oligodendrogliomas and mixed oligoastrocytomas as well [85–87].

There are several ways to test for MGMT promoter methylation in patient samples. The most common technique is PCR-based, and utilizes primers to amplify fragments of bisulfite-modified DNA sequences. Standardization and validation of this process is the next challenge to ensure that results remain clinically meaningful across clinical sites and labs [88].

IDH

As mentioned above, the Cancer Genome Atlas has uncovered mutations of isocitrate dehydrogenase (IDH) genes. IDH1 and IDH2 mutations occur in approximately

12% of glioblastomas, primarily in younger patients and in those harboring secondary glioblastomas. Further studies have implicated IDH1 mutations in the transition of low grade astrocytomas to higher grade tumors [89, 90]. The biological function of IDH in the human cell has not been extensively studied [91]. IDH1 facilitates the conversion of isocitrate to alpha-ketoglutarate and thus the regeneration of reduced glutathione to maintain an anti-oxidative state. The mutant form of IDH1 shifts alpha ketoglutarate to 2-OH glutarate and thus places the cell at risk for oxidative damage and genomic instability. It has also been implicated in the inhibition of hypoxia inducible factor 1 (HIF-1) degradation [92, 93]. Current evidence suggests that IDH1 mutations drive the progression of low grade gliomas toward glioblastomas. This may be an early mutational event in light of evidence that IDH mutations also appear during the development of oligodendrogliomas and oligoastrocytomas [94]. Further work is required to ascertain association of the IDH mutation to development of these tumors.

Traditionally, tumor DNA is used to test for the presence of an IDH mutation. Extracting a purified sample can be difficult and time-consuming, and constitutes a major barrier to routine clinical testing. Several laboratory techniques exist and new ones are being developed to isolate mutations either in fixed or unfixed tissues. Some centers prefer to identify IDH1 mutations via immunohistochemistry (IHC) and to avoid false negative results by following this test with direct DNA sequencing of any negative specimens, thus increasing both the sensitivity and the specificity of the test.

Summary

Advancements in identifying genetic factors of CNS tumors has taken clinicians past simple pathology which provides a snapshot of the characteristics of a tumor to a description of its bioactivity. While EGFR and PTEN have traditionally shown correlation to primary vs. secondary GBM, MGMT has shown to be associated with a better prognosis and a greater response to TMZ. CD 133 and IDH are newer factors which still need more studies to better determine their clinical impact on tumor prognosis. More research also needs to be done to exploit the factors being found that lead to a more aggressive nature of these tumors in the hopes of extension of life expectancy and eventually cure (Table 1).

Medulloblastomas

Introduction

Medulloblastomas are the most common form of malignant brain tumors in children [95]. Over the last two decades, advances in chemotherapy, radiotherapy, and surgical technique, as well as the increased availability of sophisticated imaging

Table 1 Oligodendroglioma and astrocytoma tumor-specific molecular profile

Marker	Alteration	Implication	Test
1p/19q[a]	Loss	Good prognosis	Microsatellite based-PCR/FISH
MGMT[a]	Methylation of promoter with decreased expression of MGMT	Good prognosis	Methylation specific-PCR
10q[a]	Loss	Poor prognosis	Microsatellite based-PCR/FISH
EGFR[a]	Amplification	Poor prognosis	IHC
PDGFR[a]	Increased activation (with combined increased expression of PDGF ligand)	Poor prognosis (with tumor progression)	FISH
CDKN2A	Deletion (may be with 9p loss)	Poor prognosis	MS-MLPA/FISH
9p	Loss	Poor prognosis (with tumor progression)	MS-MLPA/FISH
PTEN	Loss (with concurrent loss of chromosome 10)	Poor prognosis	FISH
TP53	Amplification (with p53 protein overexpression)	Poor prognosis	PCR(TP 53)/ IHC(p53)
CD 133	Elevated expression	Poor prognosis (increased rate of recurrence)	IHC
IDH1, IDH2	Mutations	Poor prognosis (progression from low to high grade glioma)	PCR/IHC

PCR polymerase chain reaction, *IHC* Immunohistochemistry, *FISH* fluorescence in situ hybridization, *MS-MLPA* methylation specific multiplex ligation-dependent probe amplification
[a] Indicates those markers currently regarded as the most reliable

modalities such as MRI, MRI spectroscopy, and MRI—based functional imaging techniques, have greatly improved long-term survival rates, with average 5-year overall survival reaching as high as 80% in some patient subgroups [96]. Unfortunately, the majority of pediatric brain tumor patients experience substantial neurologic, cognitive, and endocrinologic sequelae following therapy [97–99]. Given the greater susceptibility of young patients to the adverse effects of therapy [100], a major challenge facing pediatric neuro-oncologists is the ability to differentiate high and low-risk patients, reserve the most rigorous treatment strategies for those with the most aggressive tumors, and thereby minimize treatment-related sequelae in lower risk patients.

Traditionally patients have been divided into high and low-risk groups based on three criteria: age (<3 years), extent of surgical resection (>1.5 cm residual tumor) and metastatic stage at diagnosis, with young patients who have significant residual tumor and evidence of metastasis being at the highest risk. Tumor histology also contributes to the stratification process, with desmoplastic/nodular medulloblastomas and medulloblastomas with extensive nodularity conveying the best prognosis. Unfortunately, current procedures for risk stratification are inadequate [101] and significantly under-represent the true heterogeneity of medulloblastomas [102].

In an attempt to better account for medulloblastoma subtypes, and the unique biological milieu of each patient, molecular and genetic biomarkers are being intensively investigated. Several markers have already been identified that show more consistent correlation with patient outcome than our traditional stratification criteria, while the prognostic value of many other proposed markers hotly debated.

WNT, MYCC/MYCN, TrkC, and p53

Some of the more widely-accepted biomarkers are those associated with mutations in components of the Wnt/Wingless pathway. WNT-active mutations are found in approximately 20% of medulloblastoma [103–105], and consistently correlate with a favorable prognosis. Markers of WNT-active medulloblastoma include CTNNB1 mutations, nuclear β-catenin immunostaining, and chromosome 6 loss. Recently, Schwalbe et al. have also proposed identification of WNT-active tumors based on the presence of a 5-gene signature (CCDC46, DKK2, PYGL, TNC, and WIF1) [106]. Other markers that have begun to gain stable footing within the cannon of prognostic indicators are MYCC/MYCN amplification, TrkC expression, and p53 expression. A significant body of research has demonstrated that MYCC (c-myc) and MYCN (N-myc) oncogene amplifications correlate with poor prognosis [102, 107, 108]. Conversely, elevated expression of TrkC consistently indicates a more favorable outcome [107, 109, 110]. Expression of p53, long questioned for its prognostic significance, has emerged as a valuable marker within the subset of patients with evidence of metastatic disease. In this cohort, p53 expression is strongly correlated with poor overall survival [111].

17q

The prognostic value of many chromosomal abnormalities continues to be debated. Several studies have suggested that a gain of 17q (frequently coupled with a loss of 17p) is indicative of a poor prognosis [112–114], while other studies have failed to find such a correlation [115, 116]. Possible contributing factors to this inconsistency include the small patient numbers in many of these studies, as well as the potentially suboptimal approach used to identify chromosome 17 abnormalities. In an attempt to address these issues, Pfister et al. have conducted genomic analyses using array-based comparative genomic hybridization (aCGH). In a group of 80 medulloblastoma patients, gain of 17q was the most common genomic aberration (present in 48% of patients) and was indeed significantly correlated with both reduced progression free survival and reduced overall survival [102]. These results were then confirmed in an independent cohort of 260 medulloblastoma patients using interphase FISH. Other chromosomal abnormalities of potential prognostic value include a gain of 1q and gain of 8q, both of which have been proposed as predictors of poor prognosis [116, 117].

Table 2 Medulloblastoma tumor-specific molecular profile

Marker	Alteration	Implication	Test
CTNNB1[a]	Mutation	Good prognosis	aCGH/FISH
β-catenin[a]	Positive nuclear staining	Good prognosis	IHC
6q[a]	Loss	Good prognosis	aCGH/FISH
MYCC/MYCN[a]	Amplification	Poor prognosis	aCGH/FISH
TrkC[a]	Elevated expression	Good prognosis	IHC
p53	Positive staining	Poor prognosis (in the context of metastatic disease)	IHC
17q	Gain (with concurrent loss of 17p)	Poor prognosis	aCGH/FISH
TP53	Mutation	Good prognosis (in the context of CTNNB1 mutation)	aCGH/FISH
		Poor prognosis (in the absence of CTNNB1 mutation)	
hMOF	Downregulated expression	Poor prognosis	IHC

IHC Immunohistochemistry, *aCGH* array-based comparative genomic hybridization, *FISH* fluorescence in situ hybridization

[a] Indicates those markers currently regarded as the most reliable

TP53 and hMOF

Two recently proposed, clinically relevant prognostic markers in patients with medulloblastoma are TP53 mutations and alterations in hMOF protein expression. Recent studies examining the possible prognostic value of TP53 mutations have produced conflicting results. Some studies indicate that TP53 mutations portend rapid recurrence and reduced overall survival [118], while other studies find such mutations to be frequently associated with WNT-active status, and hence with a more favorable prognosis [119]. A possible explanation for this apparent inconsistency is the proposed interaction of TP53 and CTNNB1 mutations to facilitate the development of (good prognosis) WNT-active medulloblastomas, whereas TP53 mutations occurring in the absence of CTNNB1 (as is the case in SHH subgroup medulloblastoma) convey a poor prognosis [120]. Similarly, one study has found that downregulation of the histone acetyltransferase protein hMOF is correlated with reduced overall survival in both univariate and multivariate analyses [121], however these results have yet to be replicated.

Summary

The search for prognostically relevant biomarkers in patients with medulloblastoma is evolving rapidly. Markers of WNT activation, MYCC/MCYN amplification, and TrkC expression levels appear to be reliable prognostic indicators.However, while many of the other proposed markers, such as 17q gain, TP53 mutations, and hMOF protein expression show promise, they are, as yet, not ready for prime time (Table 2).

Serum Profiling of Central Nervous System Tumors

Many investigators have looked for potential diagnostic and prognostic serum biomarkers in the blood of patients with primary CNS tumors (Table 3). An extensive list of potentially useful biomarkers has been generated by single studies, but most of these putative biomarkers have not been corroborated in subsequent studies. The overwhelming majority of studies use Enzyme-Linked Immunosorbent Assays

Table 3 Serum markers for central nervous system tumors

Marker	Alteration	Implication	Test
Low Molecular Weight Caldesmon	Elevated expression	Presence of Glioma	ELISA with immunoblotting and immunoprecipitation to monitor specificity
YKL-40	Elevated serum levels	Presence and grade of Glioma	ELISA/Western Blot
MMP-9	Elevated serum levels	Presence of Glioma	ELISA
PBEF1/NAmRPTase/ Visfatin	Elevated serum levels	Grade of glioma	ELISA on serum samples
S100B, NPY, SCGN	Elevated expression	Future occurrence of glioma	Fluorescence bioassay
Osteopontin	Elevated expression	Presence of glioma	Western blot/ELISA
Methylated tumor-specific DNA	Presence of methylated promoter, glioma-specific DNA in plasma	Presence of glioma	Methylation-specific PCR for promoters P16/INK4a, MGMT, P73, RARbeta
α2-HS glycoprotein	Decreased levels	Grade of Glioma (Must consider impaired liver function, cardiovascular disease as alternative explanations)	Turbidimetry
GFAP	Elevated expression	Presence of glioma	ELISA
APRIL	Elevated expression	Unsure significance	ELISA
IGFBP-2	Increased serum levels	Presence and grade of glioma	ELISA
EGFR	Increased serum levels	Presence of glioma	Sandwich ELISA
Plasminogen Activator Inhibitor-1	Increased serum levels	Presence of glioma	ELISA
TNF Inhibitors	Elevated serum levels	Presence of glioma	ELISA

ELISA Enzyme-linked immunosorbent assay
Indicates those markers currently regarded as the most reliable

(ELISA) to evaluate levels of a protein of interest in the serum. Jung et al. demonstrated that supratentorial mass lesions expressed significantly elevated serum levels of glial fibrillary acidic protein (GFAP) compared to controls [121]. The finding has not been substantiated by other authors. Similarly, Lin et al. demonstrated that IGFBP-2 levels are elevated in GBM patients [122]. The sensitivity and specificity of the putative biomarkers listed in Table 3 are low, suggesting that a more sophisticated approach to interpreting multiple biomarkers and their relationships to one another will be necessary before a clinically significant biomarker can be discovered. To this end, several studies have explored machine-learning approaches to diagnostics [123, 124]. For example, Keller and colleagues developed a seroreactivity marker profile that has a specificity of 96.2% and sensitivity of 84.5% for the detection of meningiomas [124]. Ludwig and colleagues developed a cross-validated classifier for detecting glioma with sensitivity of 85.2% and specificity of 86.1% [123]. More prospective corroborative studies are needed before this technology can be applied to clinical practice, but these diagnostic panels deserve further testing in larger patient populations.

One particularly interesting diagnostic approach involves assessment of the methylation status of key promoters in tumor specimens, followed by serum monitoring for tumor-specific DNA [125]. This approach is promising particularly because the methylation status of key promoters of different tumor cell genes may be useful not only for diagnosis, but for early detection of tumor recurrence, for predicting response to specific therapies and for providing prognostic information.

Some groups have investigated serum markers to determine if patients can be identified before they develop primary CNS tumors. Gartner et al. published a prospective study of 191 atrial fibrillation patients who were tested for possible marker levels and then followed for 1 year. The authors identified two patients who subsequently developed GBMs [122]. Elevated levels of S100B, NPY, and SCGN were seen in these patients and the author suggests that these elevations may serve as prognostic indicators for development of GBM, but the study lacked the power to confirm this hypothesis. These findings definitely merit additional studies, since glioma detection a full year before the usual clinical manifestations would facilitate early intervention and possibly an l improved prognosis.

The potential for individual serum biomarkers or panels of markers to monitor response to treatment, detect recurrence, and distinguish between tumor recurrence and “pseudoprogression” has also received considerable attention. A study of astrocytic tumors and controls using turbidimetry showed that alpha2-HS glycoprotein may correlate inversely with tumor progression, although it is also decreased in sepsis, impaired liver function, and cardiovascular disease [126]. A study of a novel receptor tyrosine kinase inhibitor successfully used a variety of growth factors to track tumor progression and treatment response [127] (Table 3). Few studies have collected markers at multiple time points to address questions related to treatment response and tumor progression. Several single biomarker level elevations are correlated with survival and tumor grade in some studies, suggesting some prognostic value to those markers (Table 3).

Conclusion

Several sources of potentially informative serum biomarkers remain to be explored in the context of primary brain tumors. These untapped sources include miRNAs [128, 129] and direct assessment of the number of circulating tumor cells [127, 130]. With the rapid development of so many markers for tumors, studies are needed to evaluate the impact of each one. We are still at the beginning of this field which is bound to cause significant changes to the way treat and think about tumors of the brain.

Impact of the Tumor Microenvironment and Cancer Stem Cells

As primary brain tumors transition to higher grades they become more heterogeneous entities, collecting and utilizing a number of cell types and hence truly becoming "multiforme". The brain in its native state is a complex organ with myriad cell to cell interactions occurring over synaptic, endocrine, autocrine, paracrine and vascular cytoarchitectural frameworks. In oncologic states, evidence is mounting for hierarchical systems involving cancer stem cells [131] which appear distinct from neural stem cells. Other cells that appear to drive tumorogenesis include microglia, endothelial cells, peripheral immune cells and other sustentacular cells. To add further complexity to this environment, it is likely that specific "niches" exist that provide the required environment for brain tumor cells to grow, develop and migrate [132]. These regions may incite the ideal signaling molecules in settings such as hypoxia or provide the extra-cellular matrix for stem cell maintenance. Given the complex biology of primary brain tumors it is not surprising that they present a challenge to treat.

References

1. CBTRUS. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2004–2007. Source: Central Brain Tumor Registry of the United States, Hinsdale, IL, 2011. Website: www.cbtrus.org
2. Siegel R, Ward E, Brawley O, Jemal A. The impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA Cancer J Clin. 2011;61:212–36.
3. Paleologos NA, MacDonald DR, Vick NA. Neoadjuvant procarbazine, CCNU, and vincristine for anaplastic and aggressive oligodendroglioma. Neurology. 1999;53:1141–3.
4. Mork SJ, Halvorsen TB, Lindegaard KF, Eide GE. Oligodendrofglioma, histologic evaluation and prognosis. J Neuropathol Exp Neurol. 1986;45:65–78.
5. Shaw EG, Scheithauer BW, O'Fallon JR, Tazelaar HD, Davis DH. Oligodendrogliomas: the Mayo clinic experience. J Neurosurg. 1992;76:428–34.
6. Winger MJ, Macdonald DR, Cairncross JG. Supratentorial anaplastic gliomas in adults, the prognostic importance of extent of resection and prior low-grade glioma. J Neurosurg. 1989;71:487–93.
7. Cairncross JG, MacDonald DR. Successful chemotherapy for recurrent malignant oligodendroglioma. Ann Neurol. 1988;23:360–4.

8. Cairncross G, Berkey B, Shaw E, Jenkins R, Scheithauer B, Brachman D, Buckner J, Fink K, Souhami L, Laperierre N, Mehta M, Curran W. Phase III trial of chemotherapy plus radiotherapy compared with radiotherapy alone for pure and mixed anaplastic oligodendroglioma: Intergroup Radiation Therapy Oncology Group Trial 9402. J Clin Oncol. 2006;24:2707–14.
9. Van den Bent MJ, Carpentier AF, Brandes AA, Sanson M, Taphoorn MJ, Bernsen HJ, Frenay M, Tijssen CC, Grisold W, Sipos L, Haaxma-Reiche H, Kros JM, van Kouwenhoven MC, Vecht CJ, Allgeier A, Lacombe D, Gorlia T. Adjuvant procarbazine, lomustine, and vincristine improves progression- free survival but not overall survival in newly diagnosed anaplastic oligodendrogliomas and oligoastrocytomas: a randomized European organisation for research and treatment of cancer phase III trial. J Clin Oncol. 2006;24:2715–22.
10. Bigner SH, Matthews MR, Rasheed BK, Wiltshire RN, Friedman HS, Friedman AH, Stenzel TT, Dawes DM, McLendon RE, Bigner DD. Molecular genetic aspects of oligodendrogliomas including analysis by comparative genomic hybridization. Am J Pathol. 1999;155:375–86.
11. Von Deimling A, Louis DN, von Ammon K, Petersen I, Wiestler OD, Seizinger BR. Evidence for a tumor suppressor gene on chromosome 19q associated with human astrocytomas, oligodendrogliomas, and mixed gliomas. Cancer Res. 1992;52:4277–9.
12. Reifenberger J, Reifenberger G, Liu L, James CD, Wechsler W, Collins VP. Molecular genetic analysis of oligodendroglial tumors shows preferential allelic deletions on 19q and 1p. Am J Pathol. 1994;145:1175–90.
13. Reifenberger G, Louis DN. Oligodendroglioma: toward molecular definitions in diagnostic neuro-oncology. J Neuropathol Exp Neurol. 2003;62:111–26.
14. Jenkins R, Blair H, Flynn H, Passe S, Law M, Ballman K, et al. t(1;19)(q10;p10) mediates the combined deletions of 1p and 19q in human oligodendrogliomas. Sixteenth biennial international brain tumor research and therapy meeting, Silverado Resort and Spa, Napa Valley, CA, 2006.
15. Hartmann C, Johnk L, Kitange G, Wu Y, Ashworth LK, Jenkins RB, Louis DN. Transcript map of the 3.7 Mb D19S112-D19S246 candidate tumor suppressor region on the long arm of chromosome 19. Cancer Res. 2002;62:4100–8.
16. Griffin CA, Burger P, Morsberger L, Yonescu R, Swierczynski S, Weingart JD, Murphy KM. Identification of der(1;19)(q10;p10) in five oligodendrogliomas suggests mechanism of concurrent 1p and 19q loss. J Neuropathol Exp Neurol. 2006;65:988–94.
17. Abrey LE, Louis DN, Paleologos N. Survey of treatment recommendations for anaplastic oligodendroglioma. Neuro Oncol. 2007;9:314–8.
18. Brandes AA, Tosoni A, Cavallo G, Reni M, Franceschi E, Bonaldi L, Bertorelle R, Gardiman M, Ghimenton C, Iuzzolino P, Pession A, Blatt V, Ermani M, CIGNO. Correlation between O6-methylguanine DNA methyltransferase promoter methylation status, 1p and 19q deletions, and response to temozolomide in anaplastic and recurrent oligodendroglioma: a prospective GICNO study. J Clin Oncol. 2006;24:4746–53.
19. Van den Bent MJ, Taphoorn MJ, Brandes AA, Menten J, Stupp R, Frenay M, Chinot O, Kros JM, van der Rijt CC, Vecht CJ, Allgeier A, Gorlia T, European Organization for research and Treatment of Cancer Brain Tumor Group. Phase II study of first-line chemotherapy with temozolomide in recurrent oligodendroglial tumors: the European Organization for Research and Treatment of Cancer Brain Tumor Group Study 26971. J Clin Oncol. 2003;21:2525–8.
20. Vogelbaum MA, Berkey B, Peereboom D, Macdonald D, Giannini C, Suh JH, Jenkins R, Herman J, Brown P, Blumenthal DT, Biggs C, Schultz C, Mehta M. Phase II trial of pre-irradiation and concurrent temozolomide in patients with newly diagnosed anaplastic oligodendrogliomas and mixed anaplastic oligoastrocytomas: RTOG BR0131. Neuro Oncol. 2008;11:167.
21. Lassman AB, Cloughesy T, DeAngelis L, et al. Chemotherapy with deferred radiotherapy for newly diagnosed anaplastic oligodendroglioma and anaplastic mixed glioma. Neuro Oncol. 2006;8:445.
22. Idbaih A, Marie Y, Pierron G, Brennetot C, Hoang-Xuan K, Kujas M, Mokhtari K, Sanson M, Lejeune J, Aurias A, Delattre O, Delattre JY. Two types of chromosome 1p losses with opposite significance in gliomas. Ann Neurol. 2005;58:483–7.

23. van den Bent MJ, Looijenga LH, Langenberg K, Dinjens W, Graveland W, Uytdewilligen L, Sillevis Smitt PA, Jenkins RB, Kros JM. Chromosomal anomalies in oligodendroglial tumors are correlated with clinical features. Cancer. 2003;97:1276–84.
24. Giannini C, Burger PC, Berkey BA, Cairncross JG, Jenkins RB, Mehta M, Curran WJ, Aldape K. Anaplastic oligodendroglial tumors: refining the correlation among histopathology, 1p 19q deletion and clinical outcome in Intergroup Radiation Therapy Oncology Group Trial 9402. Brain Pathol. 2008;18:360–9.
25. Kanner AA, Staugaitis SM, Castilla EA, Chernova O, Prayson RA, Vogelbaum MA, Stevens G, Peereboom D, Suh J, Lee SY, Tubbs RR, Barnett GH. The impact of genotype on outcome in oligodendroglioma: validation of the loss of chromosome arm 1p as an important factor in clinical decision making. J Neurosurg. 2006;104:542–50.
26. McDonald JM, See SJ, Tremont IW, Colman H, Gilbert MR, Groves M, Burger PC, Louis DN, Giannini C, Fuller G, Passe S, Blair H, Jenkins RB, Yang H, Ledoux A, Aaron J, Tipnis U, Zhang W, Hess K, Aldape K. The prognostic impact of histology and 1p/19q status in anaplastic oligodendroglial tumors. Cancer. 2005;104:1468–77.
27. Hong C, Bollen AW, Costello JF. The contribution of genetic and epigenetic mechanisms to gene silencing in oligodendrogliomas. Cancer Res. 2003;63:7600–5.
28. Trouillard O, Aguirre-Cruz L, Hoang-Xuan K, Marie Y, Delattre JY, Sanson M. Parental 19q loss and PEG3 expression in oligodendrogliomas. Cancer Genet Cytogenet. 2004;151:182–3.
29. Wolf RM, Draghi N, Liang X, Dai C, Uhrbom L, Eklöf C, Westermark B, Holland EC, Resh MD. p190RhoGAP can act to inhibit PDGF-induced gliomas in mice: a putative tumor suppressor encoded on human chromosome 19q13.3. Genes Dev. 2003;17:476–87.
30. Fallon KB, Palmer CA, Roth KA, Nabors LB, Wang W, Carpenter M, Banerjee R, Forsyth P, Rich K, Perry A. Prognostic value of 1p, 19q, 9p and 10q, and EGFR-FISH analysis in recurrent oligodendroglioma. J Neuropathol Exp Neurol. 2004;63:314–22.
31. Zlatescu MC, TehraniYazdi A, Sasaki H, Megyesi JF, Betensky RA, Louis DN, Cairncross JG. Tumor location and growth pattern correlate with genetic signature in oligodendroglial neoplasms. Cancer Res. 2001;61:6713–5.
32. Mueller W, Hartmann C, Hoffman A, Lanksch W, Kiwit J, Tonn J, Veelken J, Schramm J, Weller M, Wiestler OD, Louis DN, von Diemling A. Genetic signature of oligoastrocytomas correlates with tumor location and denotes distinct molecular subsets. Am J Pathol. 2002;161:313–9.
33. Megyesi JF, Kachur E, Lee DH, Zlatescu MC, Betensky RA, Forsyth PA, Okada Y, Sasaki H, Mizoguchi M, Louis DN, Cairncross JG. Imaging correlates of molecular signatures in oligodendrogliomas. Clin Cancer Res. 2004;10:4303–6.
34. Ochs K, Kaina B. Apoptosis induced by DNA damage O6-methylguanine is Bcl-2 and caspase-9/3 regulated and Fas/caspase-8 independent. Cancer Res. 2000;60:5815–24.
35. Gerson SL. MGMT: its role in cancer aetiology and cancer therapeutics. Nat Rev Cancer. 2004;4:296–307.
36. Liu L, Markowitz S, Gerson SL. Mismatch repair mutations override alkyltransferase in conferring resistance to temozolomide but not to 1,3-bis(2-chloroethyl) nitrosourea. Cancer Res. 1996;56:5375–9.
37. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med. 2005;352:997–1003.
38. Mollemann M, Wolter M, Felsberg J, Collins VP, Reifenberger G. Frequent promoter hypermethylation and low expression of the MGMT gene in oligodendroglial tumors. Int J Cancer. 2005;113:379–85.
39. Brandes AA, Tosoni A, Cavallo G, et al. Correlations between O6-methylguanine DNA methyltransferase promoter methylation status, 1p and 19q deletions, and response to temozolomide in anaplastic and recurrent oligodendroglioma: a prospective GICNO study. J Clin Oncol. 2006;24:4746–53.
40. Kaloshi G, Everhard S, Laigle-Donadey F, Marie Y, Navarro S, Mokhtari K, Idbaih A, Ducray F, Thillet J, Hoang-Xuan K, Delattre JY, Sanson M. Genetic markers predictive of chemosensitivity and outcome in gliomatosis cerebri. Neurology. 2008;70:590–5.

41. Hoang-Xuan K, He J, Huguet S, Mokhtari K, Marie Y, Kujas M, Leuraud P, Capelle L, Delattre JY, Poirier J, Broët P, Sanson M. Molecular heterogeneity of oligodendrogliomas suggests alternative pathways in tumor progression. Neurology. 2001;57:1278–81. 30.
42. Jeuken JW, Sprenger SH, Wesseling P, Macville MV, von Deimling A, Teepen HL, van Overbeeke JJ, Boerman RH. Identifi cation of subgroups of high-grade oligodendroglial tumors by comparative genomic hybridization. J Neuropathol Exp Neurol. 1999;58:606–12.
43. Kraus JA, Koopmann J, Kaskel P, Maintz D, Bradner S, Schramm J, Louis DN, Wiestler OD, von Deimling A. Shared allelic losses on chromosomes 1p and 19q suggest a common origin of oligodendroglioma and oligoastrocytoma. J Neuropathol Exp Neurol. 1995;54:91–5.
44. Dehais C, Laigle-Donadey F, Marie Y, Kujas M, Lejeune J, Benouaich-Amiel A, Pedretti M, Polivka M, Xuan KH, Thillet J, Delattre JY, Sanson M. Prognostic stratification of patients with anaplastic gliomas according to genetic profile. Cancer. 2006;107:1891–7.
45. Idbaih A, Marie Y, Lucchesi C, Pierron G, Manié E, Raynal V, Mosseri V, Hoang-Xuan K, Kujas M, Brito I, Mokhtari K, Sanson M, Barillot E, Aurias A, Delattre JY, Delattre O. BAC array CGH distinguishes mutually exclusive alterations that define clinicogenetic subtypes of gliomas. Int J Cancer. 2008;122:1778–86.
46. Idbaih A, Crinière E, Marie Y, Rousseau A, Mokhtari K, Kujas M, El Houfi Y, Carpentier C, Paris S, Boisselier B, Laigle-Donadey F, Thillet J, Sanson M, Hoang-Xuan K, Delattre JY. Gene amplification is a poor prognostic factor in anaplastic oligodendrogliomas. Neuro Oncol. 2008;10(4):540–7.
47. Kouwenhoven K, Gorlia T, Kros J. EORTC 26951: prognosis of oligodendroglial tumors is an interplay of molecular, histological, and clinical characteristics. Neuro Oncol. 2007;9:546.
48. Trost D, Ehrler M, Fimmers R, Felsberg J, Sabel MC, Kirsch L, Schramm J, Wiestler OD, Reifenberger G, Weber RG. Identification of genomic aberrations associated with shorter overall survival in patients with oligodendroglial tumors. Int J Cancer. 2007;120:2368–76.
49. Lassman AB, Holland EC. Molecular biology and genetic models of gliomas and medulloblastomas. In: McLendon RE, Bigner DD, Rosenblum MR, Bruner JM, editors. Russell and Rubinstein's pathology of tumors of the nervous system. 7th ed. London: Arnold Health Sciences; 2006. p. 1039–91.
50. Wen PY, Yung WK, Lamborn KR, et al. Phase I/II study of imatinib mesylate for recurrent malignant gliomas: North American Brain Tumor Consortium Study 99-08. Clin Cancer Res. 2006;12:4899–907.
51. Sasaki H, Zlatescu MC, Betensky RA, et al. PTEN is a target of chromosome 10q loss in anaplastic oligodendrogliomas and PTEN alterations are associated with poor prognosis. Am J Pathol. 2001;159:359–67.
52. Collaborative Staging Task Force of the American Joint Committee on Cancer. Collaborative staging manual and coding instructions, version 1.0. Chicago, Illinois: American Joint Committee on Cancer. Bethesda, Maryland: U.S. Dept of Health and Human Services; 2004.
53. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol. 2007;114:97–109.
54. Rong Y, Durden DL, Van Meir EG, Brat DJ. 'Pseudopalisading' necrosis in glioblastoma: a familiar morphologic feature that links vascular pathology, hypoxia, and angiogenesis. J Neuropathol Exp Neurol. 2006;65:529–39.
55. Burger PC, Kleihues P. Cytologic composition of the untreated glioblastoma with implications for evaluation of needle biopsies. Cancer. 1989;63:2014–23.
56. Brennan C, Momota H, Hambardzumyan D, Ozawa T, Tandon A, Pedraza A, Holland E. Glioblastoma subclasses can be defined by activity among signal transduction pathways and associated genomic alterations. PLoS One. 2009;4:e7752.
57. Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell. 2010;17:98–110.

58. Carro MS, Lim WK, Alvarez MJ, Bollo RJ, Zhao X, Snyder EY, Sulman EP, Anne SL, Doetsch F, Colman H, et al. The transcriptional network for mesenchymal transformation of brain tumours. Nature. 2010;463:318–25.
59. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, et al. An integrated genomic analysis of human glioblastoma multiforme. Science. 2008;321:1807–12.
60. Sanai N, Alvarez-Buylla A, Berger MS. Neural stem cells and the origin of gliomas. N Engl J Med. 2005;353:811–22.
61. Xie Z, Chin LS. Molecular and cell biology of brain tumor stem cells: lessons from neural progenitor/stem cells. Neurosurg Focus. 2008;24:E25.
62. Zhou BB, Zhang H, Damelin M, Geles KG, Grindley JC, Dirks PB. Tumour-initiating cells: challenges and opportunities for anticancer drug discovery. Nat Rev Drug Discov. 2009;8:806–23.
63. Galli R, Binda E, Orfanelli U, Cipelletti B, Gritti A, De Vitis S, Fiocco R, Foroni C, Dimeco F, Vescovi A. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res. 2004;64:7011–21.
64. Ignatova TN, Kukekov VG, Laywell ED, Suslov ON, Vrionis FD, Steindler DA. Human cortical glial tumors contain neural stem-like cells expressing astroglial and neuronal markers in vitro. Glia. 2002;39:193–206.
65. Reynolds BA, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science. 1992;255:1707–10.
66. Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, Dirks PB. Identification of a cancer stem cell in human brain tumors. Cancer Res. 2003;63:5821–8.
67. Kania G, Corbeil D, Fuchs J, Tarasov KV, Blyszczuk P, Huttner WB, Boheler KR, Wobus AM. Somatic stem cell marker prominin-1/CD133 is expressed in embryonic stem cell-derived progenitors. Stem Cells. 2005;23:791–804.
68. Weigmann A, Corbeil D, Hellwig A, Huttner WB. Prominin, a novel microvilli-specific polytopic membrane protein of the apical surface of epithelial cells, is targeted to plasmalemmal protrusions of non-epithelial cells. Proc Natl Acad Sci U S A. 1997;94:12425–30.
69. Zeppernick F, Ahmadi R, Campos B, Dictus C, Helmke BM, Becker N, Lichter P, Unterberg A, Radlwimmer B, Herold-Mende CC. Stem cell marker CD133 affects clinical outcome in glioma patients. Clin Cancer Res. 2008;14:123–9.
70. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. Identification of human brain tumour initiating cells. Nature. 2004;432:396–401.
71. Wang J, Sakariassen PO, Tsinkalovsky O, Immervoll H, Boe SO, Svendsen A, Prestegarden L, Rosland G, Thorsen F, Stuhr L, et al. CD133 negative glioma cells form tumors in nude rats and give rise to CD133 positive cells. Int J Cancer. 2008;122:761–8.
72. Griguer CE, Oliva CR, Gobin E, Marcorelles P, Benos DJ, Lancaster Jr JR, Gillespie GY. CD133 is a marker of bioenergetic stress in human glioma. PLoS One. 2008;3:e3655.
73. Michalczyk K, Ziman M. Nestin structure and predicted function in cellular cytoskeletal organisation. Histol Histopathol. 2005;20:665–71.
74. Cattaneo E, McKay R. Proliferation and differentiation of neuronal stem cells regulated by nerve growth factor. Nature. 1990;347:762–5.
75. Vescovi AL, Reynolds BA, Fraser DD, Weiss S. bFGF regulates the proliferative fate of unipotent (neuronal) and bipotent (neuronal/astroglial) EGF-generated CNS progenitor cells. Neuron. 1993;11:951–66.
76. Dahlstrand J, Collins VP, Lendahl U. Expression of the class VI intermediate filament nestin in human central nervous system tumors. Cancer Res. 1992;52:5334–41.
77. Rutka JT, Ivanchuk S, Mondal S, Taylor M, Sakai K, Dirks P, Jun P, Jung S, Becker LE, Ackerley C. Co-expression of nestin and vimentin intermediate filaments in invasive human astrocytoma cells. Int J Dev Neurosci. 1999;17:503–15.
78. Jordan CT, Guzman ML, Noble M. Cancer stem cells. N Engl J Med. 2006;355:1253–61.

79. Zaidi HA, Kosztowski T, DiMeco F, Quinones-Hinojosa A. Origins and clinical implications of the brain tumor stem cell hypothesis. J Neurooncol. 2009;93:49–60.
80. von Deimling A, Korshunov A, Hartmann C. The next generation of glioma biomarkers: MGMT methylation, BRAF fusions and IDH1 mutations. Brain Pathol. 2011;21:74–87.
81. Suri V, Jha P, Sharma MC, Sarkar C. O6 -methylguanine DNA methyltransferase gene promoter methylation in high-grade gliomas: a review of current status. Neurol India. 2011;59:229–35.
82. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, Ludwin SK, Allgeier A, Fisher B, Belanger K, 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.
83. Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, Vanaclocha V, Baylin SB, Herman JG. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. N Engl J Med. 2000;343:1350–4.
84. Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med. 2005;352:997–1003.
85. Weller M, Felsberg J, Hartmann C, Berger H, Steinbach JP, Schramm J, Westphal M, Schackert G, Simon M, Tonn JC, et al. Molecular predictors of progression-free and overall survival in patients with newly diagnosed glioblastoma: a prospective translational study of the German Glioma Network. J Clin Oncol. 2009;27:5743–50.
86. Wick W, Weller M. Adjuvant therapy. Recent Results Cancer Res. 2009;171:141–53.
87. Riemenschneider MJ, Jeuken JW, Wesseling P, Reifenberger G. Molecular diagnostics of gliomas: state of the art. Acta Neuropathol. 2010;120:567–84.
88. Vogelbaum MA, Berkey B, Peereboom D, Macdonald D, Giannini C, Suh JH, Jenkins R, Herman J, Brown P, Blumenthal DT, et al. Phase II trial of preirradiation and concurrent temozolomide in patients with newly diagnosed anaplastic oligodendrogliomas and mixed anaplastic oligoastrocytomas: RTOG BR0131. Neuro Oncol. 2009;11:167–75.
89. Weller M, Stupp R, Reifenberger G, Brandes AA, van den Bent MJ, Wick W, Hegi ME. MGMT promoter methylation in malignant gliomas: ready for personalized medicine? Nat Rev Neurol. 2010;6:39–51.
90. Yan H, Bigner DD, Velculescu V, Parsons DW. Mutant metabolic enzymes are at the origin of gliomas. Cancer Res. 2009;69:9157–9.
91. Nobusawa S, Watanabe T, Kleihues P, Ohgaki H. IDH1 mutations as molecular signature and predictive factor of secondary glioblastomas. Clin Cancer Res. 2009;15:6002–7.
92. Labussiere M, Sanson M, Idbaih A, Delattre JY. IDH1 gene mutations: a new paradigm in glioma prognosis and therapy? Oncologist. 2010;15:196–9.
93. Aghili M, Zahedi F, Rafiee E. Hydroxyglutaric aciduria and malignant brain tumor: a case report and literature review. J Neurooncol. 2009;91:233–6.
94. Zhao S, Lin Y, Xu W, Jiang W, Zha Z, Wang P, Yu W, Li Z, Gong L, Peng Y, et al. Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1alpha. Science. 2009;324:261–5.
95. Yan H, Parsons W, Jin G, McLendon R, Rasheed BA, et al. IDH1 and IDH2 mutations in gliomas. N Engl J Med. 2009;360:765–73.
96. Packer RJ. Chemotherapy for medulloblastoma/primitive neuroectodermal tumors of the posterior fossa. Ann Neurol. 1990;28:823–8.
97. Fernandez-Teijeiro A, Betensky RA, Sturla LM, Kim JY, Tamayo P, Pomeroy SL. Combining gene expression profiles and clinical parameters for risk stratification in medulloblastomas. J Clin Oncol. 2004;22:994–8.
98. Danoff BF, Cowchc S, Marquette C, Mulingrew L, Kramer S. Assessment f the long-term effects of primary radiation therapy for brain tumours in children. Cancer. 1982;49:1580–6.
99. Mostow EN, Byrne, Connelly RR, Mulvihill JJ. Quality of life in longterm survivors of CNS tumours of childhood and adolescence. J Clin Oncol. 1991;9:592–9.
100. Ellenberg L, McComb JC, Siegel SE, Stowe S. Factors affecting intellectual outcome in paediatric brain tumour patients. Neurosurgery. 1987;21:638–44.

101. Packer RJ, Vezina G. Management of and prognosis with medulloblastoma. Arch Neurol. 2008;65(11):1419–24.
102. Pfister S, Remke M, Benner A, Mendrzyk F, Toedt G, Felsberg J, Wittmann A, Devens F, Gerber NU, Joos S, Kulozik A, Reifenberger G, Rutkoski S, Wiestler OD, Radlwimmer B, Scheurlen W, Lichter P, Korshunov A. Outcome prediction in pediatric medulloblastoma based on DNA copy-number aberrations of chromosomes 6q and 17q and the MYC and MYCN loci. J Clin Oncol. 2009;27(10):1627–36.
103. Eberhart CG, Tihan T, Burger PC. Nuclear localization and mutation of beta-catenin in medulloblastomas. J Neuropathol Exp Neurol. 2000;59:333–7.
104. Huang H, Mahler-Araujo BM, Sankila A, Chimelli L, Yonekawa Y, Kleihues P, Ohgaki H. APC mutations in sporadic medulloblastomas. Am J Pathol. 2000;156:433–7.
105. Dahmen RP, Koch A, Denkhaus D, Tonn JC, Sorensen N, Berthold F, Behrens J, Birchmeier W, Wiestler OD, Pietsch T. Deletions of AXIN1, a component of the WNT/Wingless pathway, on sporadic medulloblastomas. Cancer Res. 2001;61:7039–43.
106. Schwalbe EC, Lindsey JC, Straughton D, Hogg TL, Cole M, Megahed H, Ryan SL, Luscher ME, Taylor MD, Gilbertson RJ, Ellison DW, Bailey S, Clifford SC. Rapid diagnosis of medulloblastoma molecular subgroups. Clin Cancer Res. 2011;17:1883–94.
107. Eberhart CG, Kratz J, Wang Y, Summers K, Stearns D, Cohen K, Dang C, Burger P. Histopathological and molecular prognostic markersin medulloblastoma: c-myc, N-myc, TrkC, and anaplasia. J Neuropathol Exp Neurol. 2004;63(5):441–9.
108. Scheurlen WG, Schwabe GC, Joos S, Mollenhaur J, Sorensen N, Kuhl J. Molecular analysis of childhood primitive neuroectodermal tumors defines markers associated with poor outcome. J Clin Oncol. 1998;16(7):2478–85.
109. Grotzer MA, Janss AJ, Phillips PC, Trojanowski JQ. Neurotrophin receptor TrkC predicts good clinical outcome in medulloblastoma and other primitive neuroectodermal brain tumors. Klin Padiatr. 2000;212(4):196–9.
110. Segal RA, Goumnerova LC, Kwon YK, Stiles CD, Pomeroy SL. Expression of the neurotrophin receptor TrkC is linked to a favorable outcome in medulloblastoma. Proc Natl Acad Sci U S A. 1994;91:12867–71.
111. Gessi M, von Bueren AO, Rutkowski S, Pietsch T. p53 expression predicts dismal outcome for medulloblastoma patients with metastatic disease. J Neurooncol. 2011;106:135.
112. Batra SK, McLendon RE, Koo JS, Castelino-Prabhu S, Fuchs HE, Krischer JP, Friedman HS, Bigner DD, Bigner SH. Prognostic implications of chromosome 17p deletions in human medulloblastomas. J Neurooncol. 1995;24:39–45.
113. Cogen P. Prognostic significance of molecular genetic markers in childhood brain tumors. Pediatr Neurosurg. 1991;17:245–50.
114. Scheurlen WG, Seranski P, Mincheva A, Kühl J, Sörensen N, Krauss J, Lichter P, Poustka A, Wilgenbus KK. High-resolution deletion mapping of chromosome arm 17p in childhood neuroectodermal tumors reveals a common chromosomal disruption within the Smith-Magenis region, an unstable region in chromosome band 17p11.2. Genes Chromosomes Cancer. 1997;18:50–8.
115. Emadian SM, McDonald JD, Gerken SC, Fults D. Corrolation of chromosome17p loss with clinical outcome in medulloblastoma. Clin Cancer Res. 1996;2:1559–64.
116. Lo KC, Ma C, Bundy BN, Pomeroy SL, Eberhart CG, Cowell JK. Gain of 1q is a potential prognostic marker for survival in medulloblastoma. Clin Cancer Res. 2007;13(23):7022–8.
117. De Bortoli M, Castellino RC, Lu XY, Deyo J, Sturla LM, Adesina AM, Perlaky L, Pomeroy SL, Lau CC, Man TK, Rao PH, Kim JY. Medulloblastoma outcome is adversely associated with overexpression of EEF1D, RPL30, and RPS20 on the long arm of chromosome 8. BMC Cancer. 2006;6:223.
118. Tabori U, Baskin B, Shago M, Alon N, Taylor MD, Ray PN, Bouffet E, Malkin D, Hawkins C. Universal poor survival in children with medulloblastoma harboring somatic TP53 mutations. J Clin Oncol. 2010;28:1345–50.
119. Pfaff E, Remke M, Sturm D, Benner A, Witt H, Milde T, von Bueren AO, Wittmann A, Schöttler A, Jorch N, Graf N, Kulozik AE, Witt O, Scheurlen W, von Deimling A, Rutkowski S, Taylor MD, Tabori U, Lichter P, Korshunov A, Pfister SM. *TP53* mutatino is frequently

associated with CTNNB1 mutation *MYCN* amplification and is compatible with long-term survival in medulloblastoma. J Clin Oncol. 2010;28:5188–96.
120. Lindsey JC, Hill RM, Megahed H, Lusher ME, Schwalbe EC, Cole M, Hogg TL, Gilbertson RJ, Ellision DW, Bailey S, Clifford S. TP53 mutations in favorable-risk Wnt/Wingless-subtype medulloblastomas. J Clin Oncol. 2011;29(12):e344–6.
121. Pfister S, Rea S, Taipale M, Mendrzyk F, Straub B, Ittrich C, Thuerigen O, Sinn HP, Akhtar A, Lichter P. The histone acetyltransferase hMOF is frequently downregulated in primary breast carcinoma and medulloblastoma and constitutes a biomarker for clinical outcome in medulloblastoma. Int J Cancer. 2008;122:1207–13.
122. Jung CS, Foerch C, Schanzer A, Heck A, Plate KH, Seifert V, Steinmetz H, Raabe A, Sitzer M. Serum GFAP is a diagnostic marker for glioblastoma multiforme. Brain. 2007;130:3336–41.
123. Lin Y, Jiang T, Zhou K, Xu L, Chen B, Li G, Qiu X, Zhang W, Song SW. Plasma IGFBP-2 levels predict clinical outcomes of patients with high-grade gliomas. Neuro Oncol. 2009;11:468–76.
124. Ludwig N, Keller A, Comtesse N, Rheinheimer S, Pallasch C, Fischer U, Fassbender K, Steudel WI, Lenhof HP, Meese E. Pattern of serum autoantibodies allows accurate distinction between a tumor and pathologies of the same organ. Clin Cancer Res. 2008;14:4767–74.
125. Keller A, Ludwig N, Comtesse N, Hildebrandt A, Meese E, Lenhof HP. A minimally invasive multiple marker approach allows highly efficient detection of meningioma tumors. BMC Bioinformatics. 2006;7:539.
126. Weaver KD, Grossman SA, Herman JG. Methylated tumor-specific DNA as a plasma biomarker in patients with glioma. Cancer Invest. 2006;24:35–40.
127. Petrik V, Saadoun S, Loosemore A, Hobbs J, Opstad KS, Sheldon J, Tarelli E, Howe FA, Bell BA, Papadopoulos MC. Serum alpha 2-HS glycoprotein predicts survival in patients with glioblastoma. Clin Chem. 2008;54:713–22.
128. Batchelor TT, Sorensen AG, di Tomaso E, Zhang WT, Duda DG, Cohen KS, Kozak KR, Cahill DP, Chen PJ, Zhu M, Ancukiewicz M, Mrugala MM, Plotkin S, Drappatz J, Louis DN, Ivy P, Scadden DT, Benner T, Loeffler JS, Wen PY, Jain RK. AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. Cancer Cell. 2007;11:83–95.
129. Purow B. The elephant in the room: do microRNA-based therapies have a realistic chance of succeeding for brain tumors such as glioblastoma? J Neurooncol. 2010;103:429.
130. Cho WC. MicroRNAs: potential biomarkers for cancer diagnosis, prognosis and targets for therapy. Int J Biochem Cell Biol. 2010;42:1273–81.
131. Polyak K, Hahn WC. Roots and stems: stem cells in cancer. Nat Med. 2006;12(3):296–300.
132. Denysenko T, Gennero L, Roos MA, Melcarne A, Juenermann C, Faccani G, Morra I, Cavallo G, Reguzzi S, Pescarmona G, Ponzetto A. Glioblastoma cancer stem cells: heterogeneity, microenvironment and related therapeutic strategies. Cell Biochem Funct. 2010;28:343–51.

Rational Therapy for Renal Cell Carcinoma Based on its Genetic Targets

Jamie Messer, Joseph Drabick, and Matthew Kaag

Abstract Renal cell carcinoma (RCC) is not a single entity, rather it is a term defining a group of histologically distinct tumors arising in the renal parenchyma. Each histologic subtype is clinically and genetically unique. It is our understanding of the genetic basis for these cancers that has led to the variety of targeted systemic therapies now available in RCC. This review will cover the basic tumor biology behind each histology, as well as the associated therapeutic targets identified thus far. Mechanisms and associated side effects of the currently available drugs will be examined. Completed clinical trials will be discussed, leading into the rationale behind currently active trials, and future directions for drug development.

Keywords Renal cell carcinoma • RCC • CT • MRI • Clear cell • VHL • Pheochromocytoma • Hemangioblastoma • Chromophobe • Cul2 • Elongin • Asparagine hydroxylases • TGF-alpha • VEGF • GLUT1 • mTOR • Akt • PIP3 • Wnt • IFN-a • bevacizumab • Sunitinib • Sorafenib • Pazopanib • Axitinib • Tivozanib • VEGFR3 • Temsirolimus • Everolimus • Sequential therapy • Neoadjuvant therapy • Cytoreductive therapy • RECIST • Papillary renal cell cancer • MET • Leiomyoma • Fumarate hydratase • Foretinib • Oncocytoma

Introduction

Renal cell carcinoma (RCC) is the third most common genitourinary malignancy with an estimated 64,770 new cases and 13,570 cancer related deaths in 2012 [1]. Renal cell carcinomas are adenocarcinomas; each pathologic subtype is thought to

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W.S. El-Deiry (ed.), *Impact of Genetic Targets on Cancer Therapy*, Advances in Experimental Medicine and Biology 779, DOI 10.1007/978-1-4614-6176-0_13,

be derived from a specific region of the nephron [2]. RCC is generally considered to be chemo-resistant, with limited response to traditional chemotherapeutic agents [3]. During the past decade development of systemic agents for RCC has focused on the identification of molecular pathways critical in the carcinogenesis of renal tumors, and the development of agents to manipulate these pathways at critical points.

Population based studies have demonstrated that the incidence of RCC has increased 3–4% yearly since the 1970s [4]. This increase has been attributed to the incidental detection of small renal masses with the prevalent use of cross-sectional imaging modalities such as CT and MRI [4, 5], which may account for 48–66% of new renal cell carcinomas [6]. The classically described triad of flank pain, hematuria, and a palpable abdominal mass has become uncommon. Unfortunately despite this stage migration, the overall and cancer specific survival for RCC has changed little in the past three decades [7]. Patients who present with stage I RCC have an excellent 5 year survival of 96% or better; however patients with advanced metastatic disease have a 5 year survival of 26% [8]. Until recently, therapy for metastatic RCC consisted of immunotherapy with interleukin-2 (IL-2) and/or interferon. These drugs are associated with significant morbidity and mortality and produce a durable complete response in only 7–8% of patients, with an objective response in 21–23% [9–11]. More recently an improved understanding of genetic changes driving RCC has produced pharmaceuticals based on specific molecular targets.

RCC is not a single entity but a classification of several distinct pathologic subtypes of cancer occurring within the kidney. Each entity has distinct histopathology and follows a distinct clinical course [8, 12]. Only 2–3% of cases annually are related to familial patterns with several autosomal dominant syndromes responsible for distinct pathologies [13]. However, a genetic study of families with these syndromes has led to an understanding of the genetic loci responsible for many sporadic cases of RCC [12–14]. Evaluation of the gene products of these distinct loci has led to the development of pharmaceuticals that target these molecular pathways. This review will discuss the genetics of RCC and the specific molecular pathways that have been identified as viable therapeutic targets (Table 1).

Clear Cell Renal Cell Carcinoma

Von Hippel Lindau syndrome (VHL) is a highly penetrant autosomal dominant disorder present in 1 of 36,000 live births that leads to the development of vascular tumors including RCC (also hemangioblastoma and pheochromocytoma). Patients with VHL typically develop early onset multifocal clear cell RCC that historically is the ultimate cause of death in 35–45% of patients with VHL [12–14]. Therapy for VHL over the past two decades has consisted of careful surveillance with surgical intervention prompted by solid renal masses greater than 3 cm. Renal preservation is the goal whenever possible, and judicious use of partial nephrectomy and ablative techniques has lead to a significant reduction in deaths from metastatic disease [15, 16]. Genetic linkage analysis of VHL families led to the identification of the VHL gene on the short arm of chromosome 3

Table 1 Summary of histologic subtypes of RCC, their associated genetic targets and therapeutic agents

RCC subtype	Genetic syndrome	Genetic target	Therapeutic agents	Adverse effects
Clear cell	Von Hippel Lindau	*VHL* gene (chromosome 3p25–26)	TKIs (Sorafenib, Sunitinib, etc.)	Fatigue, hand foot syndrome, diarrhea, hypertension and hypothyroidism, possible depressed LV function with Sunitinib
			Bevacizumab	Bleeding, hypertension, fatigue, and proteinuria
			mTOR inhibitors (Temsirolimus, Everolimus)	Mucositis, fatigue, rash, hyperglycemia, hypophosphatemia, hypercholesterolemia, and pulmonary complications
Type I Papillary	Hereditary Papillary RCC	c-*MET* proto-oncogene (chromosome 7q31	GSK089 (foretinib)	Fatigue, hypertension, nausea, vomiting, diarrhea, and increased ALT/AST (Currently under investigation)
			mTOR inhibitors	See above
Type II Papillary	Hereditary Leiomyomatosis Renal Cell Carcinoma syndrome (HLRCC)	Fumarate hydratase (chromosome 1q42)	??VEGF targeted agents	See above
Chromophobe	Birt-Hogg-Dube Syndrome	*BHD1* gene (chromosome 17p12q11)	mTOR inhibitors	See above

[17]. Evaluation of sporadic renal cancers has led to the detection of mutations in the VHL gene in 91% of clear cell RCC; the mutation was not detected in papillary, chromophobe, or collecting duct carcinomas [18, 19]. The knowledge that the same genetic mutation was responsible for sporadic as well as hereditary clear cell RCC identifies this genetic pathway as a potential target for therapy.

VHL Gene Pathway

The VHL gene pathway is essential to our understanding of the development and mechanisms of targeted therapies for RCC. The VHL gene product forms a complex with elongins B & C, and Cul2 which targets the hypoxia inducible factors (HIF1a and HIF2a) for ubiquitin (E3 Ubiquitin Ligase) mediated degradation [20–22]. Under normoxic conditions HIF is hydroxylated by proline and asparagine hydroxylases inducing binding with the VHL gene product and eventual degradation [23, 24]. Under hypoxic conditions HIF is a transcription factor that regulates the downstream expression of several growth and angiogenic factors, such as vascular endothelial growth factor (VEGF), transforming growth factor-a (TGF-a), platelet derived growth factor (PDGF), and the epidermal growth factor receptor (EGFR) [25]. Mutation of the VHL gene can affect either the domain (inhibits binding to elongin B/C or Cul2) or b domain (targeting for ubiquitin mediated degradation) leading to accumulation of HIF and increased transcription of downstream genes (VEGF, PDGF, EGFR, TGF-a, GLUT1) [8, 12–14]. This results in disordered growth and increased angiogenesis (VEGF expression level correlates with microvessel density in RCC) [23], but also represents a potential target for therapy.

There are other molecular targets that relate to the VHL gene pathway and/or HIF1-a stability. The mTOR (mammalian Target of Rapamycin) pathway is involved in the regulation of the expression and stability of HIF1-a. Agents inhibiting mTOR are associated with inhibition of translation of mRNA encoding HIF1-a [26, 27]. Regulation of mTOR occurs through a series of interactions with PIP-3 (phosphoinositide-3) kinase and Akt, and protein kinase B which links growth factor receptor signaling (i.e. VEGF, PDGF, etc.). Signaling from growth factors such as VEGF or PDGF activates mTORC1 via Akt phosphorylation which in turn is activated by PIP3. Phosphorylation of mTORC1 leads to phosphorylation of several downstream targets such as p70 S6 kinase (p70S6K) and eukaryotic initiation factor 4 subunit E, leading to an increase in mRNA translation of cell cycle regulators such as c-myc, cyclin D1, and HIF [14, 28–30]. The pathway of Akt, PIP3 and mTOR has been reported to be constitutively active in RCC compared to normal renal tissue, but no mutations have been noted in the genes encoding these proteins. However PTEN (phosphatase and tensin homologue) inactivation by homozygous deletion has been noted in 4% of RCC tumors, which leads to activation (due to lack of inhibition by PTEN) of the PIP3, Akt, mTOR pathway [31–33].

The VHL gene is also responsible for the regulation of b-catenin, which is a molecule emerging as a key signal in the development of clear cell RCC [34].

b-catenin is degraded by the E3 ubiquitin ligase activity related to the VHL gene product, and Jade-1 is a novel E3 ubiquitin ligase that ubiquinates b-catenin leading to its degradation [35, 36]. Loss of function of the VHL gene product leads to increased activity of b-catenin. Point mutations in b-catenin are rare in clear cell RCC, over-expression of b-catenin has been demonstrated in induced renal tumors in mice [37–40]. Wnt's are a family of glycoproteins that inhibit phosphorylation of b-catenin thereby inhibiting its degradation via ubiquitination. Stabilized b-catenin can then enter the nucleus and lead to activation of transcription factors such as MYC oncogene, which is amplified in subsets of clear cell RCC and papillary RCC [41, 42]. Wnt is also thought to have an effect on cell growth via interaction with mTOR through inhibition of GSK3, which is responsible for phosphorylation of TSC2 (which inhibits mTOR) [43]. Wnt inhibitor's gene expression has been shown to be silenced in RCC, implying Wnt involvement in the pathogenesis of RCC [44–48].

Targeting the VHL Gene Pathway in Clear Cell RCC

Understanding of the VHL gene pathway provides the basis for the development of targeted systemic approaches in the treatment of patients with advanced RCC. RCC confined to the kidney can be treated with surgical extirpation alone with excellent 5-year survival rates for low stage renal tumors. Previous therapies for patients with locally advanced or metastatic RCC consisted of cytokine based therapy with IL-2 or interferon-a (IFN-a). While immunotherapy maintains a role as the only therapeutic option with the possibility of a durable complete response, cure is achieved in a minority of patients, and immunotherapy is associated with significant morbidity and mortality [49]. With research into the VHL gene pathway newer agents have been developed to target the molecular pathways involved in the pathogenesis of clear cell RCC (Fig. 1). A number of novel agents have been approved in the past decade for the systemic treatment of RCC.

Therapies Targeting VEGF

Bevacizumab. Bevacizumab is a monoclonal antibody that targets and neutralizes circulating VEGF protein [50]. This was the first agent studied that specifically targeted the VHL gene pathway [12]. The activity of this drug in RCC was identified in small randomized trials [51, 52]. A large multi-institutional phase III clinical trial involved 649 patients with metastatic RCC randomized to IFN2a versus IFN2a plus bevacizumab [53]. An advantage was noted in the bevacizumab cohort with respect to objective response rates (31% vs. 13%, $p < 0.0001$) and progression free survival (PFS) (10.2 months versus 5.4 months, $p < 0.0001$). The hazard ratio for progression in the bevacizumab group was 0.63 (95% CI 0.52–0.75, $p = 0.0001$). These findings were confirmed in a second multi-institutional trial that did not require previous nephrectomy, demonstrating an advantage in PFS versus IFN2a alone (8.5 months

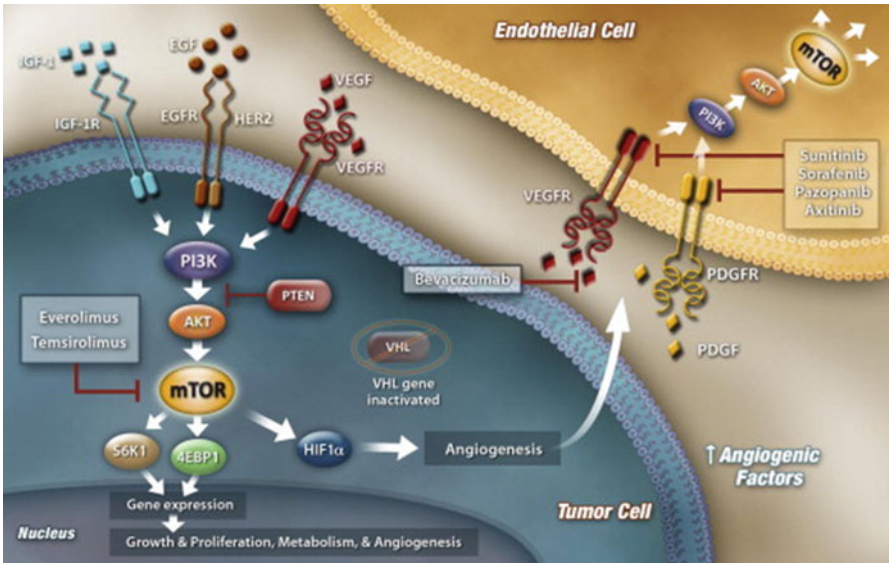

Fig. 1 Molecular targets in renal cell carcinoma (Reused with permission from Oudard et al. sequential therapy with targeted agents in advanced renal cell carcinoma: optimizing patient benefit [114]

vs. 5.2 months, $p<0.0001$) [54]. The contribution of IFN2a to the anti-neoplastic effects of this regimen is not known at present. The combination of bevacizumab and IFN2a is more toxic than either drug alone and patients should be assessed individually for risk versus benefit [14].

Sunitinib. Sunitinib is a small molecule multiple tyrosine kinase inhibitor with activity against more than 80 different kinases with antitumor and anti-angiogenic properties [55, 56]. It is a potent inhibitor of VEGF, PDGF, and the c-KIT receptors [57]. The initial evaluation of sunitinib was a two arm open label Phase II trial of 169 patients with RCC refractory to previous treatments with cytokines [55]. This trial demonstrated an objective response rate of 45% with a median PFS of 8.4 months, demonstrating a remarkable overall response rate compared to previous agents [55]. The follow-up study was a landmark phase III trial of 750 patients with untreated metastatic RCC comparing sunitinib to IFN-2α as first line therapy [56]. Compared to IFN-2α, sunitinib showed a significant advantage in objective response rate (31% vs. 6%, $p<0.001$) and PFS (median 11 months vs. 5 months, $p<0.001$) [56]. Sunitinib also demonstrated a longer overall survival of 26.4 months versus 21.8 months (p 0.05) [58]. One of the difficulties in interpreting this study is that patients randomized to interferon received either sunitinib or other targeted agents on progression, making interpretation of the differences between the two agents difficult. As with cytokine therapies, certain patient characteristics were associated with improved response to sunitinib such as an Eastern Cooperative Oncology Group (ECOG) performance status of 0, time to metastasis of >1 year, and a corrected serum calcium of 2.5 mmol/L [55]. Sunitinib is associated with adverse events

common to this class of agents including fatigue, hand foot syndrome, diarrhea, hypertension and hypothyroidism [12, 56]. Sunitinib has also been associated with decreased left ventricular function in a small subset of patients [59, 60].

Sorafenib. Sorafenib is another small molecule non-selective tyrosine kinase inhibitor with activity against VEGF, PDGF, and raf kinase [61]. The initial evidence supporting the activity of sorafenib in RCC was from a Phase II trial of 202 patients [62]. After the first 12 weeks the 65 patients who demonstrated stable disease were randomized to either placebo or continued treatment with sorafenib. Patients randomized to sorafenib demonstrated a superior PFS compared to placebo (24 weeks vs. 6 weeks, $p=0.0087$). The TARGET trial was a multi-center randomized placebo controlled trial of 903 patients with cytokine refractory RCC. This trial demonstrated a PFS advantage in the sorafenib cohort with a median PFS of 5.5 months versus 2.8 months for the placebo cohort ($p<0.001$). The hazard ratio for progression in the sorafenib group was 0.44 (95% CI 0.35–0.55, $p<0.01$). This study failed to demonstrate a difference in overall survival (17.8 months for sorafenib vs. 15.3 months for placebo, $p=0.146$). However when the subset of patients who crossed over to sorafenib is taken into account, the survival advantage is significant (17.8 vs. 14.3 months, $p=0.029$) [63]. Sorafenib was evaluated as a first line agent in a randomized phase II trial of 189 untreated patients randomized to either sorafenib or IFN-2α [64]. Patients receiving sorafenib demonstrated a higher likelihood of objective response (68% vs. 39%); however survival was similar between the two cohorts (5.7 months for sorafenib vs. 5.6 months for interferon). This study also suggested that the adverse effect profile for sorafenib, while similar to sunitinib, is not as severe.

Pazopanib. Pazopanib is an oral angiogenesis inhibitor targeting VEGFR-1, VEGFR-2, VEGFR-3, PDGFa, PDGFb, and c-kit. In a phase II study of 225 patients with metastatic RCC, 70 patients (31%) previously treated with cytokine or bevacizumab containing regimens were treated with pazopanib [65]. The overall response rate was 35%, with a median PFS of 52 weeks. Both an ECOG performance status of 0 and time to progression of >1 year correlated with prolonged PFS. In a phase III trial enrolling 435 patients, 233 (54%) treatment naïve patients were randomized to receive pazopanib versus placebo [66]. PFS was significantly prolonged in the pazopanib group (9.2 vs. 4.2 months, $p<0.0001$), especially in the treatment naïve group (11.1 vs. 2.8 months, $p<0.0001$). The objective response rate was 30% in the pazopanib group versus 3% in the placebo group. Median time until progression was 58.7 weeks (95% CI 52.1–68.1). Reported adverse events for pazopanib were consistent with but felt to be milder than adverse events for other agents in this class.

Axitinib. Axitinib is another small molecule tyrosine kinase inhibitor of VEGFR1, VEGFR2, and VEGFR3, PDGF, and c-KIT [12, 67]. Preliminary data on Axitinib comes from a small phase II trial of 52 patients with advanced RCC [68]. A partial response was noted in 21 (40%) and complete response was noted in 2 (4%), with a median time to progression of 15.7 months and median survival of 29.9 months. The appeal of this agent has been in its relatively selective inhibition of a single family of growth factor receptors as opposed to multiple kinase inhibitors such as sunitinib [69]. This agent is currently under investigation in phase III trials for metastatic RCC [67].

Axitinib reflects the current focus of drug development in this class, with researchers working to produce agents with selective activity and fewer side effects.

Tivozanib. Tivozanib is a newer inhibitor of VEGFR1 to VEGFR3, that has been preliminarily evaluated in dosing studies and found to have a tolerable side effect profile [70]. The preliminary results from a phase II clinical trial demonstrated an objective response rate of 25.4% and a median PFS of 11.4 months [71].

Therapies Targeting mTOR

The mTOR pathway regulates the expression and stability of HIF1a as previously stated [26, 27]. In preclinical studies anti-tumor activity of mTOR inhibiting agents was related to inhibition of translation of mRNA encoding HIF1a [12, 26, 27]. MTOR is situated at the convergence of several pathways that couple growth stimuli to cell cycle progression, and is felt to be critically involved in carcinogenesis [72]. There are currently two MTOR inhibitors approved for therapy in RCC, temsirolimus and everolimus.

Temsirolimus. In 2007 Hudes et al. evaluated temsirolimus versus interferon versus temsirolimus plus interferon in combination in a cohort of patients with previously untreated (predominantly clear cell) poor prognosis metastatic RCC. [73] The temsirolimus arm was associated with a PFS of 3.8 months versus 1.9 months in the interferon alone group ($p<0.001$). Overall survival also favored the temsirolimus group over the interferon alone group (10.9 vs. 7.3 months). Neither progression free nor overall survival differed significantly between the temsirolimus and combined therapy groups [73]. Temsirolimus appears to be fairly well tolerated with the most common adverse effects occurring being mucositis, fatigue, rash, hyperglycemia, hypophosphatemia, hyperlipidemia, and pulmonary complications. Pulmonary complications have been reported to occur at rates as high as 29%, leading many oncologists to avoid this agent in those with underlying pulmonary disease. Patients on temsirolimus should be monitored for pulmonary complications [74].

Everolimus. Everolimus is an orally available mTOR inhibitor. A phase III randomized controlled trial of 410 patients demonstrated a PFS of 4 months vs 1.9 months for the placebo arm, in patients with metastatic RCC that had previously failed other targeted agents. No difference could be demonstrated between everolimus and controls however, possibly due to crossover between the study arms. This agent is approved and commonly considered in patients with metastatic disease that have failed first line therapy with tyrosine kinase inhibitors [75, 12].

Evolving Uses of Targeted Therapy

Combination of Targeted Agents. Combination of agents targeting the VHL pathway can lead to increased toxicity and necessitate the reduction of doses for the agents used. Methods of combining agents have fallen into two categories vertical

and lateral inhibition. Vertical inhibition refers to combinations of agents affecting targets working in a linear pathway, while lateral inhibition refers to the inhibition of targets in non-overlapping pathways. An example of vertical inhibition would be the combination of bevacizumab with sorafenib or sunitinib which might theoretically increase therapeutic blockade of the VHL pathway, but as mentioned at the cost of increased toxicity. Lateral inhibition would refer to the combination of VEGF targeted agents with an mTOR inhibitor. Many trials are currently underway to evaluate the combination of agents in treatment of RCC. Despite the improved median PFS and reasonable tolerability reported in trials evaluating bevacizumab and IFN-a in combination [77, 78] attempts at combining two or more targeted agents have yielded little overall benefit and significantly increased morbidity. Feldman et al reported an increased rate of cardiovascular and hematologic toxicity with dose escalation in patients with metastatic RCC receiving bevacizumab in combination with escalating doses of sunitinib. The authors plan no further studies using these agents in combination [79]. Similarily a Phase I trial evaluating temsirolimus and sunitinib in combination was stopped after the first three patients experienced dose limiting toxicity at the initial dose levels [80].

Sequential use of targeted agents. Over the past decade the therapeutic options available for the systemic treatment of RCC has increased dramatically. With the many options available for the treatment of RCC the optimal sequencing of these agents is of considerable importance. Previous work, including two randomized studies, has demonstrated that many of the currently available targeted agents are active in patients who have failed cytokine therapy [51, 55, 56, 64, 81]. But there is further evidence that patients who become refractory to one VHL pathway-targeted agent, may be switched to another VHL targeted agent with renewed effect. In patients progressing on bevacizumab, sunitinib has demonstrated activity with a PFS of 29.7 weeks and 23% of patients demonstrating a partial response [82]. Similarly axitinib was associated with a PFS of 7.4 months when used in patients refractory to sorafenib, many of whom had received other agents prior to sorafenib, making axitinib a promising choice beyond the second-line setting [83]. And in a study of 90 patients refractory to either sorafenib or sunitinib, switching to the other agent was beneficial with PFS times of 22 weeks for sorafenib to sunitinib and 17 weeks for sunitinib to sorafenib [84]. mTOR inhibitors have also been shown to be effective following the development of resistance to TKI therapy. In a study randomizing TKI refractory (sorafenib, sunitinib or both) patients to everolimus versus placebo, everolimus improved PFS (4 vs. 1.9 months, HR 0.31; $p<0.001$) [85]. A small retrospective study suggests activity for temsirolimus in intermediate and poor prognosis patients failing prior VEGF-directed therapy [86]. Synergism between everolimus and bevacizumab has also been reported in the TKI-refractory setting [87]. Future clinical trials will be designed to evaluate the most effective strategy for the sequential use of targeted agents.

Neoadjuvant Use of Targeted Agents. The standard of care for patients with metastatic renal cell carcinoma in the current era of systemic therapy has been cytoreductive nephrectomy in combination with systemic therapy as demonstrated by Flanigan et al.

in 2001 [88]. However the development of targeted agents has led to the possible use of these agents in a neoadjuvant setting to decrease tumor volume [12]. One benefit of this approach is that for patients with significant metastatic burden, response can be assessed prior to cytoreductive nephrectomy and nonresponders can be spared the potential morbidity of nephrectomy [12]. While randomized trials assessing this method are still pending completion, there are several studies demonstrating that the use of neoadjuvant targeted therapy with the primary tumor in place can lead to a reduction or stabilization of tumor burden [89–92]. In 2009 the group from the Cleveland Clinic presented their results in 19 patients with advanced RCC treated with sunitinib prior to nephrectomy [89]. Only 47% had radiographic regression or stabilization of disease, and only 21% were able to undergo nephrectomy. However, this was a group with mixed histology (clear cell RCC in 53%) and locally advanced disease. Recent data is more encouraging. Hellenthal et al. reported on 20 patients treated with neoadjuvant sunitinib for biopsy proven clear cell cancers >4 cm in diameter [91]. 85% demonstrated some reduction in disease burden, though only one patient had a true partial response by RECIST. All patients tolerated nephrectomy or partial nephrectomy without undue complications. A recent publication summarized outcomes from two recent prospective trials [90]. A total of 66 patients were given sunitinib prior to nephrectomy in two different dosing regimens (two vs. three cycles). Patients with symptomatic progression on sunitinib did not undergo surgery. The median drug holiday for surgery was 29 days. 73% of patients had a clinical benefit from sunitinib by RECIST, but 36% of patients did progress while off sunitinib prior to surgery, eight with new sites of disease. The majority of these progressing patients responded to reintroduction of sunitinib. Many hoped that neoadjuvant targeted therapy would be useful in reducing inferior vena caval tumor thrombus in patients with caval involvement, making surgery safer. A study of 25 patients demonstrated that while targeted therapy arrests tumor growth, and may shrink tumor thrombus in some, it does not appear to reduce surgical complexity significantly [93]. A concern exists for inhibited wound healing in patients treated with TKIs preoperatively. A study directly addressing morbidity in pretreated patients described a statistically significant increase in superficial dehiscences (24.3% vs. 5.8%, $p<0.001$) and wound infection (12.9% vs. 2.9%, $p=0.015$) [94]. However, a third of these patients received dual agent therapy with bevacizumab and erlotinib prior to surgery, and there was no increase in overall complication rate or severe (Clavien ≥3) complication rate. Margulis et al. evaluated 44 patients treated with single-agent targeted therapy prior to cytoreductive nephrectomy compared with a matched cohort of patients undergoing surgery only. They found no difference in perioperative mortality, re-exploration, cardiovascular, embolic or other surgical complications [95]. Given our current knowledge, neoadjuvant targeted therapy seems safe and perhaps beneficial in identifying potential non-responders so that these patients may have the option of foregoing major surgery. Clinically relevant downstaging is inconsistent. Multiple randomized trials are underway aimed at better defining the role of neoadjuvant targeted therapy in RCC.

Adjuvant Targeted Therapy in Clear Cell RCC. Patients with locally advanced RCC (≥pT3) have a 20–40% chance of local or systemic recurrence, and a 5-year survival of

40–60%. The efficacy, tolerability and variety of targeted agents now available suggests a role for targeted therapy in the adjuvant setting following complete resection of a locally advanced RCC [96]. While adjuvant radiotherapy, hormonal therapy and immunotherapy have been studied, results have not yet been reported for targeted therapy in the adjuvant setting. There are several trials underway to assess the utility of adjuvant targeted therapy in patients with high risk or locally advanced RCC [12]. The ASSURE trial randomizes patients to either 1 year of Sunitinib, Sorafenib, or placebo. The SORCE trial randomizes patient to either receive placebo (3 years) or Sorafenib. The S-TRAC trial randomizes patients to 1 year of placebo or sunitinib.

Papillary Renal Cell Carcinoma

Type I Papillary Renal Cell Carcinoma. Type I papillary tumors are prevalent in patients with the aptly named hereditary papillary RCC syndrome (HPRC). Evaluation of this patient population led to the identification of the MET proto-oncogene as the genetic driver behind HPRC and type I papillary tumors [97, 98]. The MET gene encodes a tyrosine kinase membrane receptor for the hepatocyte growth factor (HGF, also located on chromosome 7). Sporadic papillary RCC has a 75% incidence of trisomy 7 [99]. Mutations of the MET proto-oncogene lead to constitutive activation resulting in a tumorigenic state [100].

Type II Papillary Renal Cell Carcinoma. The second subgroup of papillary renal cell tumors is designated Type II, and is associated with the hereditary syndrome hereditary leiomyomatosis renal cell carcinoma syndrome (HLRCC). [12, 101] Type II papillary RCC is a more aggressive subtype of RCC with a greater propensity for metastasis, even with small primary tumors. For this reason these tumors are generally resected upon discovery. [102, 103] The gene associated with HLRCC is the Krebs cycle enzyme fumarate hydratase (FH). Sequence analysis of families with cutaneous leiomyomas demonstrated FH mutations in 89% [103]. Mutations in FH lead to derangement in the TCA cycle conversion of fumarate to malate and over-accumulation of fumarate which simulates hypoxia and leads to upregulation of HIF [12, 104, 105]. Further studies have demonstrated that the accumulation of reactive oxygen species may further stabilize HIF [97, 106]. This link to HIF suggests that VEGF targeted agents may have activity in type II papillary RCC.

Targeted Therapy in Papillary RCC

One small study suggested some activity for the TKIs against papillary RCC [107]. Of 44 patients with papillary RCC treated with sunitinib or sorafenib 68% achieved stable disease with a PFS of 7.6 months. In 2008 the Global ARCC Trial Group reported on temsirolimus versus interferon as previously described [74].

Temsirolimus showed efficacy in the 20% of patients with non-clear cell histology, even in patients with adverse features. Thus, temsirolimus is thought to be active in these subtypes and remains a first line agent for papillary RCC and chromophobe RCC (discussed below). Current clinical trials are underway evaluating agents with activity against MET and/or HGF as therapeutic agents for patients with sporadic or familial **type I** papillary RCC. One such agent is GSK089 (foretinib) which is an oral dual kinase inhibitor which targets domains of MET as well as VEGFR2 [108]. Other agents currently being studied in clinical trials addressing papillary renal cell carcinoma include everolimus, sunitinib, and erlotinib alone and in combination with bevacizumab. Further information can be found at http//:clinicaltrials.gov.

Chromophobe Renal Cell Carcinoma

Birt-Hogg-Dube Syndrome is a familial syndrome characterized by the development of benign cutaneous lesions (fibrofolliculomas), pulmonary cysts (leading to potential spontaneous pneumothoraces), and renal tumors. Renal tumors tend to be bilateral and multifocal and may include chromophobe RCC, oncocytomas, and clear cell RCC [109]. Genetic linkage analysis of affected families led to the identification of the BHD gene on chromosome 17 [110]. The product of the BHD gene, folliculin (FLCN), complexes with two other FLCN interacting proteins; FNIP1 and FNIP2. The resulting complex binds to adenosine monophosphate activated protein kinase (AMPK), a molecule involved in energy sensing and regulation of the mTOR pathway [111–113]. Knockout of FLCN in mouse models activates mTOR and increases cell proliferation in the murine kidney, leading to bilateral polycystic kidneys, and death by 3 weeks of age [114]. Mice treated with rapamycin had significantly smaller kidneys and longer survival than untreated knockouts. Available data at present suggest that mTOR inhibitors may have targeted activity in BHD and chromophobe RCC.

Targeted therapy in chromophobe RCC. As previously stated, murine studies suggest activity for the mTOR inhibitor class in chromophobe tumors, a theory supported by the 2008 study published by the Global ARCC Trial Group showing efficacy for temsirolimus in non-clear cell histologies (discussed above) [74]. However, there are no prospective trials reporting on mTOR inhibitors specifically in chromophobe RCC at this time. Currently a phase II trial is recruiting patients with non-clear cell histologies including chromophobe RCC to be randomized to everolimus followed by sunitinib or sunitinib followed by everolimus. A 2008 study reported on 12 chromophobe RCC patients receiving either sorafenib or sunitinib [107]. Three patients had a partial response according to RECIST (two sorafenib, one sunitinib), and the other nine patients experienced stabilization of disease. PFS was 10.6 months. A phase II trial addressing sunitinib in chromophobe RCC is active. It is interesting to note that RET proto-oncogene expression was recently

observed in 34 of 66 papillary RCC tumors and four of ten chromophobe tumors evaluated via immunohistochemistry [115]. Sorafenib has previously been shown to have a direct inhibitory effect on RET [116], and the authors hypothesize RET as a potential target explaining the inconsistent efficacy of TKIs in papillary and chromophobe tumors. More work is needed to establish the relevance of this finding.

Conclusions

We now understand that RCC is not a single entity, rather a group comprised of diverse subtypes, each with its own molecular driver. Renal cell carcinoma variants are fundamentally metabolic diseases affecting the cellular pathways involved in energy, nutrient, and oxygen sensing. As we gain a better understanding of the genes encoding these molecular pathways the identification of novel molecular targets permits the development of highly efficacious and specific targeted therapies. Research continues to focus on identifying new agents with greater target specificity and fewer side effects, while clinical studies aim to develop new ways to use agents in combination or in sequence to optimize patient survival.

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J for Clin. 2012;62:10–29.
2. Zambrano NR, Lubensky IA, Merino MJ, Linehan WM, Walther MM. Histopathology and molecular genetics of renal tumors toward unification of a classification system. J Urol. 1999;162:1246–58.
3. Rini BI. Metastatic renal cell carcinoma: many treatment options, one patient. J Clin Oncol. 2009;27:3225–34. Official Journal of the American Society of Clinical Oncology.
4. Chow WH, Devesa SS, Warren JL, Fraumeni Jr JF. Rising incidence of renal cell cancer in the United States. JAMA. 1999;281:1628–31.
5. Jayson M, Sanders H. Increased incidence of serendipitously discovered renal cell carcinoma. Urology. 1998;51:203–5.
6. Volpe A, Panzarella T, Rendon RA, Haider MA, Kondylis FI, Jewett MA. The natural history of incidentally detected small renal masses. Cancer. 2004;100:738–45.
7. Hollingsworth JM, Miller DC, Daignault S, Hollenbeck BK. Rising incidence of small renal masses: a need to reassess treatment effect. J Natl Cancer Inst. 2006;98:1331–4.
8. Linehan WM, Grubb RL, Coleman JA, Zbar B, Walther MM. The genetic basis of cancer of kidney cancer: implications for gene-specific clinical management. BJU Int. 2005;95(Suppl 2):2–7.
9. Fyfe G, Fisher RI, Rosenberg SA, Sznol M, Parkinson DR, Louie AC. Results of treatment of 255 patients with metastatic renal cell carcinoma who received high-dose recombinant interleukin-2 therapy. J Clin Oncol. 1995;13:688–96. Official Journal of the American Society of Clinical Oncology.
10. Fyfe GA, Fisher RI, Rosenberg SA, Sznol M, Parkinson DR, Louie AC. Long-term response data for 255 patients with metastatic renal cell carcinoma treated with high-dose recombinant interleukin-2 therapy. J Clin Oncol. 1996;14:2410–1. Official Journal of the American Society of Clinical Oncology.

11. Fisher RI, Rosenberg SA, Fyfe G. Long-term survival update for high-dose recombinant interleukin-2 in patients with renal cell carcinoma. Cancer J Sci Am. 2000;6(Suppl 1):S55–7.
12. Linehan WM, Bratslavsky G, Pinto PA, et al. Molecular diagnosis and therapy of kidney cancer. Annu Rev Med. 2010;61:329–43.
13. Linehan WM, Walther MM, Zbar B. The genetic basis of cancer of the kidney. J Urol. 2003;170:2163–72.
14. Rini BI, Campbell SC, Escudier B. Renal cell carcinoma. Lancet. 2009;373:1119–32.
15. Walther MM, Choyke PL, Glenn G, et al. Renal cancer in families with hereditary renal cancer: prospective analysis of a tumor size threshold for renal parenchymal sparing surgery. J Urol. 1999;161:1475–9.
16. Walther MM, Lubensky IA, Venzon D, Zbar B, Linehan WM. Prevalence of microscopic lesions in grossly normal renal parenchyma from patients with von Hippel-Lindau disease, sporadic renal cell carcinoma and no renal disease: clinical implications. J Urol. 1995;154:2010–4. discussion 4–5.
17. Latif F, Tory K, Gnarra J, et al. Identification of the von Hippel-Lindau disease tumor suppressor gene. Science. 1993;260:1317–20.
18. Gnarra JR, Tory K, Weng Y, et al. Mutations of the VHL tumour suppressor gene in renal carcinoma. Nat Genet. 1994;7:85–90.
19. Nickerson ML, Jaeger E, Shi Y, et al. Improved identification of von Hippel-Lindau gene alterations in clear cell renal tumors. Clin Cancer Res. 2008;14:4726–34. Official Journal of the American Society of Clinical Oncology.
20. Duan DR, Pause A, Burgess WH, et al. Inhibition of transcription elongation by the VHL tumor suppressor protein. Science. 1995;269:1402–6.
21. Pause A, Lee S, Worrell RA, et al. The von Hippel-Lindau tumor-suppressor gene product forms a stable complex with human CUL-2, a member of the Cdc53 family of proteins. Proc Natl Acad Sci U S A. 1997;94:2156–61.
22. Kibel A, Iliopoulos O, DeCaprio JA, Kaelin Jr WG. Binding of the von Hippel-Lindau tumor suppressor protein to Elongin B and C. Science. 1995;269:1444–6.
23. Kaelin Jr WG. The von Hippel-Lindau protein, HIF hydroxylation, and oxygen sensing. Biochem Biophys Res Commun. 2005;338:627–38.
24. Banumathy G, Cairns P. Signaling pathways in renal cell carcinoma. Cancer Biol Ther. 2010;10:658–64.
25. Linehan WM, Pinto PA, Bratslavsky G, et al. Hereditary kidney cancer: unique opportunity for disease-based therapy. Cancer. 2009;115:2252–61.
26. Thomas GV, Tran C, Mellinghoff IK, et al. Hypoxia-inducible factor determines sensitivity to inhibitors of mTOR in kidney cancer. Natl Med. 2006;12:122–7.
27. Hudson CC, Liu M, Chiang GG, et al. Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin. Mol Cell Biol. 2002;22:7004–14.
28. Sabatini DM. mTOR and cancer: insights into a complex relationship. Nat Rev Cancer. 2006;6:729–34.
29. Rini BI. Temsirolimus, an inhibitor of mammalian target of rapamycin. Clin Cancer Res. 2008;14:1286–90. Official Journal of the American Society of Clinical Oncology.
30. Abraham RT, Gibbons JJ. The mammalian target of rapamycin signaling pathway: twists and turns in the road to cancer therapy. Clin Cancer Res. 2007;13:3109–14. Official Journal of the American Society of Clinical Oncology.
31. Forbes SA, Tang G, Bindal N, et al. COSMIC (the Catalogue of Somatic Mutations in Cancer): a resource to investigate acquired mutations in human cancer. Nucleic Acids Res. 2010;38:D652–7.
32. Lin F, Zhang PL, Yang XJ, Prichard JW, Lun M, Brown RE. Morphoproteomic and molecular concomitants of an overexpressed and activated mTOR pathway in renal cell carcinomas. Ann Clin Lab Sci. 2006;36:283–93.
33. Hara S, Oya M, Mizuno R, Horiguchi A, Marumo K, Murai M. Akt activation in renal cell carcinoma: contribution of a decreased PTEN expression and the induction of apoptosis by an Akt inhibitor. Ann Oncol. 2005;16:928–33.

34. Linehan WM, Rubin JS, Bottaro DP. VHL loss of function and its impact on oncogenic signaling networks in clear cell renal cell carcinoma. Int J Biochem Cell Biol. 2009;41:753–6.
35. Chitalia VC, Foy RL, Bachschmid MM, et al. Jade-1 inhibits Wnt signalling by ubiquitylating beta-catenin and mediates Wnt pathway inhibition by pVHL. Natl Cell Biol. 2008;10:1208–16.
36. Berndt JD, Moon RT, Major MB. Beta-catenin gets jaded and von Hippel-Lindau is to blame. Trends Biochem Sci. 2009;34:101–4.
37. Kim YS, Kang YK, Kim JB, Han SA, Kim KI, Paik SR. Beta-catenin expression and mutational analysis in renal cell carcinomas. Pathol Int. 2000;50:725–30.
38. Sansom OJ, Griffiths DF, Reed KR, Winton DJ, Clarke AR. Apc deficiency predisposes to renal carcinoma in the mouse. Oncogene. 2005;24:8205–10.
39. Qian CN, Knol J, Igarashi P, et al. Cystic renal neoplasia following conditional inactivation of apc in mouse renal tubular epithelium. J Biol Chem. 2005;280:3938–45.
40. Saadi-Kheddouci S, Berrebi D, Romagnolo B, et al. Early development of polycystic kidney disease in transgenic mice expressing an activated mutant of the beta-catenin gene. Oncogene. 2001;20:5972–81.
41. Clevers H. Wnt/beta-catenin signaling in development and disease. Cell. 2006;127:469–80.
42. Beroukhim R, Brunet JP, Di Napoli A, et al. Patterns of gene expression and copy-number alterations in von-hippel lindau disease-associated and sporadic clear cell carcinoma of the kidney. Cancer Res. 2009;69:4674–81.
43. Inoki K, Ouyang H, Zhu T, et al. TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. Cell. 2006;126:955–68.
44. Awakura Y, Nakamura E, Ito N, Kamoto T, Ogawa O. Methylation-associated silencing of SFRP1 in renal cell carcinoma. Oncol Rep. 2008;20:1257–63.
45. Hirata H, Hinoda Y, Nakajima K, et al. Wnt antagonist gene DKK2 is epigenetically silenced and inhibits renal cancer progression through apoptotic and cell cycle pathways. Clin Cancer Res. 2009;15:5678–87. Official Journal of the American Society of Clinical Oncology.
46. Hirata H, Hinoda Y, Nakajima K, et al. Wnt antagonist gene polymorphisms and renal cancer. Cancer. 2009;115:4488–503.
47. Kawakami K, Hirata H, Yamamura S, et al. Functional significance of Wnt inhibitory factor-1 gene in kidney cancer. Cancer Res. 2009;69:8603–10.
48. Urakami S, Shiina H, Enokida H, et al. Epigenetic inactivation of Wnt inhibitory factor-1 plays an important role in bladder cancer through aberrant canonical Wnt/beta-catenin signaling pathway. Clin Cancer Res. 2006;12:383–91. Official Journal of the American Society of Clinical Oncology.
49. Biswas S, Eisen T. Immunotherapeutic strategies in kidney cancer—when TKIs are not enough. Natl Rev Clin Oncol. 2009;6:478–87.
50. Presta LG, Chen H, O'Connor SJ, et al. Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. Cancer Res. 1997;57:4593–9.
51. Yang JC, Haworth L, Sherry RM, et al. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. N Engl J Med. 2003;349:427–34.
52. Bukowski RM, Kabbinavar FF, Figlin RA, et al. Randomized phase II study of erlotinib combined with bevacizumab compared with bevacizumab alone in metastatic renal cell cancer. J Clin Oncol. 2007;25:4536–41. Official Journal of the American Society of Clinical Oncology.
53. Escudier B, Pluzanska A, Koralewski P, et al. Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial. Lancet. 2007;370:2103–11.
54. Rini BI, Halabi S, Rosenberg JE, et al. Bevacizumab plus interferon alfa compared with interferon alfa monotherapy in patients with metastatic renal cell carcinoma: CALGB 90206. J Clin Oncol. 2008;26:5422–8. Official Journal of the American Society of Clinical Oncology.
55. Motzer RJ, Michaelson MD, Redman BG, et al. Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor,

in patients with metastatic renal cell carcinoma. J Clin Oncol. 2006;24:16–24. Official Journal of the American Society of Clinical Oncology.
56. Motzer RJ, Hutson TE, Tomczak P, et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med. 2007;356:115–24.
57. Mendel DB, Laird AD, Xin X, et al. In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. Clin Cancer Res. 2003;9:327–37. Official Journal of the American Society of Clinical Oncology.
58. Motzer RJ, Hutson TE, Tomczak P, et al. Overall survival and updated results for sunitinib compared with interferon alfa in patients with metastatic renal cell carcinoma. J Clin Oncol. 2009;27:3584–90.
59. Chu TF, Rupnick MA, Kerkela R, et al. Cardiotoxicity associated with tyrosine kinase inhibitor sunitinib. Lancet. 2007;370:2011–9.
60. Khakoo AY, Kassiotis CM, Tannir N, et al. Heart failure associated with sunitinib malate: a multitargeted receptor tyrosine kinase inhibitor. Cancer. 2008;112:2500–8.
61. Wilhelm SM, Carter C, Tang L, et al. BAY 43–9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res. 2004;64:7099–109.
62. Rini BI, Garcia JA, Cooney MM, et al. A phase I study of sunitinib plus bevacizumab in advanced solid tumors. Clin Cancer Res. 2009;15:6277–83. An Official Journal of the American Association for Cancer Research.
63. Escudier B, Eisen T, Stadler WM, et al. Sorafenib for treatment of renal cell carcinoma: final efficacy and safety results of the phase III treatment approaches in renal cancer global evaluation trial. J Clin Oncol. 2009;27:3312–8. Official Journal of the American Society of Clinical Oncology.
64. Escudier B, Szczylik C, Hutson TE, et al. Randomized phase II trial of first-line treatment with sorafenib versus interferon Alfa-2a in patients with metastatic renal cell carcinoma. J Clin Oncol. 2009;27:1280–9. Official Journal of the American Society of Clinical Oncology.
65. Hutson TE, Davis ID, Machiels JP, et al. Efficacy and safety of pazopanib in patients with metastatic renal cell carcinoma. J Clin Oncol. 2010;28:475–80. Official Journal of the American Society of Clinical Oncology.
66. Sternberg CN, Davis ID, Mardiak J, et al. Pazopanib in locally advanced or metastatic renal cell carcinoma: results of a randomized phase III trial. J Clin Oncol. 2010;28:1061–8. Official Journal of the American Society of Clinical Oncology.
67. Goldstein R, Pickering L, Larkin J. Does axitinib (AG-01376) have a future role in metastatic renal cell carcinoma and other malignancies? Expert Rev Anticancer Ther. 2010;10:1545–57.
68. Rixe O, Bukowski RM, Michaelson MD, et al. Axitinib treatment in patients with cytokine-refractory metastatic renal-cell cancer: a phase II study. Lancet Oncol. 2007;8:975–84.
69. Linehan WM. Targeting VEGF receptors in kidney cancer. Lancet Oncol. 2007;8:956–7.
70. Eskens FA, de Jonge MJ, Bhargava P, et al. Biological and clinical activity of tivozanib, a selective inhibitor of VEGF receptor-1, -2, and -3 tyrosine kinases, in a 4 week on, 2 week off schedule in patients with advanced solid tumors. Clin Cancer. 2011;17(22):7156–63.
71. De Luca A, Normanno N. Tivozanib, a pan-VEGFR tyrosine kinase inhibitor for the potential treatment of solid tumors. IDrugs. 2010;13:636–45.
72. Finley DS, Pantuck AJ, Belldgrun AS. Tumor biology and prognostic factors in renal cell carcinoma. Oncologist. 2011;16:4–13.
73. Hudes G, Carducci M, Tomczak P, et al. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. N Engl J Med. 2007;356:2271–81.
74. Maroto JP, Hudes G, Dutcher JP, et al. Drug-related pneumonitis in patients with advanced renal cell carcinoma treated with temsirolimus. J Clin Oncol. 2011;29:1750–6.
75. Motzer RJ, Escudier B, Oudard S, et al. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. Lancet. 2008;372:449–56.

76. Escudier B, Bellmunt J, Negrier S, et al. Phase III trial of bevacizumab plus interferon alfa-2a in patients with metastatic renal cell carcinoma (AVOREN): final analysis of overall survival. J Clin Oncol, 28 (2010), pp 2144–2150.
77. Rini BI, Halabi S, Rosenberg JE, et al. Phase III trial of bevacizumab plus interferon alfa versus interferon alfa monotherapy in patients with metastatic renal cell carcinoma: final results of CALGB 90206. J Clin Oncol, 28 (2010), pp 2137–2143.
78. Feldman DR, Baum MS, Ginsberg MS, et al. Phase I trial of bevacizumab plus escalated doses of sunitinib in patients with metastatic renal cell carcinoma. J Clin Oncol, 27 (2009), pp1432–1439.
79. Patel PH, Senico PL, Curiel RE, et al. Phase I study combining treatment with temsirolimus and sunitinib malate in patients with advanced renal cell carcinoma. Clin Genitourin Cancer, 7 (2009), pp 24–27.
80. Atkins MB, Hidalgo M, Stadler WM, et al. Randomized phase II study of multiple dose levels of CCI-779, a novel mammalian target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. J Clin Oncol. 2004;22:909–18.
81. Rini BI, Michaelson MD, Rosenberg JE, et al. Antitumor activity and biomarker analysis of sunitinib in patients with bevacizumab-refractory metastatic renal cell carcinoma. J Clin Oncol. 2008;26:3743–8.
82. Rini BI, Wilding G, Hudes G, et al. Phase II study of axitinib in sorafenib-refractory metastatic renal cell carcinoma. J Clin Oncol. 2009;27:4462–8.
83. Sablin MP, Negrier S, Ravaud A, et al. Sequential sorafenib and sunitinib for renal cell carcinoma. J Urol. 2009;182:29–34.
84. Motzer RJ, Escudier B, Oudard S, et al. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. Lancet. 2010;372:449–56.
85. MacKenzie MJ, Rini BI, Elson P, et al. Temsirolimus in VEGF-refractory metastatic renal cell carcinoma. Ann Oncol. 2011;22:145–8.
86. Hainsworth JD, Spigel DR, Burris HA, Waterhouse D, Clark BL, Whorf R. Phase II trial of bevacizumab and everolimus in patients with advanced renal cell carcinoma. J Clin Oncol. 2010;28:2131–6.
87. Flanigan RC, Salmon SE, Blumenstein BA, et al. Nephrectomy followed by interferon alfa-2b compared with interferon alfa-2b alone for metastatic renal-cell cancer. N Engl J Med. 2001;345:1655–9.
88. Thomas AA, Rini BI, Lane BR, et al. Response of the primary tumor to neoadjuvant sunitinib in patients with advanced renal cell carcinoma. J Urol. 2009;181:518–23.
89. Powles T, Blank C, Chowdhury S, et al. The outcome of patients treated with sunitinib prior to planned nephrectomy in metastatic clear cell cancer. Eur Urol. 2011;60:448–54.
90. Hellenthal NJ, Underwood W, Penetrante R, et al. Prospective clinical trial of preoperative sunitinib in patients with renal cell carcinoma. J Urol. 2010;184:859–64.
91. Cowey CL, Amin C, Pruthi RS, et al. Neoadjuvant clinical trial with sorafenib for patients with stage II or higher renal cell carcinoma. J Clin Oncol. 2010;28:1502–7.
92. Cost N, Delacroix S, Sleeper J, et al. The impact of targeted molecular therapies on the level of renal cell carcinoma vena caval tumor thrombus. Eur Urol. 2011;59:912–8.
93. Chapin B, Delacroix S, Culp S, et al. Safety of presurgical targeted tehrapy in the setting of metastatic renal cell carcinoma. Eur Urol. 2011;60:964–71.
94. Margulis V, Matin SF, Tannir N, et al. Surgical morbidity associated with administration of targeted molecular therapies before cytoreductive nephrectomy or resection of locally recurrent renal cell carcinoma. J Urol. 2008;180:94–8.
95. Haas NB, Uzzo R. Adjuvant therapy for renal cell carcinoma. Curr Oncol Rep. 2008;10:245–52.
96. Zbar B, Tory K, Merino M, et al. Hereditary papillary renal cell carcinoma. J Urol. 1994;151:561–6.
97. Schmidt L, Duh FM, Chen F, et al. Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. Nat Genet. 1997; 16:68–73.
98. Kovacs G. Molecular cytogenetics of renal cell tumors. Adv Cancer Res. 1993;62:89–124.

99. Finley DS, Pantuck AJ, Belldegrun AS. Tumor biology and prognostic factors in renal cell carcinoma. Oncologist. 2011;16(Suppl 2):4–13.
100. Launonen V, Vierimaa O, Kiuru M, et al. Inherited susceptibility to uterine leiomyomas and renal cell cancer. Proc Natl Acad Sci U S A. 2001;98:3387–92.
101. Grubb 3rd RL, Franks ME, Toro J, et al. Hereditary leiomyomatosis and renal cell cancer: a syndrome associated with an aggressive form of inherited renal cancer. J Urol. 2007;177:2074–9. discussion 9–80.
102. Toro JR, Nickerson ML, Wei MH, et al. Mutations in the fumarate hydratase gene cause hereditary leiomyomatosis and renal cell cancer in families in North America. Am J Hum Genet. 2003;73:95–106.
103. Tomlinson IP, Alam NA, Rowan AJ, et al. Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. Nat Genet. 2002;30:406–10.
104. Isaacs JS, Jung YJ, Mole DR, et al. HIF overexpression correlates with biallelic loss of fumarate hydratase in renal cancer: novel role of fumarate in regulation of HIF stability. Cancer Cell. 2005;8:143–53.
105. Pollard PJ, Briere JJ, Alam NA, et al. Accumulation of Krebs cycle intermediates and overexpression of HIF1alpha in tumours which result from germline FH and SDH mutations. Hum Mol Genet. 2005;14:2231–9.
106. Choueiri TK, Plantade A, Elson P, et al. Efficacy of sunitinib and sorafenib in metastatic papillary and chromophobe renal cell carcinoma. J Clin Oncol. 2008;26:127–31.
107. Srinivasan R, Choueiri TK, Vaishampayan U, et al. A phase II study of the dual MET/VEGFR2 inhibitor XL880 in patients (pts) with papillary renal carcinoma (PRC). ASCO Meeting Abstr. 2008;26:5103.
108. Pavlovich CP, Walther MM, Eyler RA, et al. Renal tumors in the Birt-Hogg-Dube syndrome. Am J Surg Pathol. 2002;26:1542–52.
109. Nickerson ML, Warren MB, Toro JR, et al. Mutations in a novel gene lead to kidney tumors, lung wall defects, and benign tumors of the hair follicle in patients with the Birt-Hogg-Dube syndrome. Cancer Cell. 2002;2:157–64.
110. Baba M, Hong SB, Sharma N, et al. Folliculin encoded by the BHD gene interacts with a binding protein, FNIP1, and AMPK, and is involved in AMPK and mTOR signaling. Proc Natl Acad Sci U S A. 2006;103:15552–7.
111. Hudon V, Sabourin S, Dydensborg AB, et al. Renal tumour suppressor function of the Birt-Hogg-Dube syndrome gene product folliculin. J Med Genet. 2010;47:182–9.
112. Hasumi H, Baba M, Hong SB, et al. Identification and characterization of a novel folliculin-interacting protein FNIP2. Gene. 2008;415:60–7.
113. Baba M, Furihata M, Hong SB, et al. Kidney-targeted Birt-Hogg-Dube gene inactivation in a mouse model: Erk1/2 and Akt-mTOR activation, cell hyperproliferation, and polycystic kidneys. J Natl Cancer Inst. 2008;100:140–54.
114. Flavin R, Finn SP, Choueiri TK, et al. RET protein expression in papillary renal cell carcinoma. Urol Oncol. 2011 Mar 9. [Epub ahead of print].
115. Plaza-Menacho I, Mologni L, Sala E, et al. Sorafenib functions to potently suppress RET tyrosine kinase activity by direct enzymatic inhibition and promoting RET lysosomal degradation independent of proteasomal targeting. J Biol Chem. 2007;282:29230–40.
116. Oudard S, Elaidi RT. Sequential therapy with targeted agents in patients with advanced renal cell carcinoma: optimizing patient benefit. Cancer Treat Rev. 2012;38(8):981–7.

Molecular and Genetic Markers of Follicular-Cell Thyroid Cancer: Etiology and Diagnostic and Therapeutic Opportunities

Neerav Goyal, Dhave Setabutr, Junaid Abdulghani, and David Goldenberg

Abstract Thyroid cancer has an increasing incidence in the US population and worldwide, with 95% of the cancers being of follicular cell origin—papillary, follicular, or anaplastic thyroid carcinomas. Both follicular and papillary thyroid cancers portend good survival rates, with estimated 5-year survival amongst differentiated thyroid cancer approaching 97%. On the other hand, the median survival for a patient with anaplastic thyroid carcinoma is measured in months. Despite the optimistic survival rates for papillary and follicular thyroid carcinoma, a subset of this population demonstrates resistance to radioactive iodine, and a proclivity for more aggressive tumors with higher rates of recurrence and metastasis.

As there is an increased understanding of the molecular etiology of thyroid cancer, there is also a new interest in alternative treatment methods for those nonresponsive to typical treatment. Multiple signaling pathways have been identified, including the mitogen activated protein kinase pathway, as crucial to thyroid tumor formation and progression. Additionally, particular oncogenes have been identified as prevalent in anaplastic thyroid carcinoma and thought to be involved in the transformation from differentiated to anaplastic histology.

We review the current literature and evidence describing the molecular and genetic etiology of non-medullary (follicular cell derived) thyroid carcinomas including papillary, follicular, and anaplastic thyroid carcinoma. Additionally, we evaluate the current literature on emerging and established therapies of molecular and genetic targets in these cancers.

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W.S. El-Deiry (ed.), *Impact of Genetic Targets on Cancer Therapy*, Advances in Experimental Medicine and Biology 779, DOI 10.1007/978-1-4614-6176-0_14,

Keywords Anaplastic thyroid carcinoma • Follicular thyroid cancer • Genetics • Papillary thyroid carcinoma • Chemotherapy • BRAF • Tyrosine kinase inhibitors • Thyroid neoplasm

Introduction

Thyroid cancer is on the rise with an estimated 163,000 new cases worldwide [1]. In addition to being labeled the most common endocrine cancer, US statistics from 2011 find thyroid cancer to have the fifth highest incidence amongst cancers in women [2] Differentiated thyroid cancer (DTC) includes papillary, follicular, and Hürthle cell carcinomas. They originate from follicular cells, with papillary cancer accounting for the majority of histological subtypes [3]. Medullary thyroid cancer arises from neural crest-derived parafollicular C-cells and comprise 5% of thyroid cancers, while the undifferentiated anaplastic thyroid cancer occurs even less often [4].

Certain risk factors are associated with worse prognosis such as gender, age, size of initial tumor, the presence of extension beyond the thyroid capsule, and the presence of metastases. Additionally, previous radiation exposure is associated with a higher incidence of thyroid cancer. Familial thyroid cancers do exist, most commonly medullary thyroid cancer as associated with multiple endocrine neoplasia.

Typically the only presenting symptom for patients with thyroid cancer is a palpable thyroid mass or the presence of an enlarged cervical lymph node. Progression of thyroid cancer, however may lead to more ominous signs such as hoarseness and dyspnea. Most papillary thyroid cancers are indolent tumors, while aggressive tumors such as anaplastic thyroid cancers can progress within weeks. Imaging routinely used for differentiated thyroid cancer includes a thorough ultrasound of the central and lateral neck. Sonographic characteristics of malignancy can include cystic change, hyperechogenicity, loss of hilar echogenicity and internal calcifications [5]. Ultrasound findings may alter the operative approach in 40% of initial operations and 42% of reoperations involving thyroid cancer [6].

Current therapies for differentiated thyroid cancer include surgery and radioactive iodine therapy. A total thyroidectomy is the surgical treatment of choice for most thyroid tumors although some advocate lobectomy for tumors of less than 1.0 cm in low risk patients who are diagnosed with papillary thyroid cancer [7]. Surgical treatment alone is the only effective therapy for medullary carcinoma, while palliative treatment is the general recommendation for anaplastic thyroid cancer. Radioactive iodine post surgical treatment of thyroid cancer is implemented for patients with follicular and papillary carcinoma. Regional metastasis necessitate a neck dissection at time of surgery.

For local DTC, 5 year survival is close to 100% for localized disease, 97% for regional disease, and 58% with distant metastases [2]. On the other hand, the median survival with anaplastic thyroid carcinoma is 4–6 months.

As there is an increased understanding of the molecular etiology of thyroid cancer, there is also a new interest in alternative treatment methods for those

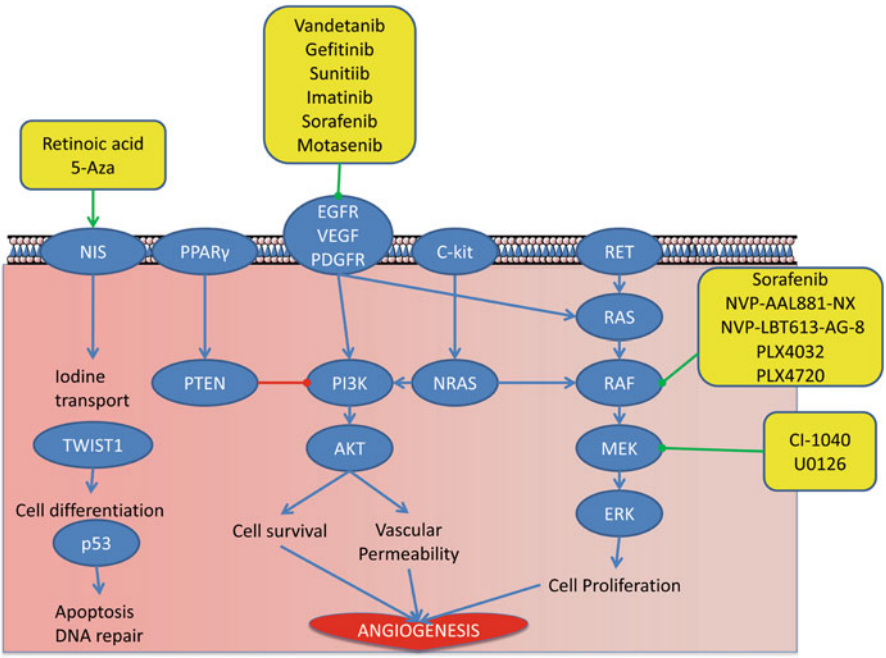

Fig. 1 Graphical depiction of molecular pathways and therapeutic targets in DTC

nonresponsive to typical treatment. Additionally, specific mutations are associated with a more aggressive disease course with higher rates or recurrence, prompting a need for different therapies for this subset of the thyroid cancer population.

In this chapter, we review the current literature and evidence describing the molecular and genetic etiology of non-medullary (follicular cell derived) thyroid carcinomas including papillary, follicular, and anaplastic thyroid carcinoma. Additionally, we will evaluate the current literature on emerging and established therapies of molecular and genetic targets in these cancers (Fig. 1).

Papillary Thyroid Carcinoma

Papillary thyroid carcinoma (PTC) encompasses more than 80% of thyroid carcinomas of follicular cell origin, and its incidence is rising by an estimated 5% per year [3]. Survival rates from this type of carcinoma are very high, with 25-year survival estimated >95% [8]. The major theory proposed for papillary tumorigenesis revolves around the MAPK (mitogen activated protein kinase) pathway which includes RAS-RAF-MEK-ERK-MAPK signaling. This pathway is crucial to major elements in the cell cycle including differentiation, proliferation, apoptosis and survival. Point, insertion, deletion and translocation mutations along this pathway may result in gain of function and permanent activation of this pathway leading to tumorigenesis [3].

BRAF

BRAF, a 94-kDa protein, represents one of three identified serine-threonine kinases (A-Raf, B-Raf, and C-Raf) within the RAF class of kinases, and is the strongest activator of the MAPK pathway. It is found on chromosome 7 at locus 7q34 and is expressed in all cell lines. In addition to thyroid carcinoma, links have been made between BRAF mutations and melanoma as well as colorectal and ovarian cancers [9]. One specific mutation that is referenced is the V600E substitution ($BRAF^{V600E}$) caused by a T1799A transversion in exon 15 the site of more than 90% of mutations associated with this gene. Xing in a literature review notes high specificity with which BRAF mutations are associated with PTC as well as PTC derived anaplastic thyroid cancer, and the specific lack of these mutations within follicular or other type of thyroid tumors. The review estimates 44% of PTCs demonstrate a mutation in this protein [10]. Bhaijee and Nikiforov note that 60% of classic PTC have BRAF mutations, while 80% of the tall cell variant, and only 10% of the follicular variant of PTC exhibit the mutation [11].

In addition to the general association with PTC, BRAF is also described as having a higher specificity with the more aggressive PTC types, and is further associated with lymph node metastases and recurrence. Its tumorigenic potential has been demonstrated in the mouse model as well as in xenograft tumor studies, and is suspected to be related to vascular endothelial growth factor (VEGF) over expression. Evidence also indicates that those tumors with a BRAF mutation have lower sensitivity and less avidity to radioiodine, likely secondary to a silencing and decreased expression of genes for the sodium iodide symporter (NIS), thyroglobulin, the thyroid stimulating hormone receptor, and thyroperoxidase [12, 13]. Tumor growth factor β may be linked to both NIS repression as well as invasive potential as demonstrated in rat thyrocytes and human PTC tissue [14]. On the other hand, cancers without the BRAF mutation have been shown to maintain differentiation closer to that of normal thyroid tissue [15].

Associations between the BRAF mutation and other mutations amongst the papillary thyroid cancer population have been attempted. A mutation found to be associated with BRAF mutants in cutaneous melanoma, a variant of the melanocortin-1 receptor, was studied in the papillary thyroid cancer population without any significant correlation. Oler et al. identified increased expression of CST6 and CXCL14, chemokines found in association with other cancer lines, with BRAF mutations [16]. Watanabe demonstrated correlations with BRAF and increased expression of extracellular matrix proteins including fibronectin, vimentin, and CITED1 [17]. Guan et al. discovered that higher rates of BRAF mutation are associated with those populations exposed to higher iodine content [18].

A second locus (B-raf-2) at Xq13 has also been identified as relating to the BRAF protein, but is predicted to be a pseudogene—a gene with a similar DNA sequence yet without the ability to produce a functional protein. Zou et al. studied the presence and mutation of this second locus within thyroid benign and malignant pathologies. The study revealed the pseudogene was less frequently detected in those

samples with mutation of the BRAF protein, yet it was still present in both benign and malignant pathology with a higher preponderance in multinodular goiters. Additionally, the pseudogene was able to activate the MAP kinase signaling pathway, likely by interacting with the wild type BRAF protein [19].

RET/PTC

The RET protein serves as a ligand activator of the MAPK pathway, usually activated by the glial cell line derived neurotrophic factor (GNDF) family. Wild-type RET is expressed in parafollicular C-cells, but is not expressed in the follicular cells of the thyroid [20]. In the RET/PTC mutation, chromosomal rearrangement causes the end of the RET gene, located at 10q11.2, to be spliced with an unrelated gene, causing release of tyrosine kinase without the tyrosine kinase receptor stimulus; this in turn causes unabated activation of MAPK [10]. The common rearrangements are RET/PTC1 and RET/PTC3 which involve fusion with CCDC6 (H4) at locus 10q21 and NCOA4 (ELE1) at locus 10q11.2, respectively. While radiation exposure is implicated in the formation of these rearrangements, most adult tumors are sporadic and of the RET/PTC1 variety, which account for 70% of RET/PTC tumors [21, 22]. Gandhi suggests that these mutations all occur at fragile sites within chromosomes, specifically noting that the RET gene as well as the CCDC6 and NCOA4 loci contain fragile sites within their sequence [22]. The population of PTC patients found to have RET/PTC rearrangements tend to be younger, with classic papillary thyroid cancer features, yet have a higher likelihood of metastasis to lymph nodes [11].

In looking at RET/PTC mutations as well as BRAF mutations, Henderson et al. discovered that a higher rate of recurrence is noted amongst those populations that have both mutations, though noting that this specific population tended to be much older than those typically seen with RET/PTC re-arrangements [23].

RAS

The family of RAS genes includes HRAS, KRAS and NRAS—G-proteins that signal various intracellular targets via the MAPK and PI3K/AKT pathways. Mutations of these genes in thyroid cancer often involves codon 61 in either the NRAS or HRAS genes, leading to an inability to cleave the bound GTP and deactivate the protein. As a result, these mutations lead to permanent RAS activation, and thus stimulation of the MAPK and PI3K/AKT signaling pathways. Other mutations involving either codon 12 or 13 are also described, which cause an increased affinity for GTP [24]. Analysis of papillary thyroid carcinomas shows that 15–20% of this population has evidence of RAS mutations, most of which are follicular variant PTCs. Additionally, PTC patients with RAS mutations tend to have more encapsulated tumors with lower rates of lymph node metastases [11, 25].

NTRK1

The NTRK1 at locus 1q21-22 gene encodes a high affinity receptor for Nerve Growth Factor (NGF) and thus is crucial in the development of the central and peripheral nervous system as well as the proliferation of lymphocytes, keratinocytes and prostate cells. The TRK class of oncogenes is a result of this gene rearranging with other loci on chromosome 1 or chromosome 3, and these oncogenes were initially identified in colon carcinoma. The rearranged oncogene, similar to BRAF, yields a constitutively active tyrosine kinase. While not as thoroughly studied as BRAF, it is estimated that 12% of PTCs may contain TRK rearrangements [26]. A mouse transgenic model has shown the TRK-T1 oncogene to cause both follicular hyperplasia and lead to papillary thyroid cancer in 54% of the transgenic population before 7 months of age, and the entire population after that age [27]. Similar to patients with RET/PTC rearrangements, patients with NTRK1 rearrangements are believed to be younger and have a poorer prognosis with a higher likelihood of lymph node metastasis [26, 28].

HLA Associations

Few studies have evaluated the presence of HLA markers in PTC or determined any HLA associations or susceptibilities with PTC. The largest study to date compared patients in Portugal with benign thyroid pathology, follicular thyroid carcinoma, and healthy non-related controls to a papillary thyroid carcinoma population (n = 180), finding HLA-DR8 and HLA-DQ4 to be significantly higher in the PTC group [29]. Research from Iran in PTC populations (n = 54 and n = 70) compared to healthy controls found associations with HLA-Cw*4 and HLA-Cw*15 in one population and HLA-DB1*04 in the second population [30, 31]. Jo et al. evaluated the expression of MHC class II antigens on the surface of PTC cells (n = 77), and found a correlation between HLA-DR and HLA-DQ expressing cells and decreased nodal metastasis and decreased risk of recurrence [32].

Follicular and Hürthle Cell Carcinoma

Follicular thyroid carcinoma (FTC) is the second most common thyroid cancer accounting for 10–15% of thyroid malignancies and is characterized by translocations or fusion of genes and expression of specific proteins [12]. FTC's are morphologically similar to follicular adenomas (FA) and can only be distinguished histologically by their capsular invasion into veins or extra-thyroidal tissue. They can be further classified based on follicular size, the presence or lack of follicular lumina, and whether the morphology of the mass is solid or trabecular [33]. The most commonly identified genetic alterations associated with follicular thyroid carcinoma have been identified as point mutations of the RAS genes and PAX8-*PPAR*γ

rearrangements. Point mutations of the RAS genes, PAX8-*PPAR*γ and aberrant methylation of tumor suppressor and thyroid specific genes can activate mitogen-activated protein (MAP) kinase and P13K/AKT pathways in thyroid cancer.

*PAX8/PPAR*γ

FTC have increased rates of imbalance in chromosome number and acquired chromosomal rearrangements. It shares its two main chromosomal patterns with follicular thyroid adenoma [34]. Kroll et al. found that one such pattern involves a translocation that leads to an in-frame fusion between the coding sequences of thyroid transcription factor PAX8 (2q13) and the peroxisome proliferator activated receptor PPARγ (3p15) [35]. This develops from a translocation that is present in 5–11% of FA and 30–35% of FTC as well as 13% of follicular variant PTC and 2% of Hürthle cell carcinoma. PAX8 plays a key role in normal thyroid differentiation and becomes fused to form the protein PAX8/PPARγ. PPARγ normally encodes a transcription factor and it is hypothesized that despite its fusion with PAX8 it retains the transcriptional activity of its wild-type counterpart [36]. PAX8 plays a role in thyrocyte differentiation and is specifically involved in NIS, thyroglobulin and TSH receptor expression. Though this fusion gene's presence in FA raises the question as to whether it is a true oncogene, current suspicions indicate that the gene may play a role in the progression of FA to FTC. Additionally, studies looking at transfection of the PAX8/PPARγ gene show reduced apoptosis and increased cell growth that is attachment-independent—an indicator of malignancy [37].

PTEN

The loss of PTEN function can activate the P13K/Akt signaling pathway, as it is a direct inhibitor of this pathway. This pathway plays an important role in cell growth, proliferation and survival. Mutations of this gene are associated with Cowden syndrome, a familial benign disease involving hamartomas of the thyroid. PTEN gene mutations have also been shown to occur in a small proportion of follicular carcinomas and has a higher prevalence amongst follicular adenomas [38]. Methylation of the gene is linked to its inactivation.

RAS

The connection between that of RAS activation and chromosomal instability in thyroid tumors has been supported by associations with between H-Ras 81 T-C polymorphism

together with increased p21 expression and aneuploidy [39]. As stated before, the most commons sites of mutation involve codon 61 of H-Ras or N-Ras. Ras mutations have been found to correlate with an older age of diagnosis while that of PAX8-*PPAR*γ has been associated with a younger age of diagnosis signifying importance given that age of diagnosis can be a molecular identifier of the tumor [40].

Hürthle Cell Carcinoma

Hürthle cell tumors consist predominantly of Hürthle cells, synonymous with oxyphilic, eosinophilic, Ashkenazi or oncocytic cells . Criteria differentiating benign from malignant Hürthle cell tumors are the as those used for follicular carcinomas: capsular invasion into veins or extra-thyroidal tissues. Genomic alterations including trisomy 5, 7, and 12 and loss of heterozygosity in 10q have been described in this population. One subset was noted to have p53 over expression. Ras mutations have been found in these carcinomas, but not with high prevalence [41]. Mitochondrial DNA (mtDNA) has been suggested in the formation of Hürthle cell tumors—specifically a 4,977 base pair deletion known as the common deletion. It is found in oncocytic thyroid tumors including PTC, FTC and FA tumors with oncocytic features, but is occasionally found in non-oncocytic tumors. Additionally, a significantly higher quantity of mtDNA deletions occurs in oncocytic tumors as compared to non-oncocytic tumors [42].

Anaplastic Thyroid Cancer

Anaplastic thyroid cancer (ATC) or undifferentiated thyroid cancer is very invasive and aggressive, accounting for nearly a third of the mortality attributed to thyroid neoplasms. While almost always lethal with a median survival of 4 months, ATC fortunately comprises less than 5% of diagnosed thyroid cancers. It is suspected that ATC arises from a pre-existing differentiated thyroid carcinoma such as PTC or FTC [43, 44]. Several genes are implicated patients with ATC, many of which are shared with the differentiated thyroid carcinomas. However, ATC are also more likely to be aneuploid, have a loss of heterozygosity, and involve multiple genetic alterations. Unfortunately, individual molecular/genetic studies suffer from small sample sizes due to the low incidence of ATC, and thus the frequency of certain mutations is variable from one population to the next.

MAPK Pathway

Xing describes the incidence of BRAF mutations amongst ATC as 24%, specifically noted that it is found in PTC derived ATC; however, other studies

report rates between 0 and 50% [10, 45]. RAS mutations are also variably present in ATC, usually with mutations at codon 61 [46]. RET/PTC rearrangements on the other hand are not well associated with ATC and studies of poorly differentiated carcinomas estimated only 10% were positive for RET/PTC. Similarly, cases of ATC with PAX8-PPARγ mutations have not been well-reported in the literature [47].

P53

Unlike the previously discussed mutations, mutations involving the tumor suppressor p53 in the thyroid are mainly associated with poorly differentiated carcinoma and ATC. The p53 gene encodes transcription factors that regulate cell cycle, DNA repair and apoptosis by helping induce G_1 arrest—important in allowing DNA repair to take place. When DNA damage is too significant, it induces apoptosis. Point mutations or deletions can result in unchecked DNA damage allowing for further mutations to occur [47]. Kim et al. looked at the KAT-18 cell line and found that the anti-apoptotic activity of the p53 mutation seemed to be linked to a transcriptor STAT3. When inhibitors against STAT3 were utilized, there was a correlating decrease in cell viability [48].

TWIST1

TWIST1 is a transcription factor that plays a role in cell differentiation, and its over expression is discussed in many human tumors in association with advanced tumor stage and poor prognosis. It not only promotes transition between epithelial and mesenchymal cell types, but it is also associated with chemotherapy resistance and inhibits apoptosis and senescence. Salerno et al. evaluated the presence and expression of TWIST1 in normal thyroid tissue, PTC, poorly differentiated thyroid carcinoma, ATC, and cancer cell lines. The group also evaluated the effects of siRNA developed to repress TWIST1. TWIST1 expression correlated with increased cell proliferation and tumor aggressiveness and also correlated with the presence of a p53 mutation. Additionally, its repression induced apoptosis, and reduced cell migration and invasiveness of ATC cells. Introducing a vector with TWIST1 into PTC cells increased their migration and protected them from apoptosis. The results of this trial point to the important role TWIST1 has on tumor progression and also its viability as a therapeutic target [49].

Multiple Hit Hypothesis

While each of the aforementioned mutations plays a role in anaplastic thyroid carcinogenesis, these same mutations are found alone in less aggressive neoplastic processes.

Researchers suggest that ATC requires multiple mutations in a stepwise fashion, resulting in a poorly differentiated carcinoma initially that then progresses to ATC. Gauchotte et al. evaluated a series of PTC and ATC tumor cells, finding that those ATC tumors with BRAF shared characteristics with PTC. Additionally, the group noted that the PTC derived ATC had further mutation of p53 and increased SOX2 expression [50].

Radiation Exposure and Its Relation to Thyroid Cancer

With iatrogenic radiation exposure populations, one of the more common cancers found are thyroid carcinomas, especially PTC. Previous large scale exposures sites include Nagasaki, Hiroshima, Chernobyl, and most recently Fukushima [51–53]. In the United States, Three Mile Island, considered one of the worst national nuclear incidents, has not been causally linked to increases in thyroid cancer as seen in these other populations [54]. Amongst the different previously identified mutations, the RET/PTC3 rearrangement has been strongly associated with radiation exposure and a solid variant of PTC. In contrast, studies looking at the NTRK1 rearrangement's prevalence in Chernobyl noted no difference between the radiation exposed population and the sporadic PTC population [26].

Amongst atomic bomb survivors from Hiroshima and Nagasaki, researchers found that most thyroid carcinogenesis was dose related to the amount of gamma radiation received. Additionally, RET/PTC rearrangements were more common in the higher dose population, and had a lower latency of presentation. In contrast, BRAF mutations were present in patients who had received lower doses of radiation, and their presentation with PTC was much more delayed. In contrast to Chernobyl, the histology of the PTC tended to be classical variant as opposed to solid variant [51, 53].

The population affected by Chernobyl also had a heavy prevalence of PTC, with an increased incidence amongst the pediatric and adult population correlating with the degree of radioactive iodine ground contamination. In comparing this population to sporadic PTCs, Ermak et al. notes different p53 mutations, a higher frequency of RET/PTC3 mutations, and a lack of Ras point mutations. Interestingly, the initially presenting tumors had RET/PTC3 rearrangements while those presenting 10 years or longer after the incident had more RET/PTC1 mutations [52].

Genetic and Molecular Targets

Diagnosis and Surveillance

With many available targets identified as having a major role in thyroid carcinogenesis, current research is devoted to using these targets to aid in diagnosis and medical treatment of thyroid tumors. The use of genetic and molecular markers in

diagnosis can aid histopathologists in classifying thyroid tumors. Fine needle aspiration biopsies are a diagnostic modality used in evaluating thyroid nodules and masses to determine the need for further intervention and help establish the risk of malignancy. In those samples where there aren't clear histological features to identify the specimen and they are judged to be indeterminate nodules, 15–20% of these nodules can be malignant—necessitating another biopsy or definitive surgical management. However, with the use of genetic markers, more of these indeterminate nodules can be appropriately classified. Xing described $BRAF^{V600E}$ detection with 100% specificity, and 54% sensitivity of PTC [55]. Compiling currently published studies, Nikiforov noted a 0.2% false positive rate of BRAF testing, with 15–40% of the positive samples initially classified as indeterminate by cytology [56]. Guerra et al. performed Southern blot analysis looking for RET/PTC rearrangements, and noted 36% of PTC in their sample showed the mutation, however 13.3% of benign nodules also had evidence of the mutation. When they used RT-PCR, 14.3% of PTC and 3.6% of benign nodules were positive for RET/PTC rearrangements, improving on the accuracy of conventional cytology [57].

Another area where genetic targets show promise is in differentiating follicular adenoma from follicular adenocarcinoma. While Ras mutations are prevalent in both populations, the PAX8/PPARγ mutation is more likely in the follicular adenocarcinoma population, yielding a potential diagnostic marker. Kitano et al. looked at micro RNA—fragments of noncoding RNA—to differentiate between follicular adenoma and adenocarcinoma and found miR-125 and miR-7 to be differentially expressed between the two groups, though the study was limited by low sample size [58].

Beyond initial diagnosis, genetic markers may be useful in assessing residual disease or recurrence. $BRAF^{V600E}$ mutations were detectable in varying concentrations from 1:325 to lower than 1:100,000 ($BRAF^{V600E}$ mutation containing cell to BRAF homozygous wild type cell) in a Mayo Clinic study. Twenty of 193 patients with previously diagnosed PTC had $BRAF^{V600E}$ positive blood, eight of which were also known to be currently alive disease; yielding a relative risk of 2.55 of being alive with disease if the blood tested positive. Of note, patients who recently underwent surgery also tested positive for the mutation, with a trend towards higher concentration of mutations the more recent the surgery was. As noted by the authors, this testing could prove valuable in individuals where thyroglobulin level monitoring is not reliable either secondary to large remnant thyroid or the presence of thyroglobulin antibodies [59].

MAPK/ERK Inhibition

As previously described, mutations of BRAF represent a major portion of papillary thyroid cancer and as such make an attractive target for experimental therapies. Additionally, PTCs with BRAF mutations tend to be more aggressive and less radioiodine sensitive. Beyond PTC and ATC, BRAF—as well as the other RAF kinases—are downstream in the MAPK signaling cascade of Ras as well as Ret,

Table 1 Drugs affecting the MAP/ERK pathway currently in clinical trials involving follicular cell derived thyroid cancer

Drug name	Targets affected	Current phase of study
Motesanib (AMG 706)	VEGFR, PDGFR, c-kit	II
Sorafenib (BAY 43-9006)	VEGFR, PDGFR, RET, BRAF	II,III
Sunitinib (SU 11248)	VEGFR, PDFGR, c-kit	II
Axitinib (AG-01 3736)	VEGFR	II
Pazopanib	VEGFR, PDFGR	II
Imatinib (ST 1571)	PDGFR, c-kit, BCR-ABL, RET	II
Gefitinib	EGFR	II

oncogenes that are involved in follicular variant PTC as well as FTC. Pre-clinical trials have evaluated molecular inhibition of BRAF using small interfering RNA sequences, or siRNA, with promising results. Additionally, RAF kinase inhibitors such as Sorafenib (Nexavar, Bayer HealthCare Pharmaceuticals, New Haven, CT, USA), NVP-AAL881-NX, NVP-LBT613-AG-8 (Novartis, Cambridge, MA, USA), PLX4032 (RG7204 or vemurafenib), and PLX4720 (Plexxikon Inc, Berkeley, CA, USA) have been tested in vitro and in vivo xenograft mouse models [60–63]. These studies show that the $BRAF^{V600E}$ mutation is sensitive to the previously mentioned inhibitors leading to inhibited growth and enhanced apoptosis. Vandetanib, sunitinib, and gefitinib are other tyrosine kinase inhibitors that work on epithelial growth factor receptors and have been shown to block proliferation in PTC cell lines. Additionally, they have noted activity against RET/PTC rearrangements [64].

Sorafenib is currently approved for renal cell carcinoma and is in trials for melanoma and thyroid carcinoma. A recent Phase I trial evaluated the combination of sorafenib and tipifarnib (a Ras inhibitor) and determined that the combination therapy did seem effective, though no control group was included [65]. Multiple phase II trials have shown promising results with degrees of partial response and stable disease in patients with progressive radioiodine resistant differentiated thyroid cancers (DTC). Additionally, a phase III trial is currently underway to evaluate the role of sorafenib in progressive metastatic DTC [66]. Sunitinib underwent phase II trials showing partial response in 13% and stable disease in 68% of progressive DTC patients [66]. Gefitinib, a drug studied for non-small cell lung cancer, underwent a phase II trial involving 27 patients with only 18% showing stable disease, and a mean progression free survival of 4 months [64]. Axitinib, another medication used in renal cell carcinoma, showed progression free survival of 18 months with 30% of patients showing partial response and 38% showing stable disease amongst a population with advanced thyroid cancer[64]. Pazonpanib in a phase II trial demonstrated 71% progression free survival at 6 months, and 32% of confirmed partial responses amongst rapidly progressing DTC patients [66]. Table 1 lists the drugs involving the MAPK/ERK pathway and their current phase of clinical study.

Schweppe et al. evaluated the role of CI-1040 (Calbiochem, Gibbstown, NJ, USA) and U0126 (Pfizer, Ann Arbor, MI, USA) in PTC and ATC cell lines to determine

the role of active MEK inhibitors. Their study determined that these two compounds not only inhibited growth of BRAF mutated cell lines but also cells with RET/PTC1 rearrangements. Additionally, CI-1040 was shown to inhibit the degree of invasion of the cell lines. Neither compound, however, promoted apoptosis, suggesting the need for the drugs to be used in combination therapy. [67]. Henderson et al. quantified a decrease of 47.5% in RET/PTC1 tumor volume in xenografted mice, and a 31.3% reduction in volume in xenografted mice with BRAF mutations [68].

NIS

The decreased radio sensitivity of certain thyroid carcinomas is thought to be linked to repression of NIS channels in thyrocytes. Techniques to re-establish NIS expression include exposure to agents to cause re-differentiation of malignant thyrocytes as well as the introduction of recombinant DNA. Agents that have been tested include retinoic acid, 5-Aza, valproic acid, suberoylanilide hydroxamic acid (SAHA, vorinostat), troglitazone, rosiglitazone, thyroid hormone, and TSH [69–71]. Of those tested, retinoic acid and 5-Aza show improvement in radioactive iodine uptake in some studies, while others demonstrate an increase NIS expression without a correlating increase in function. Riesco-Eizaguirre et al. described the use of recombinant telomerase sequences that were injected into xenografted cancer cell lines including TPC-1, a thyroid cancer cell line. The study demonstrated an increase in NIS expression as well as an increased sensitivity to ^{131}I [72].

Tumor Microenvironment and Cancer Stem Cells

Dumont et al. (1992) first postulated the existence of thyroid stem cell populations in the mature thyroid, partly since the growth of thyroid transplants in recipient animal requires the injection of minimum number of cells and also because foci formation in cloning assays is inefficient [73, 74]. A population of adult stem cells co-expressing the pluripotent marker Oct-4, endodermal marker, HNF4a, TTF and Pax8, exist within human goiter [75]. The Insulin like growth factor IGF increases Oct-4 activity, a stem cell marker that is highly expressed in papillary thyroid cancer [76]. In one study, stem cells were isolated from primary thyroid culture and grown as monolayer or embedded in collagen. Cells induced with TSH in serum enriched medium expressed PAX8, TG, NIS, TSHR, and TPO mRNA [77].

Of late, the existence of special types of cancer cells has been hypothesized, which includes cells in epithelial mesenchymal transmission (EMT) [78]. This idea is based on the observation that tumor progresses by cell migration; some of these cells detach and undergo EMT, migrating separately with the appearance of mesenchymal stem cells. Reversible EMT is caused in vitro by extracellular matrix/factors [79]. In dogs and rat erthyrocytes the progress is enhanced by hepatocyte growth factor (HGF),

Epidermal growth factor (EGF) or by culturing cells [80] . TGFb plays a role in both the CSC and EMT phenotypes, specifically in the invasive front of PTC [81]. In medullary thyroid carcinoma, the cells were positive for CD133 and demonstrated the role for RET protooncogene, bFGF and EGF in CSC cell renewal [82, 83]. This evidence suggests avenues for MTC should aim to inhibit tyrosine kinase receptors, bFGF and EGF receptors [83].

Conclusion

Significant research has been conducted in determining the etiology and progression of thyroid cancer on a molecular biology and genetics level. Mutation and alteration of several major pathways including the MAPK as well as PI3K signaling cascades are crucial to the tumorigenesis of thyroid cancer. BRAF, RET/PTC, and up-regulation of tyrosine kinase receptors are strongly implicated in papillary thyroid cancer. Recent research also suggests the involvement of PTEN, and PAX8/PPARγ in follicular thyroid cancer.

With information regarding the genomics of thyroid cancer, recent research has paved the way for the development of inhibitors of these tumor pathways. Additionally, current drugs on the market have been used in the treatment of anaplastic and papillary thyroid cancer with promising results. Continued research will elucidate the role of molecular genetics in not only diagnosing thyroid cancer, but also aid in predicting its course in the individual patient and thus allowing for more directed and targeted treatment.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61(2):69–90.
2. Cancer Facts & Figures. 2011. Atlanta: American Cancer Society. Available from: http://www.cancer.org/Research/CancerFactsFigures/CancerFactsFigures/cancer-facts-figures-2011.
3. Nucera C, Lawler J, Hodin R, Parangi S. The BRAFV600E mutation: what is it really orchestrating in thyroid cancer? Oncotarget. 2010;1(8):751–6.
4. Sherman SI. Thyroid carcinoma. Lancet. 2003;361(9356):501–11.
5. Choi JS, Kim J, Kwak JY, Kim MJ, Chang HS, Kim EK. Preoperative staging of papillary thyroid carcinoma: comparison of ultrasound imaging and CT. AJR Am J Roentgenol. 2009;193(3):871–8.
6. Stulak JM, Grant CS, Farley DR, Thompson GB, van Heerden JA, Hay ID, et al. Value of preoperative ultrasonography in the surgical management of initial and reoperative papillary thyroid cancer. Arch Surg. 2006;141(5):489–94. discussion 94–6.
7. Bilimoria KY, Bentrem DJ, Ko CY, Stewart AK, Winchester DP, Talamonti MS, et al. Extent of surgery affects survival for papillary thyroid cancer. Ann Surg. 2007;246(3):375–81. discussion 81–4.
8. LiVolsi VA. Papillary thyroid carcinoma: an update. Mod Pathol. 2011;24(Suppl 2):S1–9.
9. Puxeddu E, Durante C, Avenia N, Filetti S, Russo D. Clinical implications of BRAF mutation in thyroid carcinoma. Trends Endocrinol Metab. 2008;19(4):138–45.

10. Xing M. BRAF mutation in papillary thyroid cancer: pathogenic role, molecular bases, and clinical implications. Endocr Rev. 2007;28(7):742–62.
11. Bhaijee F, Nikiforov YE. Molecular analysis of thyroid tumors. Endocr Pathol. 2011;22(3):126–33.
12. Carpi A, Mechanick JI, Saussez S, Nicolini A. Thyroid tumor marker genomics and proteomics: diagnostic and clinical implications. J Cell Physiol. 2010;224(3):612–9.
13. Xing M. Recent advances in molecular biology of thyroid cancer and their clinical implications. Otolaryngol Clin North Am. 2008;41(6):1135–46.
14. Riesco-Eizaguirre G, Rodriguez I, De la Vieja A, Costamagna E, Carrasco N, Nistal M, et al. The BRAFV600E oncogene induces transforming growth factor beta secretion leading to sodium iodide symporter repression and increased malignancy in thyroid cancer. Cancer Res. 2009;69(21):8317–25.
15. Durand S, Ferraro-Peyret C, Joufre M, Chave A, Borson-Chazot F, Selmi-Ruby S, et al. Molecular characteristics of papillary thyroid carcinomas without BRAF mutation or RET/PTC rearrangement: relationship with clinico-pathological features. Endocr Relat Cancer. 2009;16(2):467–81.
16. Oler G, Camacho CP, Hojaij FC, Michaluart Jr P, Riggins GJ, Cerutti JM. Gene expression profiling of papillary thyroid carcinoma identifies transcripts correlated with BRAF mutational status and lymph node metastasis. Clin Cancer Res. 2008;14(15):4735–42.
17. Watanabe R, Hayashi Y, Sassa M, Kikumori T, Imai T, Kiuchi T, et al. Possible involvement of BRAFV600E in altered gene expression in papillary thyroid cancer. Endocr J. 2009;56(3):407–14.
18. Guan H, Ji M, Bao R, Yu H, Wang Y, Hou P, et al. Association of high iodine intake with the T1799A BRAF mutation in papillary thyroid cancer. J Clin Endocrinol Metabol. 2009;94(5):1612–7.
19. Zou M, Baitei EY, Alzahrani AS, Al-Mohanna F, Farid NR, Meyer B, et al. Oncogenic activation of MAP kinase by BRAF pseudogene in thyroid tumors. Neoplasia. 2009;11(1):57–65.
20. Nikiforov YE. RET/PTC rearrangement in thyroid tumors. Endocr Pathol. 2002;13(1):3–16.
21. Nikiforova MN, Nikiforov YE. Molecular genetics of thyroid cancer: implications for diagnosis, treatment and prognosis. Expert Rev Mol Diagn. 2008;8(1):83–95.
22. Gandhi M, Dillon LW, Pramanik S, Nikiforov YE, Wang YH. DNA breaks at fragile sites generate oncogenic RET/PTC rearrangements in human thyroid cells. Oncogene. 2010;29(15):2272–80.
23. Henderson YC, Shellenberger TD, Williams MD, El-Naggar AK, Fredrick MJ, Cieply KM, et al. High rate of BRAF and RET/PTC dual mutations associated with recurrent papillary thyroid carcinoma. Clin Cancer Res. 2009;15(2):485–91.
24. Rivera M, Ricarte-Filho J, Knauf J, Shaha A, Tuttle M, Fagin JA, et al. Molecular genotyping of papillary thyroid carcinoma follicular variant according to its histological subtypes (encapsulated vs infiltrative) reveals distinct BRAF and RAS mutation patterns. Mod Pathol. 2010;23(9):1191–200.
25. Nikiforov YE. Molecular diagnostics of thyroid tumors. Arch Pathol Lab Med. 2011;135(5):569–77.
26. Greco A, Miranda C, Pierotti MA. Rearrangements of NTRK1 gene in papillary thyroid carcinoma. Mol Cell Endocrinol. 2010;321(1):44–9.
27. Russell JP, Powell DJ, Cunnane M, Greco A, Portella G, Santoro M, et al. The TRK-T1 fusion protein induces neoplastic transformation of thyroid epithelium. Oncogene. 2000;19(50):5729–35.
28. Bongarzone I, Fugazzola L, Vigneri P, Mariani L, Mondellini P, Pacini F, et al. Age-related activation of the tyrosine kinase receptor protooncogenes RET and NTRK1 in papillary thyroid carcinoma. J Clin Endocrinol Metab. 1996;81(5):2006–9.
29. Porto T, Coelho I, Boavida J, Pereira C, Nunes JM, Mendonca D, et al. Association of HLA DQ4-DR8 haplotype with papillary thyroid carcinomas. Clin Endocrinol (Oxf). 2006;64(2):179–83.
30. Haghpanah V, Khalooghi K, Adabi K, Amiri P, Tavangar SM, Amirzargar A, et al. Associations between HLA-C alleles and papillary thyroid carcinoma. Cancer Biomark. 2009;5(1):19–22.
31. Amoli MM, Yazdani N, Amiri P, Sayahzadeh F, Haghpanah V, Tavangar SM, et al. HLA-DR association in papillary thyroid carcinoma. Dis Markers. 2010;28(1):49–53.

32. Jo YS, Lee JC, Li S, Choi YS, Bai YS, Kim YJ, et al. Significance of the expression of major histocompatibility complex class II antigen, HLA-DR and -DQ, with recurrence of papillary thyroid cancer. Int J Cancer. 2008;122(4):785–90.
33. Zitzelsberger H, Thomas G, Unger K. Chromosomal aberrations in thyroid follicular-cell neoplasia: in the search of novel oncogenes and tumour suppressor genes. Mol Cell Endocrinol. 2010;321(1):57–66.
34. Caria P, Vanni R. Cytogenetic and molecular events in adenoma and well-differentiated thyroid follicular-cell neoplasia. Cancer Genet Cytogenet. 2010;203(1):21–9.
35. Kroll TG, Sarraf P, Pecciarini L, Chen CJ, Mueller E, Spiegelman BM, et al. PAX8-PPARgamma1 fusion oncogene in human thyroid carcinoma [corrected]. Science. 2000;289(5483): 1357–60.
36. Giordano TJ. Genome-wide studies in thyroid neoplasia. Endocrinol Metab Clin North Am. 2008;37(2):311–31.
37. Eberhardt NL, Grebe SKG, McIver B, Reddi HV. The role of the PAX8/PPARgamma fusion oncogene in the pathogenesis of follicular thyroid cancer. Mol Cell Endocrinol. 2010;321(1):50–6.
38. Learoyd DL, Messina M, Zedenius J, Robinson BG. Molecular genetics of thyroid tumors and surgical decision-making. World J Surg. 2000;24(8):923–33.
39. Castro P, Soares P, Gusmao L, Seruca R, Sobrinho-Simoes M. H-RAS 81 polymorphism is significantly associated with aneuploidy in follicular tumors of the thyroid. Oncogene. 2006;25(33):4620–7.
40. Antonelli A, Ferrari SM, Fallahi P, Berti P, Materazzi G, Marchetti I, et al. Evaluation of the sensitivity to chemotherapeutics or thiazolidinediones of primary anaplastic thyroid cancer cells obtained by fine-needle aspiration. Eur J Endocrinol. 2008;159(3):283–91.
41. Maximo V, Sobrinho-Simoes M. Hurthle cell tumours of the thyroid. A review with emphasis on mitochondrial abnormalities with clinical relevance. Virchows Archiv. 2000;437(2): 107–15.
42. Sobrinho-Simoes M, Preto A, Rocha AS, Castro P, Maximo V, Fonseca E, et al. Molecular pathology of well-differentiated thyroid carcinomas. Virchows Arch. 2005;447(5):787–93.
43. Pacifico F, Crescenzi E, Mellone S, Iannetti A, Porrino N, Liguoro D, et al. Nuclear factor-{kappa}B contributes to anaplastic thyroid carcinomas through up-regulation of miR-146a. J Clin Endocrinol Metabol. 2010;95(3):1421–30.
44. Kojic SL, Strugnell SS, Wiseman SM. Anaplastic thyroid cancer: a comprehensive review of novel therapy. Expert Rev Anticancer Ther. 2011;11(3):387–402.
45. Smallridge RC, Marlow LA, Copland JA. Anaplastic thyroid cancer: molecular pathogenesis and emerging therapies. Endocr Relat Cancer. 2009;16(1):17–44.
46. Volante M, Rapa I, Gandhi M, Bussolati G, Giachino D, Papotti M, et al. RAS mutations are the predominant molecular alteration in poorly differentiated thyroid carcinomas and bear prognostic impact. J Clin Endocrinol Metab. 2009;94(12):4735–41.
47. Nikiforov YE. Genetic alterations involved in the transition from well-differentiated to poorly differentiated and anaplastic thyroid carcinomas. Endocr Pathol. 2004;15(4):319–27.
48. Kim TH, Lee SY, Rho JH, Jeong NY, Soung YH, Jo WS, et al. Mutant p53 (G199V) gains antiapoptotic function through signal transducer and activator of transcription 3 in anaplastic thyroid cancer cells. Mol Cancer Res. 2009;7(10):1645–54.
49. Salerno P, Garcia-Rostan G, Piccinin S, Bencivenga TC, Di Maro G, Doglioni C, et al. TWIST1 plays a pleiotropic role in determining the anaplastic thyroid cancer phenotype. J Clin Endocrinol Metabol. 2011;96(5):E772–81.
50. Gauchotte G, Philippe C, Lacomme S, Leotard B, Wissler MP, Allou L, et al. BRAF, p53 and SOX2 in anaplastic thyroid carcinoma: evidence for multistep carcinogenesis. Pathology. 2011;43:447–52.
51. Nakachi K, Hayashi T, Hamatani K, Eguchi H, Kusunoki Y. Sixty years of follow-up of Hiroshima and Nagasaki survivors: current progress in molecular epidemiology studies. Mutat Res. 2008;659(1–2):109–17.

52. Ermak G, Figge JJ, Kartel NA, Davies KJA. Genetic aberrations in Chernobyl-related thyroid cancers: implications for possible future nuclear accidents or nuclear attacks. IUBMB Life. 2003;55(12):637–41.
53. Hamatani K, Eguchi H, Ito R, Mukai M, Takahashi K, Taga M, et al. RET/PTC rearrangements preferentially occurred in papillary thyroid cancer among atomic bomb survivors exposed to high radiation dose. Cancer Res. 2008;68(17):7176–82.
54. Levin RJ. Incidence of thyroid cancer in residents surrounding the Three Mile Island nuclear facility. Laryngoscope. 2008;118(4):618–28.
55. Xing M. Prognostic utility of BRAF mutation in papillary thyroid cancer. Mol Cell Endocrinol. 2010;321(1):86–93.
56. Nikiforov YE. Molecular analysis of thyroid tumors. Mod Pathol. 2011;24(2):34–43.
57. Guerra A, Sapio MR, Marotta V, Campanile E, Moretti MI, Deandrea M, et al. Prevalence of RET/PTC rearrangement in benign and malignant thyroid nodules and its clinical application. Endocr J. 2011;58(1):31–8.
58. Kitano M, Rahbari R, Patterson EE, Xiong Y, Prasad NB, Wang Y, et al. Expression profiling of difficult-to-diagnose thyroid histologic subtypes shows distinct expression profiles and identify candidate diagnostic microRNAs. Ann Surg Oncol. 2011;18:3443–52.
59. Cradic KW, Milosevic D, Rosenberg AM, Erickson LA, McIver B, Grebe SK. Mutant BRAF(T1799A) can be detected in the blood of papillary thyroid carcinoma patients and correlates with disease status. J Clin Endocrinol Metabol. 2009;94(12):5001–9.
60. Espinosa AV, Porchia L, Ringel MD. Targeting BRAF in thyroid cancer. Br J Cancer. 2007;96(1):16–20.
61. Salerno P, De Falco V, Tamburrino A, Nappi TC, Vecchio G, Schweppe RE, et al. Cytostatic activity of adenosine triphosphate-competitive kinase inhibitors in BRAF mutant thyroid carcinoma cells. J Clin Endocrinol Metabol. 2010;95(1):450–5.
62. Nucera C, Nehs MA, Nagarkatti SS, Sadow PM, Mekel M, Fischer AH, et al. Targeting BRAFV600E with PLX4720 displays potent antimigratory and anti-invasive activity in preclinical models of human thyroid cancer. Oncologist. 2011;16(3):296–309.
63. Xing J, Liu R, Xing M, Trink B. The BRAFT1799A mutation confers sensitivity of thyroid cancer cells to the BRAFV600E inhibitor PLX4032 (RG7204). Biochem Biophys Res Commun. 2011;404(4):958–62.
64. Woyach JA, Shah MH. New therapeutic advances in the management of progressive thyroid cancer. Endocr Relat Cancer. 2009;16(3):715–31.
65. Hong DS, Cabanillas ME, Wheler J, Naing A, Tsimberidou AM, Ye L, et al. Inhibition of the Ras/Raf/MEK/ERK and RET kinase pathways with the combination of the multikinase inhibitor sorafenib and the farnesyltransferase inhibitor tipifarnib in medullary and differentiated thyroid malignancies. J Clin Endocrinol Metab. 2011;96(4):997–1005.
66. Sherman SI. Targeted therapy of thyroid cancer. Biochem Pharmacol. 2010;80(5):592–601.
67. Schweppe RE, Kerege AA, Sharma V, Poczobutt JM, Gutierrez-Hartmann A, Grzywa RL, et al. Distinct genetic alterations in the mitogen-activated protein kinase pathway dictate sensitivity of thyroid cancer cells to mitogen-activated protein kinase kinase 1/2 inhibition. Thyroid. 2009;19(8):825–35.
68. Henderson YC, Ahn SH, Clayman GL. Inhibition of the growth of papillary thyroid carcinoma cells by CI-1040. Arch Otolaryngol Head Neck Surg. 2009;135(4):347–54.
69. Vivaldi A, Miasaki FY, Ciampi R, Agate L, Collecchi P, Capodanno A, et al. Re-differentiation of thyroid carcinoma cell lines treated with 5-Aza-2'-deoxycytidine and retinoic acid. Mol Cell Endocrinol. 2009;307(1–2):142–8.
70. Akagi T, Luong QT, Gui D, Said J, Selektar J, Yung A, et al. Induction of sodium iodide symporter gene and molecular characterisation of HNF3 beta/FoxA2, TTF-1 and C/EBP beta in thyroid carcinoma cells. Br J Cancer. 2008;99(5):781–8.
71. Fernandez CA, Puig-Domingo M, Lomena F, Estorch M, Camacho Marti V, Bittini AL, et al. Effectiveness of retinoic acid treatment for redifferentiation of thyroid cancer in relation to recovery of radioiodine uptake. J Endocrinol Invest. 2009;32(3):228–33.

72. Riesco-Eizaguirre G, Santisteban P. New insights in thyroid follicular cell biology and its impact in thyroid cancer therapy. Endocr-Relat Cancer. 2007;14(4):957–77.
73. Dumont JE, Lamy F, Roger P, Maenhaut C. Physiological and pathological regulation of thyroid cell proliferation and differentiation by thyrotropin and other factors. Physiol Rev. 1992;72(3):667–97.
74. Watanabe H, Gould MN, Mahler PA, Mulcahy RT, Clifton KH. The influence of donor and recipient age and sex on the quantitative transplantation of monodispersed rat thyroid cells. Endocrinology. 1983;112(1):172–7.
75. Thomas T, Nowka K, Lan L, Derwahl M. Expression of endoderm stem cell markers: evidence for the presence of adult stem cells in human thyroid glands. Thyroid. 2006;16(6):537–44.
76. Malaguarnera R, Frasca F, Garozzo A, Giani F, Pandini G, Vella V, et al. Insulin receptor isoforms and insulin-like growth factor receptor in human follicular cell precursors from papillary thyroid cancer and normal thyroid. J Clin Endocrinol Metab. 2011;96(3):766–74.
77. Lan L, Cui D, Nowka K, Derwahl M. Stem cells derived from goiters in adults form spheres in response to intense growth stimulation and require thyrotropin for differentiation into thyrocytes. J Clin Endocrinol Metab. 2007;92(9):3681–8.
78. Cannito S, Novo E, di Bonzo LV, Busletta C, Colombatto S, Parola M. Epithelial-mesenchymal transition: from molecular mechanisms, redox regulation to implications in human health and disease. Antioxid Redox Signal. 2010;12(12):1383–430.
79. Coclet J, Lamy F, Rickaert F, Dumont JE, Roger PP. Intermediate filaments in normal thyrocytes: modulation of vimentin expression in primary cultures. Mol Cell Endocrinol. 1991;76(1–3):135–48.
80. Coclet J, Foureau F, Ketelbant P, Galand P, Dumont JE. Cell population kinetics in dog and human adult thyroid. Clin Endocrinol (Oxf). 1989;31(6):655–65.
81. Riesco-Eizaguirre G, Rodriguez I, De la Vieja A, Costamagna E, Carrasco N, Nistal M, et al. The BRAFV600E oncogene induces transforming growth factor beta secretion leading to sodium iodide symporter repression and increased malignancy in thyroid cancer. Cancer Res. 2009;69(21):8317–25.
82. Zhu W, Hai T, Ye L, Cote GJ. Medullary thyroid carcinoma cell lines contain a self-renewing CD133+ population that is dependent on ret proto-oncogene activity. J Clin Endocrinol Metab. 2010;95(1):439–44.
83. Santarpia L, Ye L, Gagel R. Beyond RET: potential therapeutic approaches for advanced and metastatic medullary thyroid carcinoma. J Intern Med. 2009;266(1):99–113.

Genetic Targets in Pediatric Acute Lymphoblastic Leukemia

Chandrika Gowda and Sinisa Dovat

Abstract Acute leukemia represents 31% of all cancers diagnosed in children and 80% of it is of Lymphoblastic type. Multiple genetic lesions in the hematopoietic progenitor cells prior to or during differentiation to B and T cell lead to development of leukemia. There are several subtypes of Acute Leukemia based on chromosome number changes, the presence of certain translocations and gene mutations, each of which has different clinical, biological and prognostic features. High throughput genomic technologies like array-based comparative genomic hybridization (array-CGH) and single nucleotide polymorphism microarrays (SNP arrays), have given us insight through a very detailed look at the genetic changes of leukemia, specifically, ALL. Here, we discuss various genetic mutations identified in Acute Lymphoblastic Leukemia. We also explore various genetic targets and currently available as well as upcoming targeted therapies for ALL.

Keywords Pediatric ALL • B-cell • Immunophenotype • Fanconi anemia • Down's syndrome • Bloom's syndrome • Ataxia telangiectasia • Neurofibromatosis • Leukemia • ALL • Array CGH • SNP • RUNX1 • MLL • BCR-ABL • IKZF1 • CRLF2 • E2A-PBX1 • E2A-HLF • FLT3 • Ras • Gamma sectretase • TKI • ETV6-RUNX1 • Dasatinib • Ikaros • TdT • JAK mutation • STAT • PAX5 • NOTCH • FBXW7 • PTEN • PI3K • Akt • LMO1 • TAL1 • HOX11 • MYB

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W.S. El-Deiry (ed.), *Impact of Genetic Targets on Cancer Therapy*, Advances in Experimental Medicine and Biology 779, DOI 10.1007/978-1-4614-6176-0_15,

Introduction

Acute leukemia is the most common malignancy of childhood. It represents 31% of all cancers diagnosed in children [1]. About 3,250 cases of acute leukemia are diagnosed per year in United States. Approximately 80% of the childhood acute leukemia is lymphoblastic. 80% of Lymphoblastic leukemia in children between ages 2–10 years is of Pre B- cell immunophenotype and the rest are T cell lineage. Adolescents and young adults tend to have myeloid malignancies. There are several subtypes within these broad subgroups based on chromosome number changes, presence of certain translocations and gene mutations. Each of these subtypes have different clinical, biological and prognostic features.

Etiology and Pathogenesis

Exact etiology and pathogenesis of all types of childhood leukemia is still unknown. Only less than 5% cases are explained by inherited, predisposing genetic syndromes, such as Down's syndrome, Neurofibromatosis, Fanconi anemia, Bloom's syndrome, ataxia-telangiectasia, and Nijmegen breakage syndrome, or exposure to ionizing radiation or to specific chemotherapeutic drugs. There is evidence suggesting a prenatal origin for some types of childhood leukemia [2, 3]. Multiple genetic lesions in the hematopoietic progenitor cells prior to or during differentiation to B and T cell lead to development of leukemia. These mutations affect their ability of unlimited self renewal which leads to arrest at that specific developmental stage. Understanding the outcomes of frequently arising genetic lesions and their effects on cell survival, proliferation and differentiation will help researchers then to devise selectively targeted treatments against the altered gene products to which the leukemic clones have become addicted.

Current Treatment and Need for Targeted Therapy

About 60 years back, acute leukemia was universally fatal. Thanks to multicenter, national and international clinical trials, collaborations and basic science research, tremendous progress has been made in this field which has made childhood leukemia a success story of twentieth century. Cure rate for leukemia has increased from 10% to nearly 85% [4].

Current treatment of leukemia is based on intense multiagent chemotherapy and prophylaxis of central nervous system. Risk assessment and treatment allocation is made based on clinical features (age and white cell count at diagnosis), biological features (B or T cell immunophenotype) and response to initial treatment (morphological and minimal residual disease in bone marrow at the end of induction therapy) [5].

Despite high cure rate, nearly one quarter of children with leukemia of certain molecular subtypes, high risk clinical features and those who relapse, have poor outcome. Significant proportions of the children who fall into standard risk category (age 1–10 years and total white count at diagnosis <50,000 and Precursor B cell Immunophenotype) have treatment failure or relapse [6]. Outcome of these children is poor, despite intense chemotherapy and/or allogenic hematopoietic stem cell transplant. Relapsed ALL is a leading cause of cancer related death. There is little room for intensification of already intense chemotherapy due to dose limiting toxicities and related morbidity and mortality. There is need for development of new targeted therapies which can improve outcome in this group of patients and have less side effects [7].

Molecular Genetics of Acute Lymphoblastic Leukemia (ALL)

It is very important to indentify genetic and epigenetic abrasions of prognostic importance in order to assign the patients to modern classification protocol and offer treatment [8, 9]. About 25% of the primary genetic lesions in ALL cannot be detected by standard genetic analysis. Currently, high throughput genomic technologies like array-based comparative genomic hybridization (array-CGH) and single nucleotide polymorphism microarrays (SNP arrays), have given us insight into very detailed look at the genetic changes of leukemia, specifically, ALL. Multiple novel submicroscopic genetic alterations in ALL samples which are not detectable by cytogenetic analysis have been identified [10]. Highly informative array-CGH using bacterial artificial chromosomes (BACs) typically use probes derived from large (up to 200 kb) fragments of human DNA cloned into BAC vectors [11]. Oligo nucleotide arrays use smaller probes (20–100 bp) for more detailed look at the genomic regions. Oligo CGH array is used for detection of copy number abnormality (CNA) and Single nucleotide polymorphism (SNP) array is used to detect both CNA and copy neutral Loss of Heterozygosity (LOH).

Table 1 shows important genetic alterations seen in B-cell and Table 2 shows important genetic alterations indentified in T cell ALL. Figure 1 shows important intracellular pathways, targets and corresponding therapeutic agents that are under investigation. We will discuss below in detail about some of the most important genetic alterations.

ETV6-RUNX1

ETV6-RUNX1 formerly known as TEL-AML1, is translocation (12; 21) resulting in fusion of the *ETV6* gene from chromosome band 12p13 to the *RUNX1* gene from chromosome band 21q21. It is associated with recruitment of complexes containing

Table 1 Genetic abnormalities identified in B cell ALL

Genetic sub type	Clinical relevance
Hyperdiploidy (>50 chromosomes)	Good prognosis with therapy
ETV6-RUNX1 t(12;21)	Prenatal translocation, good prognosis with chemotherapy
MLL rearrangement t(4,11)(q23;p13); t(11:19); t(9:11)	Eighty percent infant leukemia, poor prognosis, over expression of FLT3
BCR-ABL t(9:22)	Poor prognosis; associated *IKZF1* or *CDKN2A* deletions
IKZF1 deletion/mutation	25 to 30% of B cell ALL and 80% of BCR-ABL + ALL; increased risk of relapse
JAK mutations	Predominantly in High risk leukemia; potential response to JAK 2 inhibitors
CRLF2 overexpression	Poor prognosis; 55% of Down syndrome ALL
PAX 5	Mutations found in 31% of pediatric ALL (43)
E2A-PBX1 t(1:19)	Associated with poor prognosis
MYC t(8,14);t(2,8);t(8,22)	Favorable prognosis
Internal amplification of Chromosome 21	Common in older children, poor outcome
E2A-HLF	Adolescent presentation, hypercalcemia, and disseminated intravascular coagulation

Table 2 Important genetic alterations identified in T cell ALL

Genetic sub type	Clinical relevance
TAL1/SCL t(1;14)	~30% of ALL; Good prognosis
HOX11L2 (5q35)(TLX3)	Poor prognosis in some studies
HOX11(10q24)	Favorable prognosis
NOTCH/FBXW7	Intrageneic gain of function mutation in ~55%; potentially responsive to NOTCH inhibitor
PTEN-P13K-AKT	Resistance to Gamma secretase inhibitor
CDKN2A/2B	?response to DNA methylation inhibitors
LMO1 & LMO2	Good prognosis in some studies, response to HDAC inhibitors
IKAROS	Mutation/deletion in 5–10% T cell ALL

histone deacetylases to AML1 target genes, causing aberrant transcriptional repression [11–15]. It is the most common chromosomal translocation seen in children with 'Common Precursor B cell ALL' (25%) but rarely observed in T cell ALL [12]. It is cryptic by conventional karyotyping but detected by FISH or molecular analysis. Translocation (12;21) [12, 16] was noted in a large number of archived neonatal blood samples suggesting prenatal origin but, only 1% actually developed T cell leukemia indicating that additional mutations later in life are necessary for leukemogenesis [2, 3]. ETV6 -RUNX1 is known to be associated with favorable outcome [12].

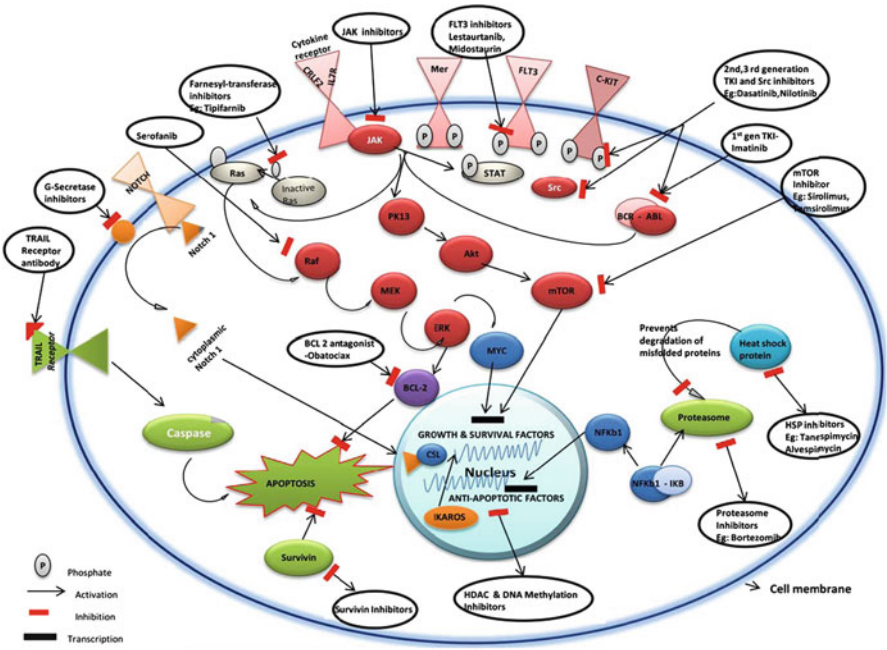

Fig. 1 Cellular pathways and genetic targets with corresponding inhibitors under investigation for targeted therapy of leukemia

BCR-ABL

The Philadelphia chromosome is characterized by the abnormal transposition of the q34 portion of chromosome 9 and the q11 portion of chromosome 22. A reciprocal translocation causes a head to-tail fusion of the breakpoint cluster region (*BCR*) gene on chromosome 22 with the cellular homolog of the Abelson (*c-ABL*) viral oncogene on chromosome 9, thereby placing the *BCR-ABL* oncogene under the control of the ubiquitously expressed *BCR* promoter. *BCR-ABL* encodes two main BCR-ABL fusion oncoproteins of distinct molecular weights, p190 and p210, that arise from different translocation breakpoints in the *BCR* gene. The p210 isoform is expressed in nearly one third of adult Ph+ B-ALL, with the other 2/3rd of adult Ph+ B-ALL expressing the p190 isoform. Approximately 90% of childhood Ph+ B-ALL cases express p190 [17]. BCR-ABL1 positive ALL is highly aggressive and has a poor prognosis [18, 19]. BCR-ABL is seen in 25–40% of adult CML and 3–5% of pediatric B cell -ALL.CML typically responds well to kinase inhibitors. BCR-ABL is a deregulated, constitutively active non receptor tyrosine kinase, and this kinase activity is required for cell transformation. BCR-ABL promotes leukemia mainly through two signal transduction pathways (RAS-MAPK and

PI3K-AKT) that control cell proliferation, size, survival, and activation [20]. The constitutively active BCR-ABL1 cell impedes programmed cell death by keeping pro apoptotic protein in phosphorylated state and impeding it from localizing to mitochondria [21].

Targeted Therapy for BCR-ABL

Prior to use of tyrosine kinase inhibitors, BCR-ABL positive ALL was one of the worst prognostic groups in pediatric ALL [16]. Imatinib Mesylate is a small orally available molecule which acts by binding to the ATP binding site of tyrosine kinase and stabilizing the inactive conformation. Imatinib showed remarkable a result in adults with CML. It is the best available first line therapy for CML in chronic phase [22]. Combination of Imatinib with chemotherapy in adults with Ph+ ALL showed encouraging results but the results were short lived when used as single agent. Children's oncology group (COG) clinical trial COGALL0031 conducted between 2002 and 2006 used Imatinib in children with Ph+ ALL starting after induction chemotherapy. It showed 3 years EFS of 80% which is more than double the EFS of historic control group treated without tyrosine kinase inhibitor (TKI) in the past [23]. The outcome has remained stable in this patient cohort .

Dasatinib is a second generation TKI with potent BCR-ABL kinase inhibitor activity and active against most Imatinib resistant BCR-ABL-mutants (except T3135). Dasatinib also inhibits SRC kinase and is an attractive therapy in Ph+ ALL . Unlike CML, signaling through Src family kinases is required for development of leukemia. COG study AALL0622 is now testing addition of Dasatinib to same intense chemotherapy regimen.

MLL Rearrangement

The *mixed lineage leukemia* (*MLL*) gene encodes a large complex oncoprotein that regulates transcription.MLL methylates histone H3 lysine 4 (H3K4) and regulates gene expression (especially *HOX* family gene expression) to control early hematopoietic progenitor cell development. MLL gene rearrangements are seen in over 80% of Infant leukemia and 10% of childhood ALL cases [24, 25]. More than 40 different balanced chromosomal translocations have been identified as partners for *MLL* in ALL. The five most common *MLL* rearrangements, seen in *MLL*-translocated leukemia are,

t (4; 11)(q21;q23)-encoding MLL-AF4 (seen in 70% cases)
t (11; 19) (q23;p13.3)-encoding MLL-ENL (seen in 13% cases),
t (9; 11)(p22;q23)-encoding MLL-AF9,
t (10; 11) (p12;q23)-encoding MLL-AF10,
t (6;11)(q27;q23)-encoding MLL-AF6.

FLT3

FLT3 in-frame deletions and internal tandem duplications (ITDs) in the juxtamembrane region and point mutations in the activation loop of the kinase domain results in FLT3 protein over expression and constitutive activation of FLT3 signaling pathways through STAT5, MAP kinase, and AKT. FLT3-ITD mutations are found in approximately 2% of childhood ALL and are associated with poor prognosis. Lestaurtanib is a selective FLT3 inhibitor which has shown promising results in primary infant leukemia and ALL cells with high expression of constitutively activated FLT3. In COG phase three study AALL0631, Lestaurtanib followed by chemotherapy is being tested in infants with MLL rearranged leukemia.

IKZF1

Ikaros encodes a tumor suppressor zinc finger protein that is involved in heritable gene silencing. In hematopoietic cells, Ikaros localizes to pericentromeric heterochromatin (PC-HC) where it recruits its target genes, resulting in their activation or repression via chromatin remodeling [26–28]. The function of Ikaros is controlled by posttranslational modifications. Ikaros is shown to be phosphorylated by CK2 kinase at its C terminus, affecting cell cycle progression [29–31]. Reversible phosphorylation of Ikaros at specific amino acids controls its sub cellular localization as well as its ability to regulate TdT expression during thymocyte differentiation. PP1 regulates thymocyte differentiation by controlling Ikaros' association with chromatin remodeling complexes and its ability to repress the transcription of developmentally regulated genes [32, 33].

Deletion or sequence mutation of the IKZF1 gene, is a hallmark of HR childhood ALL [34, 35]. Deletion of IKZF1 is present in over 80% of cases of BCR-ABL+ lymphoid leukemia, either de novo Ph+ ALL or chronic myeloid leukemia (CML) at progression to lymphoid blast crisis. The deletions either involve entire IKZF1 locus, resulting in loss of function, or delete an internal subset of IKZF1 exons, resulting in the expression of dominant negative IKZF1 alleles. Expression of such dominant negative IKZF1 alleles in hematopoietic progenitors impairs lymphoid development, and loss of IKZF1 accelerates the onset of Ph+ ALL in a retroviral BM transplant and transgenic models of this disease [36]. BCR-ABL negative ALL cases with deletion or sequential mutation of IKZF1 have are shown to have higher chance of treatment failure [37, 38].

JAK Mutations

The Janus kinase (JAK) family of tyrosine kinases is activated by cytokine binding to a Type I cytokine receptor. Activation of JAK leads to phosphorylation of STAT, and subsequent activation of both the RAS/RAF and PI3K/AKT pathways, ultimately

leading to cell proliferation. In ALL cell lines, members of this JAK family are abundantly expressed. JAK2 has been noted to be expressed more frequently than JAK1 or JAK [39, 40]. Constitutively active JAK/STAT results in uncontrolled proliferation of leukemia cells and has been associated with poor prognosis [41]. Activating mutations of JAK also correlate with other gene abnormalities, IKZF1 deletion or mutation and genomic rearrangement involving the Cytokine receptor-like factor 2 gene (CRLF2) which results in its over expression, both of which confer poor prognosis. JAK family of kinases, are mutated in Down syndrome-ALL and High risk non-DS ALL. Inhibitors targeting JAK pathways are currently being tested in clinical trials for adults. INCB018424 is a competitive ATP inhibitor that binds to the catalytic domain of JAK1/2. This agent is known to inhibit both wild-type and mutated JAK proteins. COG trial ADVL1011 is a single-agent phase I trial for children with relapsed/refractory solid tumors, leukemias, and myeloproliferative neoplasms.

CRLF2 is a subunit of the type I cytokine receptor, which forms a heterodimer with interleukin seven receptor (IL7R). Cytokine binds to the receptor and stimulates B-cell proliferation. Rearrangements involving CRLF2 have causes constitutive dimerization with IL7R, resulting in cytokine-independent activation of JAK2 and STAT5. This leads to subsequent B-cell proliferation, and possibly cell transformation, especially in the presence of a constitutively activated JAK mutation [41]. Targeting cells with activated JAK mutations may help to improve prognosis for patients with IKAROS mutations and CRLF-2 over expression because of the known high-frequency association of these abnormalities. 30% of childhood 'BCR-ABL1-like' ALL cases harbor rearrangements of the lymphoid cytokine receptor gene CRLF2, either alone or with concomitant mutation of the Janus kinase genes JAK1 and JAK2 [40–42].

PAX 5 Mutations

PAX5 encodes a gene required for B lymphoid lineage maturation. Recent SNP array and genomic DNA sequencing on B cell ALL samples have shown deletion and point mutation in 32% of cases [43]. Altered PAX5 may cause differentiation blockade in B cell development by targeting transcription factor genes known to be involved in B and T cell differentiation (IKAROS -IKZF1, and AIOLOS -IKZF3) [44–46].

E2A-PBX1

Translocation (1;19) is found in 3–5% of B-ALL cases. *E2A* encodes class I b Helix-loop -Helix (HLH) E47 and E12 E-box transcription factors that regulate the common lymphoid progenitor (CLP) to pre-pro-B cell transition in early B cell development. At (1; 19) (q23; p13) fuses the *PBX1* class II divergent *HOX* gene to *E2A* which encodes a chimeric transcription factor that binds and sequesters normal

PBX partners leading to repression of E2A target genes. This leads to uncontrolled cell-cycle progression [47]. This translocation is mostly seen in cytoplasmic Immunoglobulin positive (cIg+) Pre B ALL rather than cIg negative B -ALL and is associated with poor prognosis in those cases.

E2A-HLF

Translocation (17; 19) *E2A* variant translocation occurs in 1% of cases of childhood B-cell precursor ALL, which creates an *E2A-HLF* (hepatic leukemia factor) fusion gene. The novel chimeric transcription factor E2A-HLF promotes aggressive, treatment-resistant pro–B cell stage ALL that shows unique clinical associations including adolescent presentation, hypercalcemia, and disseminated intravascular coagulation [48].

TAL1/SCL

TAL1 (SCL) gene at Chromosome band 1p34 encodes a class II basic Helix loop helix (bHLH) transcription factor that is a master regulator of hematopoietic lineage commitment. *SCL* is a target for translocation or mutation in nearly 25–30% of childhood T-ALL cases. Translocation t(1;14)(p34;q11), and deletions aberrantly activating *SCL* during thymocyte maturation causes leukemia by promoting transformation.

Homeobox (HOX) Genes

Homeobox genes regulate axial patterning and cellular differentiation during embryonic development. HOX A cluster which belongs to Class I HOX is implicated in T cell leukemia.

HOX11 (also known as *T cell leukemia*, *homeobox 1* and *TLX1*) is a class II orphan *HOX* gene that is normally required for survival of splenic precursors during organogenesis. Translocation t(10;14)(q24;q11) or t(7;10)(q34;q24), causes juxtaposition of HOX11 to *TCR α/δ*- or *TCR β*-loci regulatory elements leading to increased expression of HOX11. Over expression of HOX11 is found in about 5% of pediatric T cell-ALL. Loss of negative regulatory elements with cytogenetic rearrangements or by loss of silencing DNA methylation also causes aberrant HOX expression. HOX11-containing T-ALL has a better prognosis than other T-ALL subtypes [17, 49–51].

HOX11L2 (*TLX3*) is another well-studied class II orphan *HOX* gene that undergoes a t(5;14)(q35;q32), bringing it under the influence of *TCRα-/δ*-regulatory elements downstream of *BCL11B* (a gene expressed throughout T cell development) in ~20% of children with T-ALL and these cases have less favorable prognosis compared to HOX11 positive T cell ALL [16–52].

NOTCH1

NOTCH is a transmembrane heterodimeric receptor. Sequentially cleavage of NOTCH by an ADAM metalloproteinase and then c-secretase, releases the intracellular domain Notch1 (ICN1). There it forms a transcription complex which functions as a transcription activator that regulates T-cell development in normal cells, and has been shown to activate transcription of genes such as MYC and NFKB1. Translocation t (7; 9) (q34; q34.3), fuses *TCRB* to the gene encoding the NOTCH1 and is extremely uncommon. It is found in less than 1% of T cell ALL. Gain-of-function intrageneic mutation in NOTCH1 were recently discovered in ~55% of translocation negative T-ALL cases, which results in ligand-independent cleavage of Notch1 [53, 54]. This process still needs gamma secretase proteolysis to release active ICN1 which makes Gamma secretase Inhibitors (GSI) attractive therapy for NOTCH1 altered T cell ALL. GSIs are under development, and being tested in phase I trials [55–57].

PTEN

PTEN is a tumor suppressor with lipid and protein phosphatase activity that opposes the receptor tyrosine kinase–PI3K-induced activation of AKT. *PTEN* is mutated and is the most consistently down regulated gene in GSI-resistant T-ALL cell lines. Gain-of-function *NOTCH1* mutations and mutational loss of *PTEN* are associated with resistance to GSIs in T-ALL. This is because the malignant clone transfers its oncogene addiction from constitutive NOTCH1 signaling to constitutive PI3K-AKT signaling.

FBXW7

FBW7 (F-box- and WD repeat domain–containing 7) is a protein substrate recognition subunit of the SCF-type E3 ubiquitin ligases. It is mutated in a wide range of human cancers, where it functions as a tumor suppressor. FBW7 mutation block FBW7-mediated ICN1 and possibly MYC degradation, leading to excessive NOTCH pathway signaling [58, 59]. FBW7 mutations make T-ALL cell lines and relapsed T-ALL insensitive to GSIs. Mechanism for drug resistance that is potentially related to stabilization of MYC expression. FBW7 mutations may also coexist with NOTCH1 heterodimerization–domain mutations to further augment NOTCH pathway signaling [59].

LYL1

LYL1 encodes another class II basic helix loop helix transcription factor that forms heterodimers with class I bHLH proteins, such as E2A (E47 and E12) and HEB. *LYL1* was identified from a t(7;19)(q35;p13) in a T cell leukemia line and is aberrantly expressed in only a few T-ALL cases [17, 59, 60]. LYL1 has an unknown cellular function, but it has an overlapping expression pattern with TAL1.

MYB

MYB is the cellular homolog of the *v-Myb* oncogene which is essential for T cell development in mouse. Translocation and duplication involving MYB is detected in 8–15% of T cell ALL cases leading to *MYB* over expression and a blockade in T cell differentiation. Translocation t(6; 7)(q23;q34), juxtaposes the *C-MYB* gene at chromosome band 6q23 with the *TCRB* locus. Interestingly, translocation t(6; 7) is noted in younger children with T cell ALL. These cases also contain NOTCH1 mutations and CDNK2A p16 ARF deletions. This translocation is readily detectable by FISH but not by conventional karyotyping due to subtelomeric location of C-MYB and TCRB.

LMO1 and LMO2

LMO1 and LMO2 are oncogenic transcription factors, when fused to different TCR loci lead to unscheduled expression of the respective transcription protein. *LMO1* (e.g., *RBTN1, TTG1*) and *LMO2* (e.g., *RBTN2, TTG2*) genes encode cysteine-rich tandem LIM–only domain-containing proteins that interact with a variety of nuclear factors, including TAL1 in erythroid cells. LMO 2 translocations occur in 10–20% T cell ALL cases.

Conclusion

Detailed information about genetic alterations in Leukemia is being generated as a result of high throughput genomic analysis tools and many potential targets for therapy have been identified. Ideal ‘target’ is a protein or pathway which is specific to the tumor cell, not shared by normal cells, essential for tumor cell maintenance and/or proliferation and is easily accessible by therapeutic agent. Understanding these targets will help us identify and develop best targeted therapies for childhood leukemia.

Acknowledgement This work was supported in part by an R01 HL095120 grant, The Four Diamonds Fund of the Pennsylvania State University College of Medicine, John Wawrynovic Leukemia Research Scholar Endowment (SD), St.Baldrick’s foundation grant.

References

1. Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. Lancet. 2008;371(9617):1030–43. Epub 2008/03/25.
2. O’Leary M, Krailo M, Anderson JR, Reaman GH, Children’s Oncology G. Progress in childhood cancer: 50 years of research collaboration, a report from the Children’s oncology group. Semin Oncol. 2008;35(5):484–93. Epub 2008/10/22.

3. Wiemels JL, Cazzaniga G, Daniotti M, Eden OB, Addison GM, Masera G, et al. Prenatal origin of acute lymphoblastic leukaemia in children. Lancet. 1999;354(9189):1499–503. Epub 1999/11/07.
4. Armstrong SA, Look AT. Molecular genetics of acute lymphoblastic leukemia. J Clin Oncol. 2005;23(26):6306–15. Epub 2005/09/13.
5. 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. Epub 2006/09/28.
6. Borowitz MJ, Devidas M, Hunger SP, Bowman WP, Carroll AJ, Carroll WL, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's oncology group study. Blood. 2008;111(12):5477–85. Epub 2008/04/05.
7. Weinstein IB, Joe AK. Mechanisms of disease: oncogene addiction—a rationale for molecular targeting in cancer therapy. Nat Clin Pract Oncol. 2006;3(8):448–57. Epub 2006/08/09.
8. Pui CH, Carroll WL, Meshinchi S, Arceci RJ. Biology, risk stratification, and therapy of pediatric acute leukemias: an update. J Clin Oncol. 2011;29(5):551–65. Epub 2011/01/12.
9. Wiemels J. Perspectives on the causes of childhood leukemia. Chem Biol Interact. 2012;196(3):59–67. Epub 2012/02/14.
10. Mullighan CG. Genomic analysis of acute leukemia. Int J Lab Hematol. 2009;31(4):384–97. Epub 2009/06/03.
11. Mullighan CG, Downing JR. Genome-wide profiling of genetic alterations in acute lymphoblastic leukemia: recent insights and future directions. Leukemia. 2009;23(7):1209–18. Epub 2009/02/27.
12. Zelent A, Greaves M, Enver T. Role of the TEL-AML1 fusion gene in the molecular pathogenesis of childhood acute lymphoblastic leukaemia. Oncogene. 2004;23(24):4275–83. Epub 2004/05/25.
13. Ford AM, Bennett CA, Price CM, Bruin MC, Van Wering ER, Greaves M. Fetal origins of the TEL-AML1 fusion gene in identical twins with leukemia. Proc Natl Acad Sci USA. 1998;95(8):4584–8. Epub 1998/05/16.
14. Pine SR, Wiemels JL, Jayabose S, Sandoval C. TEL-AML1 fusion precedes differentiation to pre-B cells in childhood acute lymphoblastic leukemia. Leuk Res. 2003;27(2):155–64. Epub 2003/01/16.
15. Greaves MF, Wiemels J. Origins of chromosome translocations in childhood leukaemia. Nat Rev Cancer. 2003;3(9):639–49. Epub 2003/09/03.
16. Arico M, Schrappe M, Hunger SP, Carroll WL, Conter V, Galimberti S, et al. Clinical outcome of children with newly diagnosed philadelphia chromosome-positive acute lymphoblastic leukemia treated between 1995 and 2005. J Clin Oncol. 2010;28(31):4755–61. Epub 2010/09/30.
17. Teitell MA, Pandolfi PP. Molecular genetics of acute lymphoblastic leukemia. Annu Rev Pathol-Mech. 2009;4:175–98. Epub 2008/09/12.
18. Clark SS, McLaughlin J, Crist WM, Champlin R, Witte ON. Unique forms of the abl tyrosine kinase distinguish Ph1-positive CML from Ph1-positive ALL. Science. 1987;235(4784):85–8. Epub 1987/01/02.
19. Ribeiro RC, Abromowitch M, Raimondi SC, Murphy SB, Behm F, Williams DL. Clinical and biologic hallmarks of the philadelphia chromosome in childhood acute lymphoblastic leukemia. Blood. 1987;70(4):948–53. Epub 1987/10/01.
20. Kharas MG, Fruman DA. ABL oncogenes and phosphoinositide 3-kinase: mechanism of activation and downstream effectors. Cancer Res. 2005;65(6):2047–53. Epub 2005/03/23.
21. Salomoni P, Condorelli F, Sweeney SM, Calabretta B. Versatility of BCR/ABL-expressing leukemic cells in circumventing proapoptotic BAD effects. Blood. 2000;96(2):676–84. Epub 2000/07/11.
22. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2003;348(11):994–1004. Epub 2003/03/15.

23. Arico M, Valsecchi MG, Camitta B, Schrappe M, Chessells J, Baruchel A, et al. Outcome of treatment in children with philadelphia chromosome-positive acute lymphoblastic leukemia. N Engl J Med. 2000;342(14):998–1006. Epub 2000/04/06.
24. Armstrong SA, Staunton JE, Silverman LB, Pieters R, den Boer ML, Minden MD, et al. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. Nat Genet. 2002;30(1):41–7. Epub 2001/12/04.
25. Armstrong SA, Mabon ME, Silverman LB, Li A, Gribben JG, Fox EA, et al. FLT3 mutations in childhood acute lymphoblastic leukemia. Blood. 2004;103(9):3544–6. Epub 2003/12/13.
26. Brown KE, Guest SS, Smale ST, Hahm K, Merkenschlager M, Fisher AG. Association of transcriptionally silent genes with ikaros complexes at centromeric heterochromatin. Cell. 1997;91(6):845–54. Epub 1997/12/31.
27. Dovat S, Payne KJ. Tumor suppression in T cell leukemia—the role of ikaros. Leuk Res. 2010;34(4):416–7. Epub 2009/11/07.
28. Payne KJ, Dovat S. Ikaros and tumor suppression in acute lymphoblastic leukemia. Crit Rev Oncog. 2011;16(1–2):3–12. Epub 2011/12/14.
29. Dovat S, Song C, Payne KJ, Li Z. Ikaros, CK2 kinase, and the road to leukemia. Mol Cell Biochem. 2011;356(1–2):201–7. Epub 2011/07/14.
30. Song C, Li Z, Erbe AK, Savic A, Dovat S. Regulation of Ikaros function by casein kinase 2 and protein phosphatase 1. World J Biol Chem. 2011;2(6):126–31. Epub 2011/07/19.
31. Li Z, Song C, Ouyang H, Lai L, Payne KJ, Dovat S. Cell cycle-specific function of ikaros in human leukemia. Pediatr Blood Cancer. 2012;59(1):69–76.
32. Gurel Z, Ronni T, Ho S, Kuchar J, Payne KJ, Turk CW, et al. Recruitment of ikaros to pericentromeric heterochromatin is regulated by phosphorylation. J Biol Chem. 2008;283(13): 8291–300. Epub 2008/01/29.
33. Popescu M, Gurel Z, Ronni T, Song C, Hung KY, Payne KJ, et al. Ikaros stability and pericentromeric localization are regulated by protein phosphatase 1. J Biol Chem. 2009;284(20):13869–80. Epub 2009/03/14.
34. 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. Epub 2008/04/15.
35. Mullighan CG, Su X, Zhang J, Radtke I, Phillips LA, Miller CB, et al. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. N Engl J Med. 2009;360(5):470–80. Epub 2009/01/09.
36. Tonnelle C, Dijon M, Moreau T, Garulli C, Bardin F, Chabannon C. Stage specific overexpression of the dominant negative ikaros 6 reveals distinct role of ikaros throughout human B-cell differentiation. Mol Immunol. 2009;46(8–9):1736–43. Epub 2009/03/17.
37. Waanders E, van der Velden VH, van der Schoot CE, van Leeuwen FN, van Reijmersdal SV, de Haas V, et al. Integrated use of minimal residual disease classification and IKZF1 alteration status accurately predicts 79 % of relapses in pediatric acute lymphoblastic leukemia. Leukemia. 2011;25(2):254–8. Official Journal of the Leukemia Society of America, Leukemia Research Fund, UK. Epub 2010/11/26.
38. Den Boer ML, van Slegtenhorst M, De Menezes RX, Cheok MH, Buijs-Gladdines JG, Peters ST, et al. A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. Lancet Oncol. 2009;10(2):125–34. Epub 2009/01/14.
39. Hunger SP, Raetz EA, Loh ML, Mullighan CG. Improving outcomes for high-risk ALL: translating new discoveries into clinical care. Pediatr Blood Cancer. 2011;56(6):984–93. Epub 2011/03/04.
40. Mullighan CG, Collins-Underwood JR, Phillips LA, Loudin MG, Liu W, Zhang J, et al. Rearrangement of CRLF2 in B-progenitor- and down syndrome-associated acute lymphoblastic leukemia. Nat Genet. 2009;41(11):1243–6. Epub 2009/10/20.
41. Mullighan CG, Zhang J, Harvey RC, Collins-Underwood JR, Schulman BA, Phillips LA, et al. JAK mutations in high-risk childhood acute lymphoblastic leukemia. Proc Natl Acad Sci USA. 2009;106(23):9414–8. Epub 2009/05/28.

42. Russell LJ, Capasso M, Vater I, Akasaka T, Bernard OA, Calasanz MJ, et al. Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. Blood. 2009;114(13):2688–98. Epub 2009/07/31.
43. Mullighan CG, Goorha S, Radtke I, Miller CB, Coustan-Smith E, Dalton JD, et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature. 2007;446(7137):758–64. Epub 2007/03/09.
44. Bousquet M, Broccardo C, Quelen C, Meggetto F, Kuhlein E, Delsol G, et al. A novel PAX5-ELN fusion protein identified in B-cell acute lymphoblastic leukemia acts as a dominant negative on wild-type PAX5. Blood. 2007;109(8):3417–23. Epub 2006/12/21.
45. Cortes M, Wong E, Koipally J, Georgopoulos K. Control of lymphocyte development by the ikaros gene family. Curr Opin Immunol. 1999;11(2):167–71. Epub 1999/05/14.
46. Georgopoulos K. Haematopoietic cell-fate decisions, chromatin regulation and ikaros. Nat Rev Immunol. 2002;2(3):162–74. Epub 2002/03/27.
47. Aspland SE, Bendall HH, Murre C. The role of E2A-PBX1 in leukemogenesis. Oncogene. 2001;20(40):5708–17. Epub 2001/10/19.
48. Hunger SP, Li S, Fall MZ, Naumovski L, Cleary ML. The proto-oncogene HLF and the related basic leucine zipper protein TEF display highly similar DNA-binding and transcriptional regulatory properties. Blood. 1996;87(11):4607–17. Epub 1996/06/01.
49. Dear TN, Colledge WH, Carlton MB, Lavenir I, Larson T, Smith AJ, et al. The Hox11 gene is essential for cell survival during spleen development. Development. 1995;121(9):2909–15. Epub 1995/09/01.
50. Roberts CW, Shutter JR, Korsmeyer SJ. Hox11 controls the genesis of the spleen. Nature. 1994;368(6473):747–9. Epub 1994/04/21.
51. Hatano M, Roberts CW, Minden M, Crist WM, Korsmeyer SJ. Deregulation of a homeobox gene, HOX11, by the t(10;14) in T cell leukemia. Science. 1991;253(5015):79–82. Epub 1991/07/05.
52. Su XY, Della-Valle V, Andre-Schmutz I, Lemercier C, Radford-Weiss I, Ballerini P, et al. HOX11L2/TLX3 Is transcriptionally activated through T-cell regulatory elements downstream of BCL11B as a result of the t(5;14)(q35;q32). Blood. 2006;108(13):4198–201. Epub 2006/08/24.
53. Weng AP, Ferrando AA, Lee W, Morris JP, Silverman LB, Sanchez-Irizarry C, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science. 2004;306(5694):269–71. Epub 2004/10/09.
54. Palomero T, Lim WK, Odom DT, Sulis ML, Real PJ, Margolin A, et al. NOTCH1 directly regulates c-MYC and activates a feed-forward-loop transcriptional network promoting leukemic cell growth. Proc Natl Acad Sci USA. 2006;103(48):18261–6. Epub 2006/11/23.
55. Milano J, McKay J, Dagenais C, Foster-Brown L, Pognan F, Gadient R, et al. Modulation of notch processing by gamma-secretase inhibitors causes intestinal goblet cell metaplasia and induction of genes known to specify gut secretory lineage differentiation. Toxicol Sci. 2004;82(1):341–58 An official Journal of the Society of Toxicology. Epub 2004/08/21.
56. Real PJ, Tosello V, Palomero T, Castillo M, Hernando E, de Stanchina E, et al. Gamma-secretase inhibitors reverse glucocorticoid resistance in T cell acute lymphoblastic leukemia. Nat Med. 2009;15(1):50–8. Epub 2008/12/23.
57. Ciofani M, Zuniga-Pflucker JC. Notch promotes survival of pre-T cells at the beta-selection checkpoint by regulating cellular metabolism. Nat Immunol. 2005;6(9):881–8. Epub 2005/08/02.
58. O'Neil J, Grim J, Strack P, Rao S, Tibbitts D, Winter C, et al. FBW7 mutations in leukemic cells mediate NOTCH pathway activation and resistance to gamma-secretase inhibitors. J Exp Med. 2007;204(8):1813–24. Epub 2007/07/25.
59. Thompson BJ, Buonamici S, Sulis ML, Palomero T, Vilimas T, Basso G, et al. The SCFFBW7 ubiquitin ligase complex as a tumor suppressor in T cell leukemia. J Exp Med. 2007;204(8):1825–35. Epub 2007/07/25.
60. Rubnitz JE, Pui CH. Recent advances in the treatment and understanding of childhood acute lymphoblastic leukaemia. Cancer Treat Rev. 2003;29(1):31–44. Epub 2003/03/14.

Emerging Molecular Therapies for the Treatment of Acute Lymphoblastic Leukemia

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Abstract The improved molecular understanding of cancer initiation, progression, and therapeutic resistance has yielded several novel molecular events that are being targeted by emerging therapies. While the treatment of ALL is a success story in the pediatric population, achieving a sustained remission in the adult population remains an area of investigation. Nevertheless, certain therapies have significantly improved the overall survival for adult ALL patients that should continue to improve with the discovery of better molecular targets and targeted agents. Here, we discuss novel therapeutic options under clinical investigation for the treatment of Philadephia chromosome negative ALL including immunotherapy, monoclonal antibodies, and small molecules that may be used as single agent or adjuvant therapy in the management of adult ALL.

Key words ALL • CD20 • Ofatumumab • Rituximab • Hyper CVAD • Anti-CD22 • CD22 • Epratuzumab • Anti-CD19 • CD19 • Blinatumumab • CD3 • SAR3419 • Cell death • Tubulin • Anti-CD52 • CD52 • Alemtuzumab • CART-19 • Notch inhibitor • Aurora kinase inhibitor • mTOR inhibitor • Polo-like kinase inhibitor • MDM2 inhibitor • p53 • MEK inhibitor • Proteasome inhibitor • Nucleoside analogue • Clofarabine • Forodesine • HDAC inhibitor • Demethylating agent

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W.S. El-Deiry (ed.), *Impact of Genetic Targets on Cancer Therapy*, Advances in Experimental Medicine and Biology 779, DOI 10.1007/978-1-4614-6176-0_16,

Introduction

Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer, with 6,050 newly diagnosed patients each year in the United States and 1,440 succumbing to the disease (SEER Database) [1]. ALL is characterized by impaired early lymphoid development and can be classified as either B-cell or T-cell ALL. B-cell ALL is more frequent than T-cell ALL, accounting for 83 % of ALL diagnoses and 30–40 % of all childhood cancers. Overall response rates are as high as 80 % in children, now approaching as high as 90 %, while series of adults rarely reach above 40 % long term survivorship [2, 3]. In adults, achieving first remission with standard chemotherapy regimens is feasible, whereas preventing or treating relapse is challenging. Unfortunately, the rate of relapse is as high as 70–80 % for ALL in the adult population [1, 4].

With the increasing availability of advanced molecular technology that has enabled genetic and genomic analysis, studies of the various stages of lymphoid cell development have revealed specific genetic mutations that eventually lead to ALL. This information has been of paramount importance in stratifying certain high-risk groups and identifying potential drug targets for targeted agents. Such novel drug targets include surface antigens that can be targeted by monoclonal antibodies and other proteins intimately involve in the oncogenesis and progression of ALL targeted by small molecules, as well immune response mechanisms that can be harnessed (Fig. 1). These new therapies have improved efficacy and safety profiles over traditional chemotherapies. Here, we review some of these agents that have been incorporated into the standard-of-care treatment for Philadephia chromosome negative ALL and several others that are being investigated in clinical trials (Table 1).

Monoclonal Antibodies

Anti-CD20

CD20 is a non-glycosylated integral membrane phosphoprotein that is specific to B lymphocytes and performs an important role in calcium transport across the cell membrane [5]. CD20 is expressed on normal and malignant B cells at varying levels but is not expressed on normal stem cells. The presence of CD20 in ALL has been known to portend a poor prognosis. Borowitz et al. studied 1,231 children >1 year of age with newly diagnosed precursor B-cell ALL and concluded that the level of CD20 expression measured by immunofluorescence was associated with prognostic significance and treatment failure [6]. Ofatumumab is a high affinity anti-CD20 antibody that is currently in a phase II clinical trial that is studying the drug in combination with standard chemotherapy in ALL (NLM Identifier NCT01363128, Clinicaltrials.gov).

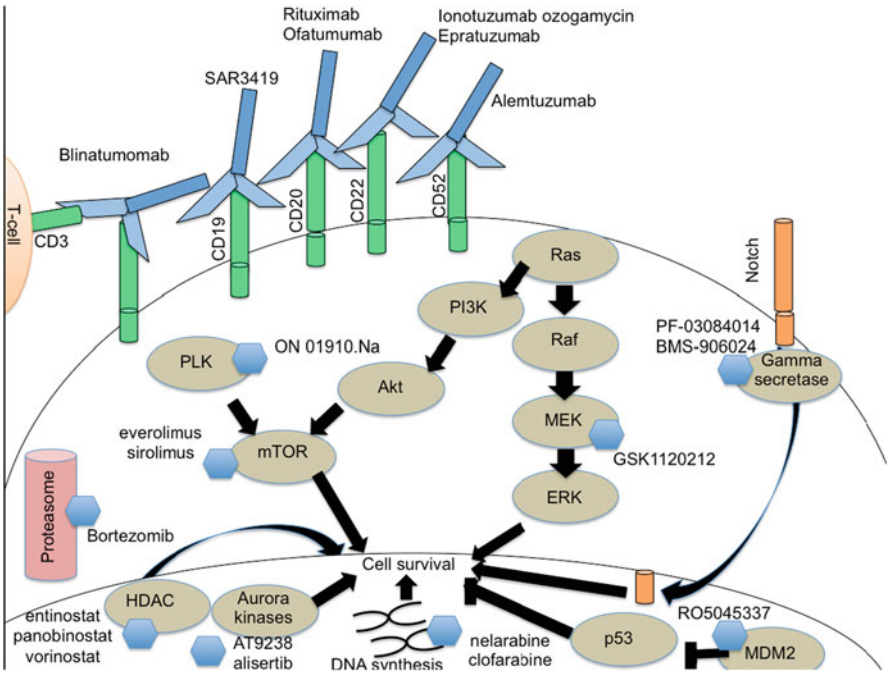

Fig. 1 Molecular targets of small molecules and antibodies being investigated in clinical trials for the treatment of ALL

Rituximab is a monoclonal chimeric mouse/human IgG antibody that binds to CD20. The mechanism of action for rituximab involves several events that include antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity, and apoptosis. Rituximab has changed the face of Non-Hodgkin's lymphoma (NHL) treatment, increasing overall survival by >20 % [7], [8]. Because CD20 is expressed on most mature ALL blasts, rituximab has been investigated as a treatment for ALL as well. Although CD20 is expressed in less than half of B-cell precursor ALL, it is interesting to note that after induction chemotherapy CD20 expression increases along with rituximab efficacy [9]. Rituximab is under investigation in large multicenter trials as a part of induction, salvage, and maintenance therapies for B-cell ALL.

Thomas et al. studied 282 adult and adolescent patients with precursor B-cell ALL that were Philadelphia chromosome-negative and a median age of 41 years. Patients with CD20 expression $\geq$ 20 % received rituximab as part of their initial chemotherapy along with standard Hyper CVAD. Though the complete remission (CR) was similar across all treatment groups, complete remission duration (CRD) in CD20-positive young patients was 70 % with an overall survival (OS) of 75 % at 3 years, as compared to the cohort that did not receive rituximab with a CRD of 38 % and an OS of 47 %. Further subset analysis revealed that rituximab improved the CRD and OS only in the younger group and that the older group (>60 years) did not show any significant improvement in CRD or OS despite

being CD20-positive [10]. A German study group performed a similar trial with rituximab as a part of initial chemotherapy. In this GMALL study 07/2003 protocol, young patients who were CD20-positive were treated with rituximab after being stratified into risk groups. Rituximab improved the CR rate from 57 % to 89 % in the "standard" risk group and also improved the OS from 54 % to 75 %. The high-risk group who had received rituximab were able to proceed to allogeneic stem cell transplant and had an improved OS [11].

Table 1 Open clinical trials investigating new treatments for ALL. Studies enriching for pediatric patients are highlighted in light blue

Type	Target	Therapy	Combination therapy	Phase	Malignancy	Age (years)	Sponsor	Target enrollment	Identifier
Antibody	CD20	Rituximab	mVPDL	II	Untreated Ph-negative ALL or LL	≥15	Asan Medical Center	77	NCT01429610
			Prednisone + etoposide + ifosfamide	-	Refractory/relapsed ALL	≥ 1 and ≤ 30	Emory University	15	NCT01230788
			Hyper CVAD	IV	CD20+ ALL	≥ 18 and ≤ 60	Ruijin Hospital	100	NCT01358253
			Standard chemotherapy	III	Newly diagnosed, untreated ALL	≥ 25 and ≤ 65	University College London Hospitals	720	NCT01085617
			Modified Hyper CVAD	II	Newly diagnosed, untreated ALL or LL	all	M.D. Anderson Cancer Center	280	NCT00671658
				II	B-ALL or Burkitt's NHL	≥15	Northern Italy Leukemia Group	113	NCT01290120
			Standard chemotherapy	II/III	B-ALL or other B-cell malignancies	≤ 18	Children's Oncology Group	640	NCT01595048
		Ofatumumab	Standard induction chemotherapy	III	Newly diagnosed, untreated ALL	≥ 25 and ≤ 65	University College London	720	UKALL 14
			Hyper CVAD	II	Newly diagnosed, previously untreated CD20+ Ph-negative ALL	all	M.D. Anderson Cancer Center	80	NCT01363128
	CD22	Ionotuzumab ozogamycin	Rituximab	I	Refractory/relapsed ALL	≥16	M.D. Anderson Cancer Center	90	NCT01134575
			Hyper CVAD + rituximab	I/II	Untreated Ph-negative pre-B ALL	> 60	M.D. Anderson Cancer Center	66	NCT01371630
		Epratuzumab	-	I/II	Refractory/relapsed ALL	≥ 18 and ≤ 70	Nantes University Hospital	9	NCT01354457
	CD19	Blinatumomab	-	II	Relapsed/Refractory B-Precursor ALL	≥18	Micromet GmbH	25	NCT01209286
				II	Minimum residual disease B-Precursor ALL	≥18	Micromet GmbH	21	NCT00560794
				I/II	Relapsed/Refractory B-Precursor ALL	<18	Micromet GmbH	84	NCT01471782
				II	Minimal Residual Disease of B-precursor ALL	≥18	Micromet GmbH	130	NCT01207388
				II	Relapsed/refractory Ph-negative B-precursor ALL	≥18	Micromet GmbH	61	NCT01466179
		SAR3419	-	II	Refractory/relapsed ALL	≥16	Sanofi-Aventis	51	NCT01440179
	CD52	Alemtuzumab	Methotrexate + vincristine + asparaginase + dexamethasone	II	Refractory/relapsed ALL	≥18	Eastern Cooperative Oncology Group	85	NCT00262925
			Fludarabine + busulfan + AlloSCT	I/II	ALL or other advanced hematological malignancies	≤ 70	Baylor College of Medicine	40	NCT01256398
			G-CSF	I/II	Refractory/relapsed ALL	≥15	Assistance Publique - Hôpitaux de Paris	15	NCT00773149
	Gamma secretase	PF-03084014	-	I	Refractory/relapsed ALL and/other refractory advanced cancers	≥16	Pfizer	60	NCT00878189
		BMS-906024	-	I	Refractory/relapsed T-ALL or T-cell LL	≥18	Bristol-Myers Squibb	30	NCT01363817
	Aurora kinase inhibitor	AT9238	-	I	Refractory/relapsed ALL, AML, or other acute leukemia	<18	Cancer Research UK	15	NCT01431664
		Alisertib	-	II	Refractory/relapsed ALL, AML, or refractory solid malignancies	≥ 1 and ≤ 21	Children's Oncology Group	228	NCT01154816
	mTOR	Everolimus	Re-Induction chemotherapy	I	ALL in first bone marrow relapse	≥ 1.5 and ≤ 21	Dana-Farber Cancer Institute	42	NCT01523977
			Hyper-CVAD or methotrexate + cytarabine	I/II	Refractory/relapsed ALL or LL	≥10	M.D. Anderson Cancer Center	42	NCT00968253
		Sirolimus	Methotrexate	II	Refractory/relapsed ALL or NHL	≤ 25	Children's Hospital of Philadelphia	17	NCT01162551
	PLK	ON 01910.Na	-	I/II	Refractory/relapsed ALL or AML	≥ 18	Onconova Therapeutics, Inc.	34	NCT01167166
	MDM2	RO5045337	-	I	ALL, AML, CML in blast phase, refractory CLL	≥ 18	Hoffmann-La Roche	140	NCT00623870
	MEK	GSK1120212	-	I/II	Refractory/relapsed ALL or other leukemias	≥ 18	GlaxoSmithKline	120	NCT00920140
	Proteasome	Bortezomib	Reinduction chemotherapy	II	Refractory/relapsed ALL or LL	> 1 and ≤ 31	Children's Oncology Group	60	NCT00873093
			Reinduction chemotherapy	I/II	Refractory/relapsed ALL	≥ 1 and ≤ 21	Therapeutic Advances in Childhood Leukemia Consortium	31	NCT00440726
			Vorinostat + dexamethasone	II	Refractory/relapsed ALL	≥ 1 and ≤ 30	Masonic Cancer Center, University of Minnesota	33	NCT01312818
			Belinostat	I	Refractory/relapsed ALL, AML, MDS, or CML	≥ 18	Virginia Commonwealth University	24	NCT01075425
			Mitoxantrone	I/II	Refractory/relapsed ALL or AML	≥ 18	Thomas Jefferson University	55	NCT00410423
			Nelfinavir Mesylate	I	Refractory/relapsed ALL or other advanced hematological malignancies	≥ 18	Swiss Group for Clinical Cancer Research	24	NCT01164709
	PNP	nelarabine	Etoposide + Cyclophosphamide	I/II	Refractory/relapsed T-ALL or LL	≥ 1 and ≤ 21	Therapeutic Advances in Childhood Leukemia Consortium	36	NCT00981799
			Hyper CVAD	II	Untreated T-ALL or LL	-	M.D. Anderson Cancer Center	60	NCT00501826
			-	IV	Untreated T-ALL or T-LBL	≤ 21	GlaxoSmithKline	40	NCT00866671
			Standard chemotherapy	III	Newly diagnosed T-ALL or T-NHL	≥ 1 and ≤ 30	Children's Oncology Group	1580	NCT00408005
			Standard chemotherapy	III	Newly diagnosed, untreated ALL	≥ 25 and ≤ 65	University College London Hospitals	720	NCT01085617

(continued)

Table 1 (continued)

Small molecule	DNA polymerases and ribonucleotide reductase	Clofarabine	Cimetidine	-	Ph-negative ALL, AML, MDS, CMML	≥ 18	UNC Lineberger Comprehensive Cancer Center	22	NCT01169012
			Cytarabine + radiation + AlloSCT	I/II	Refractory/relapsed ALL or AML	≤ 30	New York Medical College	40	NCT00529360
			TLI + ATG	I	ALL, AML, MDS, or refractory/relapsed NHL, HD, MM, CML	≥ 18 and ≤ 75	Beth Israel Deaconess Medical Center	30	NCT00697684
			Busulfan + AlloSCT	II	ALL, acute lymphoblastic lymphoma, or BL	≤ 65	M.D. Anderson Cancer Center	100	NCT00990249
			Thiotepa + busulfan + AlloSCT	II	Refractory/relapsed ALL and other hematological malignancies	≤ 60	M.D. Anderson Cancer Center	60	NCT00857389
			VANDA without cytarabine	I	Newly diagnosed ALL	≥ 1 and ≤ 23	University Hospital, Lille	20	NCT01279096
			Busulfan + ATG	II	ALL, AML, or MDS	≥ 18 and ≤ 65	Nantes University Hospital	30	NCT00863148
			Sorafenib + cytarabine	I	Refractory/relapsed ALL, AML, APL, MDS, or BL	≤ 31	St. Jude Children's Research Hospital	18	NCT00908167
			Cytarabine	II	MRD of relapsed/refractory ALL or AML	≥ 1 and ≤ 21	Therapeutic Advances in Childhood Leukemia Consortium	36	NCT01158885
			Cyclophosphamide	II	Refractory/relapsed ALL	≥ 18 and ≤ 60	Gruppo Italiano Malattie EMatologiche dell'Adulto	27	NCT01462253
			PEG-Asparaginase or cytarabine	II/III	ALL	≥ 1 and ≤ 17	Universitätsklinikum Hamburg-Eppendorf	660	NCT01228331
			Cytarabine	I/II	Refractory/relapsed ALL or AML	≥ 1 and ≤ 30	Children's Oncology Group	87	NCT00372619
			Entinostatin	I	Ph-negative ALL or BL	≥ 21	Sidney Kimmel Comprehensive Cancer Center	38	NCT01132573
			Cytarabine + epratuzumab	II	Refractory/relapsed Ph-negative T-ALL	≥ 16	Southwest Oncology Group	35	NCT00945815
			Melphalan + thiotepa + AlloSCT	I/II	ALL or other advanced hematological malignancies	≤ 54	Memorial Sloan-Kettering Cancer Center	42	NCT00423514
			Topotecan hydrochloride, vinorelbine ditartrate, thiotepa, and dexamethasone	I	Refractory/relapsed ALL or AML	≤ 28	Memorial Sloan-Kettering Cancer Center	23	NCT00462787
			AlloSCT	II	Refractory/relapsed ALL or AML	≥ 1 and ≤ 21	Lund University Hospital	10	NCT01025778
			Cyclophosphamide + etoposide + methotrexate + vincristine + PEG-Asparaginase	III	Ph-negative NCI high-risk ALL	≥ 1 and ≤ 30	Children's Oncology Group	4450	NCT01406756
			Prednisone + vincristine + cytarabine, doxorubicin + 6-mercaptopurine + methotrexate + PEG-Asparaginase	II	Untreated T-ALL or LL	≥ 51 and ≤ 75	Massachusetts General Hospital	30	NCT00973752
	HDAC1 and HDAC3	Entinostat	Imatinib	I/II	Refractory/relapsed Ph-positive ALL or AML	≥ 18	Sidney Kimmel Comprehensive Cancer Center	50	NCT01383447
	pan-HDAC	Panobinostat	-	I	Refractory/relapsed ALL, AML, HD, or NHL	≥ 8 and ≤ 21	Therapeutic Advances in Childhood Leukemia Consortium	36	NCT01321346
	pan-HDAC	Vorinostat	Bortezomib + dexamethasone	II	Refractory/relapsed ALL	≥ 2 and ≤ 30	Masonic Cancer Center, University of Minnesota	33	NCT01312818
			Decitabine	I/II	Refractory/relapsed ALL	≥ 1 and ≤ 21	Therapeutic Advances in Childhood Leukemia Consortium	16	NCT01483690
			Decitabine + vincristine + prednisone +doxorubicin + PEG-Asparaginase	II	Refractory/relapsed ALL or LL	≥ 2 and ≤ 60	Masonic Cancer Center, University of Minnesota	40	NCT00882206
CAR-modified T cells	-	CART-19	-	I	CD19+ ALL relapsed after AlloSCT	≥18	Abramson Cancer Center of the University of Pennsylvania	10	NCT01551043
				I	Refractory/relapsed ALL	≥18	Memorial Sloan-Kettering Cancer Center	18	NCT01044069
				-	CD19+ B-cell leukemia or lymphoma	≥18	Abramson Cancer Center of the University of Pennsylvania	10	NCT01029366
				I	CD19+ leukemia or lymphoma	≥18	University of Pennsylvania	10	NCT00891215
				I	CD19+ ALL relapsed after AlloSCT	≥18	Abramson Cancer Center of the University of Pennsylvania	10	NCT01551043

Based on these data, rituximab may be a promising targeted agent to improve the poor prognosis associated with CD20-positivity, though this benefit in the older population is less clear. A pilot study by Thomas et al. added rituximab to standard Hyper CVAD regimen in 76 patients. Unlike the previously discussed studies, patients were not differentiated on basis of CD20-positivity. There was no overall change in CR rate, but a significantly reduced relapse rate was observed and an improved 3-year OS especially in population over 60 years of age [12]. Rituximab appears safe and very promising as a frontline agent in combination with standard chemotherapy in ALL. Results from the UK National Cancer research Institute UKALL14 and French Group for research in Adult Acute Lymphoblastic Leukemia

GRALL2005 studies may provide sufficient data to enable the incorporation of rituximab into standard protocols for ALL in the future.

Anti-CD22

CD22 is an adhesion molecule that leads to down regulation of the B-cell receptor and CD19 expression upon phosphorylation. Expression of CD22 is associated with good prognosis in ALL [13]. CD22 is present on virtually all malignant B cells, making the cell surface protein another attractive target for antibody therapy. CD22 undergoes rapid internalization after binding an antibody, potentiating the conjugation of a cytotoxin such as calicheamicin to an anti-CD22 antibody to gain cytotoxicity. This delivery mechanism allows toxins such as calicheamicin to exert their cytotoxic effects selectively on tumor cells after being internalized without causing side effects on normal cells [14].

Inotuzumab ozogamycin is one such "immunotoxin" conjugate that is an anti-CD22 antibody coupled with calicheamicin. Calicheamicin is an extremely potent and toxic antibiotic that binds to the minor groove DNA, which results in free radical formation and DNA double-strand breaks. Results from a phase II trial evaluating inotuzumab were encouraging. Kantarjian and colleagues at MD Anderson recruited 49 adults and children with refractory or relapsed ALL to study efficacy of inotuzumab ozogamycin. CD22 was expressed in more than 50 % of blasts of all patients. There was a 57 % overall response with a median OS of 5.1 months. However, the OS was unchanged in patients with or without minimal residual disease (MRD). The most common adverse effects were fevers, hypotension, and liver function abnormalities (65 %) that were mostly reversible, though 13 % had severe liver impairment. Nevertheless, 20 of the patients proceeded to allogeneic stem cell transplant (SCT) and 25 % of these patients had veno-occlusive disease, which may be related to the liver function abnormalities caused by inotuzumab ozogamycin. Thus, Kantarjian and colleagues concluded that inotuzumab ozogamycin improves the response rate and may be a strong candidate for initial therapy of relapsed or refractory adult ALL [15]. A phase I clinical trial studying Inotuzumab ozogamycin in combination with rituximab as front-line therapy for refractory/relapsed ALL is underway (NLM Identifier NCT01134575; Clinicaltrials.gov).

Epratuzumab is an unconjugated anti-CD22 antibody under clinical investigation. This antibody has been studied in the pediatric population by Raetz and the Children's Oncology Group (COG) evaluating single-agent epratuzumab at four doses of 360 mg/m^2 administered intravenously twice weekly followed by four weekly doses of epratuzumab in combination with standard reinduction chemotherapy. CD22 expression was determined by flow cytometry using two distinct epitopes. Fifteen patients with relapsed or refractory ALL were enrolled. Morphologic and MRD responses were determined at the end of this 6-week period. All patient but one had no detectable CD22-positive cells by flow cytometry within 24 h following epratuzumab infusion, indicating effective depletion of the target cell population by the agent. Nine patients achieved complete remission and seven

were MRD negative. Toxicities were moderate with one patient experiencing a grade 4 seizure and another with grade 3 elevation in ALT [16].

Given these encouraging results with epratuzumab, this study was expanded to include a total of 116 children with relapsed ALL. Epratuzumab was added to an established chemotherapy regimen to improve the rates of second complete remission. The rate of MRD negativity increased from 25 % in controls to 42 % in the study group, indicating benefit with the addition of epratuzumab as measured by length of remission [17]. Southwest Oncology Group (SWOG) S0910 is also studying epratuzumab together with clofarabine and cytarabine for adult patients with relapsed and refractory B-ALL [18]. Another novel anti-CD22 immunotoxin, moxetumomab pasudotox, is currently being assessed in young adults and adolescents in a phase I trial to establish a dosing regimen for future trials [19].

Anti-CD19

CD19 is a pan B-cell antigen that acts as a B-cell receptor, controlling the proliferation and differentiation of B cells. It is also present on the earliest precursor B lymphocyte, making it amenable for the detection of cells in bone marrow to enable evaluation of therapeutic response in such specimens [20]. The current anti-CD19 antibodies under clinical study are unique from conventional monoclonal antibodies in their mechanism of action. Blinatumomab is a bispecific, single-chain antibody containing an anti-CD3 arm and an anti-CD19 arm. This allows the antibody to engage T-cells via its anti-CD3 arm upon binding to CD19 arm, causing T-cell activation. Upon stimulation by the antibody, T-cells are activated and exert cytotoxic effects via perforin-mediated cell death. T-cells are activated only when both the CD3 and CD19 arms are engaged and when the T-cells and B-cells make physical contact. This interesting concept forms the foundation of "Bi specific T cell engineering" [21], [22].

A phase II clinical trial evaluated blinatumomab in 21 ALL patients with MRD persistence or relapse. Blinatumomab was administered as a 4-week continuous intravenous infusion at 15 μg/m^2 over 24 h. Eighty percent of 20 patients changed to MRD-negative. Twelve of the 16 patients that were MRD-negative had been refractory in past, even with aggressive chemotherapy regimens. The relapse-free survival probability was 78 % with sustained responses in the majority of patients at 405 days follow up. Adverse events with this agent included lymphopenia, one patient experienced seizure, and another patient had a syncopal episode. No side effects from cytokine release were observed. It was noted that few patients had relapse in extramedullary sites such as cerebrospinal fluid and the testis, which are protected from T-cell responses. It was encouraging to see that these patients achieved remission with further blinatumomab therapy [23]. In a small pilot study by Handgretinger et al., three pediatric patients were treated with blinatumomab after SCT. This treatment induced a rapid change to MRD-negative status, was well tolerated and did not cause any signs of graft-versus-host disease despite recruitment of donor CD3 cells [24].

The German study group GMALL evaluated blinatumomab in adult patients with relapsed or refractory ALL. Interim analysis on 18 patients that received blinatumomab reported that 12 patients achieved CR, of which 3 patients had the t(4;11) chromosomal translocation and 1 patient was Philadelphia-positive. Four patients proceeded to SCT and one patient had extramedullary relapse. Side effects were modest with cytokine release syndrome in one patient and four patients with neurologic side effects [25]. Blinatumomab appears promising in achieving remission in patients with chemotherapy-resistant ALL and extensive MRD burden. The US intergroup and the European Union are in the process of recruiting patients for a larger scale study to evaluate blinatumomab.

SAR3419 is another novel anti-CD19 humanized monoclonal antibody conjugated via a cleavable linker to a maytansine derivate that is a tubulin inhibitor. SAR3419 is similar to immunotoxins that are internalized after their binding and deliver toxic small molecules to cause cell death [26]. This agent is being evaluated in a phase II trial for its activity against ALL (NLM Identifier NCT01440179, Clinicaltrials.gov).

Anti-CD52

CD52 is a costimulatory molecule that plays an important role in inducing CD4 regulatory T-cells. This cell surface antigen is present on mature B lymphocytes, macrophages, monocytes, and most lymphoproliferative malignant cells including more than half of ALL [27]. Alemtuzumab is an anti-CD52, humanized monoclonal antibody that is under investigation for the treatment of various leukemias and lymphomas. In a pilot study by the Central Oncology Group (COG), 13 patients with refractory or relapsed ALL who received alemtuzumab as an intravenous infusion over 2 h, five times per week for 1 week, then three times per week for three additional weeks. Only one of the 13 patients had a CR, while four had stable disease [28]. Alemtuzumab was not further studied as a single agent, but was combined with other chemotherapy regimens. Parnes et al. reported three cases of refractory ALL patients that were treated with fludarabine and alemtuzumab. Although all three cases achieved marrow complete responses, the patients exhibited complications with Staphylococcus aureus bacteremia, vancomycin-resistant enterococci bacteremia, and listeria bacteremia. One patient relapsed and one other was able to proceed to allogeneic SCT [29].

A phase I trial of alemtuzumab with CALGB 10102 chemotherapy in newly diagnosed ALL reported at least a 1-log reduction in MRD in eight patients, with a median OS of 55 months and median disease-free survival of 53 months in 24 patients. Infections were commonly noted with two involving cytomegalovirus and one involving a Staphylococcus abscess. After treatment, viral infections were common with eight patients developing CMV viremia, two patients with herpes simplex infections, and three patients with reactivation of the herpes zoster virus [30]. Though the agent is active, infections may be a major limiting factor in the use of alemtuzumab.

Immunotherapy

Chimeric Antigen Receptor-Modified T-Cell (CART)-19

T-cells can only recognize antigens that have been processed by antigen-presenting cells and are MHC restricted. T-cells can be modified to express chimeric antigen receptors (CARs) so that upon activation by a specific antigen of interest, these engineered T-cells can exert cytotoxic effects in an HLA-independent manner. CARs combine an antigen recognition domain of a specific antibody with intracellular domain of CD3-zeta chain or FcγRI protein into a single chimeric protein. Porter et al. have developed a potent T-cell culture with lentiviral-mediated transduction of human T-cells to express the pan B-cell antigen CD19, thereby creating CART-19 cells. By including the costimulatory molecule CD137 (4-1BB) the in vivo antitumor activity and persistence of CART-19 cells has been greatly enhanced, yielding an exciting novel modality of treatment for B-cell malignancies [31]

In a single reported case, a patient with chronic lymphocytic leukemia (CLL) was treated with autologous T-cells that were obtained after leukapheresis. This patient underwent lymphocyte depletion therapy with pentostatin. After 4 days, the patient received a total of 3×10^8 T-cells, of which $1.42 \times 10^{7/KG}$ were transduced (5 %) with a lentiviral vector to produce CART-19 cells which were given over 3 days via intravenous infusions (10 % on day 1, 30 % on day 2, and 60 % on day 3). No toxic infusion-related reactions were noted. The patient developed tumor lysis syndrome on day 22 but recovered completely after fluid resuscitation and rasburicase. By day 28, the patient had achieved molecular and morphologic remission and has remained in remission until now [32]. The safety of these transduced T cells remains a concern. Recently, Scholler et al. studied the transduced cell lines and determined its safety in a follow up of more than 500 patient years of follow up [33]. CART-19 therapy is being studied in adults with ALL in a phase I clinical trial (NLM Identifier NCT01551043). CART-19 may serve as a new modality of treatment for ALL that harnesses the powerful antitumor potential of the immune system.

Small Molecules

Notch Inhibitors

Notch is a membrane-bound protein that is cleaved to liberate a cytoplasmic domain upon ligand binding, which then translocates to nucleus to alter gene transcription. Notch signaling mediates self-renewal and plays a key role in the various stages of T-cell development. When the hematopoietic cells migrate to thymus, enhanced Notch signaling drives the cells to commit to the T-cell lineage and inhibits other non-T-cell signals. Of the four Notch family members, Notch1 is the

most critical in the commitment and development of T-cells until pre-TCR activation takes over [34]. Over 50 % of T-cell ALL (T-ALL) possesses mutations in Notch1 signaling making Notch inhibition an area of interest for hematologists and a potential drug target in ALL [35].

Gamma secretase is a key enzyme that can cleave and therefore activate Notch1. Hence gamma secretase inhibitors (GSIs) have been studied in T-ALL. Preclinical studies were initially encouraging, finding that GSIs could induce G_0/G_1 cell cycle arrest, reduce cell proliferation, and increase apoptosis in tumor cells. GSI-treated cells were also found to be more sensitive to other therapeutics such as dexamethasone and imatinib, though these results were seen in only a small subset of the population [35].

A phase I clinical trial by Deangelo et al. evaluated the GSI MK-0752 in relapsed T-ALL patients yielded disappointing results [36]. Of eight patients studied, one patient had transient clinical response but also had significant gastrointestinal toxicity, as the intestinal mucosa seems to be very sensitive to gamma secretase inhibition. Many theories, mainly lack of specificity among GSIs and an incomplete understanding of complex Notch receptor regulation have been put forth to explain the failure of this pathway that was a promising therapeutic target. Many phase I and II clinical trials are underway to evaluate the benefit of other GSIs for Notch inhibition in T-ALL such as PF-03084014 in advanced cancers including leukemia (NLM Identifier NCT00878189, Clinicaltrials.gov). Alternative approaches under investigation for Notch inhibition include antibodies that antagonize Notch or Delta/Jagged ligands, inhibitors of molecular components of the signaling pathway, ADAM10/17 protease inhibition, and concurrent inhibition of the PI3K/Akt/mTOR pathway to enhance efficacy [37]. GSIs may also reverse acquired resistance to steroid therapy in ALL and steroids may be able to alleviate gastrointestinal toxicity associated with GSIs. Based on this potential, the combination of GSIs and steroids is being explored in ALL [38].

Aurora Kinase Inhibitors (AKIs)

Aurora kinases play an essential role in regulating mitosis. These proteins are serine/threonine kinases that regulate cell cycle progression from G2, mediating the formation of the mitotic spindle, and causing centromere maturation, separation, and cytokinesis [39]. Three Aurora kinases (A, B, and C) have been identified with various mutations in a variety of malignancies. A number of small molecule Aurora kinase inhibitors (AKIs) have been developed that bind to the ATP-binding pocket of the kinase to compete with the substrate, ATP. Because ATP-binding pockets have high structural homology across the kinome, most AKIs have off-target effects. Therefore, several small molecule kinase inhibitors including AKIs act as multikinase inhibitors that target other kinases such as ABL, JAK2, and FLT3 [40].

Most AKIs have been evaluated in ALL due to their additional activity against the BCR-ABL kinase in Philadelphia-positive ALL. Research has primarily focused

on their ability to overcome tyrosine kinase inhibitor (TKI)-resistance due to mutations of the ABL1 kinase, typically involving the T315I mutation seen in 43 % of Philadelphia leukemia patients that are resistant to TKIs [41]. Many AKIs are in clinical trials in patients with leukemia. The AKI MK0457 in combination with vorinostat was studied by Okabe et al. in Philadelphia+ ALL cell lines [42]. Following this, a study was performed involving two patients with chronic myeloid leukemia (CML) harboring the T315I ABL1 mutation and one patient with relapsed ALL. Significant BCR-ABL inhibition was evident and the reported side effect was reversible pancytopenia (Giles et al. 2007). The multi-kinase inhibitor AT9238 is being studied in a phase I trial in the UK in relapsed/refractory acute leukemia (NLM Identifier NCT01431664, Clinicaltrials.gov). Danusertib is a small molecule that inhibits all Aurora kinases and ABL and is being investigated in a phase I trial of 23 patients with relapsed CML or ALL with positive response in six patients reported in a preliminarily analysis [43].

mTOR Inhibitors

The mammalian target of rapamycin (mTOR) is a downstream member of the PI3K/AKT signaling pathway mediating prosurvival signaling and regulating cell growth, cell cycle, and protein synthesis . mTOR is often involved in oncogenesis. Sirolimus, everolimus, and temsirolimus are well-known examples of mTOR inhibitors that are under investigation in several malignancies. Everolimus is being investigated in a phase I/II clinical trial studying patients with relapsed or refractory ALL using alternating cycles with standard chemotherapy (NLM Identifier NCT00968253, Clinicaltrials.gov). PP-242 and OSI-027 are other mTOR inhibitors in preclinical development that have demonstrated significant in vitro inhibition of mTORC1 and mTORC2 signaling pathways and associated cytotoxicity in T-ALL cells [44].

Suppression of mTORC1 signaling that involves mTOR can be achieved by activating AMP-activated protein kinase (AMPK). Metformin, an anti-diabetic medication that activates AMPK, has been shown to induce profound apoptosis and autophagy in T-ALL and putative leukemia-initiating cells without toxicity to CD4 lymphocytes. These results strongly suggest that metformin may serve as a treatment for ALL in future [45].

Polo-Like Kinase Inhibitors

Polo-like kinase (PLK) is a serine/threonine kinase that regulates mitosis by controlling spindle formation and centromere maturation. PLK is also an upstream regulator of SYK kinase, which activates pro-survival transcription nuclear factor kB (NFkB) and PI3K/AKT pathway under oxidative stress. Selective inhibition of PLK1 with LFM-A12 in vitro and in mouse xenografts have demonstrated increased

oxidative stress and induction of apoptosis in B-ALL [46]. The safety and efficacy of the PLK1 inhibitor ON 01910.Na is currently being studied in a phase I/II clinical trial in AML and ALL (NLM Identifier NCT01167166, Clinicaltrials.gov).

Murine Double Minute 2 (MDM2) Inhibitors

Mdm2 is an E3 ubiquitin ligase that associates with the tumor suppressor gene p53 to promote its proteasomal degradation. Thus inhibition of the MDM2-p53 interaction by small molecules such as Nutlin-3 stabilizes p53 to cause cell death [47]. A phase I study is evaluating the maximum tolerated dose of the MDM2 inhibitor RO5045337 in various leukemias (NLM Identifier NCT00623870, Clinicaltrials.gov).

MEK Inhibitors

MAPK signaling is a prosurvival growth factor signaling pathway that involves the Ras/Raf/MEK/ERK signaling cascade and is frequently overactivated in cancers including leukemia. Activating this signaling pathway has several pro-survival and anti-apoptotic effects such as ERK-mediated phosphorylation and degradation of the pro-apoptotic protein Bim. Activation of MAPK signaling is a pro-survival mechanism utilized by leukemia cells to evade the effects of steroid treatment, causing resistance to such therapy. Thus inhibition of members of MAPK signaling such as MEK and ERK may restore sensitivity to steroid therapy [48]. While a number of MEK inhibitors are being studied in various malignancies, a phase I/II clinical with the MEK Inhibitor GSK1120212 is currently recruiting patients with relapsed or refractory leukemia (NLM Identifier NCT00920140, Clinicaltrials.gov).

Proteasome Inhibitors

Bortezomib is a proteasome inhibitor that is used for the treatment of certain hematological malignancies such as multiple myeloma and mantle cell lymphoma. In a phase I study, bortezomib combined with chemotherapy induced remission in two patients with relapsed ALL. This therapeutic response was associated with reduced NF-kB activity, which is an effect of bortezomib [49]. Bortezomib has also been studied in another phase I trial for relapsed and refractory acute leukemia. Fifteen patients were treated with escalating doses of bortezomib to establish the MTD. Sixty-eight percent proteasome inhibition was seen at 1.5 mg/m^2 and three out of five patients had in vitro evidence of apoptosis in blast cells. Orthostatic hypotension, nausea, diarrhea and fluid retention were the dose-limiting toxicities. Five of

15 patients showed evidence of hematologic improvement [50]. The COG (Children's Oncology Group) also studied this agent in 12 leukemia patients to determine the maximum tolerated dose. Toxicities included confusion, febrile neutropenia, hypotension and high creatinine [51].

The results of a phase I/II trial of combined bortezomib with intensive reinduction chemotherapy in young patients with ALL or lymphoblastic lymphoma are currently being analyzed (NLM Identifier NCT00873093, Clinicaltrials.gov). Another phase II trial is combining bortezomib with vorinostat and dexamethasone for the treatment of relapsed/refractory ALL. (NLM Identifier NCT01312818, Clinicaltrials.gov). The acceptable toxicity profile and the previously reported synergy of bortezomib and vorinostat together make this a promising combination [52].

Nucleoside Analogues

Forodesine is a small molecule inhibitor of purine nucleoside phosphorylase (PNP), which causes an intracellular accumulation of dGTP that in turn leads to cell cycle arrest and apoptosis. A phase I clinical trial in five patients with relapsed T-ALL reported tolerability and neutropenia as the most common side effect. This study was followed by a phase II trial in 34 patients that found a 32 % response rate, with seven CR and four partial responses [53].

Nelarabine is a purine analogue that is phosphorylated and incorporated into DNA, stalling DNA replication and inducing apoptosis. Krutzberg et al. established the maximum tolerated dose of nelarabine and reported its association with neurotoxicity including weakness, coma, ataxia, and confusion [54]. A larger phase II trial was conducted by the GMALL group in adults with relapsed or refractory T-ALL/LBL with nelarabine. After one or two cycles, CR was seen in 36 %, partial response in 10–52 % were refractory. OS was 24 % after 1 year with one mortality and two withdrawals reported. Eighty percent of the patients who achieved CR were able to proceed to SCT. Post-transplant survival was 31–37 % of patients were disease-free at 3 years. Neurotoxicity was a significant side effect of the drug but was generally well tolerated [55]. This study was encouraging and further analysis of results from this trial is anticipated for the combination of nelarabine with other chemotherapeutic agents for induction chemotherapy. Based on these promising results, nelarabine is on fast-track approval for the treatment of T-ALL patients that failed or relapsed on at least two previous chemotherapeutic regimens in the pediatric population.

Clofarabine is a second-generation purine analogue that is used in salvage chemotherapy for relapsed/refractory pediatric ALL. Clofarabine is widely used in CLL and AML, however the data in adult ALL is limited. Kantarjian et al. conducted phase I and II trials investigating clofarabine that reported two CR out of the 12 patients. Hepatotoxicity was the prevalent toxicity, unlike other purine analogues that typically cause neurotoxicity. Eighty one percent of the patients developed febrile neutropenia and 50 % had documented infection [56]. The Spanish PETHEMA

group studied 31 patients with relapsed/refractory acute lymphoblastic leukemia and lymphoma that were treated with clofarabine-based regimens. Five patients received clofarabine as a single agent while the rest received combination regimens. OS at 1 year was 10 % and CR was achieved in 31 % patients with a median CR duration of 3 months. Grade 3 hematologic toxicity was seen in all patients, and infectious complications in 67 % of patients. While clofarabine may yield good response rates in combination therapies, its associated toxicity may limit its use [57]

Histone Deacetylase Inhibitors and Hypomethylating Agents

Post-translational modifications to histones can affect its interactions with DNA, altering the genomic structure and thereby changing the expression of genes. Epigenetic modifications including DNA hypermethylation in gene promoter regions commonly occur in ALL. In ALL, hypermethylation commonly occurs in CpG islands in the promoter region of tumor suppressor genes that results in gene silencing [58]. Decitabine is a pyrimidine analogue that inhibits DNA methyltransferase activity and therefore can reactivate silenced genes by reducing hypermethylation. In a phase I trial of decitabine in relapsed and refractory ALL involving 39 patients, 23 achieved a transient CR with decitabine alone. Half of the patients who had received decitabine alone later received Hyper CVAD as well and 52 % achieved a response with a median duration of 4 months. No significant toxicities were reported but the responses were transient [59].

Histone acetylation is another modality of gene regulation controlled by several proteins including histone acetyltransferases and histone deacetylases (HDACs). HDAC inhibitors are a class of small molecules that effectively increases the acetylation of histones, altering chromatin structure to enhance DNA transcription [60]. Vorinostat, for example, is an HDAC inhibitor in phase I study in combination with bortezomib in ALL (NLM Identifier NCT01312818, Clinicaltrials.gov).

Conclusion

The investigation of treatment of Philadephia chromosome negative ALL in adults has yielded several promising therapies in development. Some agents such as rituximab have already proven effective in other hematologic malignancies and may extend their therapeutic benefits to ALL. In addition, there are several novel monoclonal antibodies and small molecules emerging to target unique molecular aspects of ALL in an effort to improve outcome with minimal toxicity. Early phase clinical data for several monoclonal antibodies targeting surface antigens such as inotuzumab, blinatumomab, and alemtuzumab appear promising and are being further investigated. Numerous small molecules have been developed to target molecular aberrancies in ALL that include Notch inhibitors, Aurora kinase inhibitors, PLK inhibitors, and

mTOR inhibitors. Though in early stages of clinical development, other classes of encouraging small molecule antitumor agents have emerged with preliminary activity against ALL including proteasome inhibitors, purine analogues, and HDAC inhibitors among others. Together, a multimodal approach that integrates immunotherapy, targeted small molecules and antibodies, and conventional therapies may ultimately provide maximum benefit to patients with ALL and other malignancies. New therapies and biomarkers that predict response are expected to emanate from ongoing genomic and genetic studies of ALL aimed at identifying and elucidating the numerous and diverse molecular events that initiate and propagate the disease.

References

1. SEER incidence and NCHS mortality statistics [database on the Internet]. 2011. Available from http://seer.cancer.gov/statfacts/html/alyl.html#survival. Contract No.: Report.
2. Onciu M. Acute lymphoblastic leukemia. Hematol/Oncol Clin North Am. 2009;23(4):655–74. doi:10.1016/j.hoc.2009.04.009.
3. Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. N Engl J Med. 2006;354(2):166–78. doi:10.1056/NEJMra052603.
4. Nishiwaki S, Miyamura K. Allogeneic stem cell transplant for adult Philadelphia chromosome-negative acute lymphoblastic leukemia. Leuk Lymphoma. 2012 Apr;53(4):550–6.
5. 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. doi:10.1074/jbc.M708459200.
6. 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.
7. Coiffier B, Thieblemont C, Van Den Neste E, Lepeu G, Plantier I, Castaigne S, et al. Long-term outcome of patients in the LNH-98.5 trial, the first randomized study comparing rituximab-CHOP to standard CHOP chemotherapy in DLBCL patients: a study by the Groupe d'Etudes des Lymphomes de l'Adulte. Blood. 2010;116(12):2040–5. doi:10.1182/blood-2010-03-276246.
8. Habermann TM, Weller EA, Morrison VA, Gascoyne RD, Cassileth PA, Cohn JB, et al. Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma. J Clin Oncol. 2006;24(19):3121–7. doi:10.1200/jco.2005.05.1003. Official Journal of the American Society of Clinical Oncology.
9. Dworzak MN, Schumich A, Printz D, Potschger U, Husak Z, Attarbaschi A, et al. CD20 up-regulation in pediatric B-cell precursor acute lymphoblastic leukemia during induction treatment: setting the stage for anti-CD20 directed immunotherapy. Blood. 2008;112(10):3982–8. doi:10.1182/blood-2008-06-164129.
10. 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. doi:10.1200/jco.2009.26.9456. Official Journal of the American Society of Clinical Oncology.
11. Hoelzer D, Heuttmann A, Kaul F, et al. Immunochemotherapy with rituximab in adult CD20+ B -precursor ALL improves molecular CR rate and outcome in standard as well as in high risk patients with stem cell transplant. Haematologica. 2009;94 Suppl 2:481. Abst.
12. 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. doi:10.1002/cncr.21776.
13. Paietta E LX, Richards S et al. Implications for the use of monoclonal antibodies in future adult ALL trials :analysis of antigen expression in 505 B-lineage all patients on the MRC UKALLXII/ECOG2993 intergroup trial. Proc ASH. 2008;112(1907).
14. 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. doi:10.1038/leu.2011.206 Official Journal of the Leukemia Society of America, Leukemia Research Fund, UK.
15. 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. The Lancet Oncol. 2012;13(4):403–11. doi:10.1016/s1470-2045(11)70386-2.
16. 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. doi:10.1200/jco.2007.15.3528. Official Journal of the American Society of Clinical Oncology.
17. Raetz EA, Cairo MS, Borowitz MJ, Lu X, Devidas M, Reid JM, et al. 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. Blood. 2011;118(573): Abst.
18. Portell CA, Advani AS. Antibody therapy for acute lymphoblastic leukemia. Curr Hematol Malig Rep. 2012;7(2):153–9. doi:10.1007/s11899-012-0120-7.
19. Kreitman RJ, Pastan I. Antibody fusion proteins: anti-CD22 recombinant immunotoxin moxetumomab pasudotox. Clin Cancer Res. 2011;17(20):6398–405. doi:10.1158/1078-0432.ccr-11-0487. An Official Journal of the American Association for Cancer Research.
20. Raponi S, De Propris MS, Intoppa S, Milani ML, Vitale A, Elia L, et al. Flow cytometric study of potential target antigens (CD19, CD20, CD22, CD33) for antibody-based immunotherapy in acute lymphoblastic leukemia: analysis of 552 cases. Leuk Lymphoma. 2011;52(6):1098–107. doi:10.3109/10428194.2011.559668.
21. Nagorsen D, Baeuerle PA. Immunomodulatory therapy of cancer with T cell-engaging Bite antibody blinatumomab. Exp Cell Res. 2011;317(9):1255–60. doi:10.1016/j.yexcr.2011.03.010.
22. Nagorsen D, Bargou R, Ruttinger D, Kufer P, Baeuerle PA, Zugmaier G. Immunotherapy of lymphoma and leukemia with T-cell engaging bite antibody blinatumomab. Leuk Lymphoma. 2009;50(6):886–91. doi:10.1080/10428190902943077.
23. 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. doi:10.1200/jco.2010.32.7270. Official Journal of the American Society of Clinical Oncology.
24. Handgretinger R, Zugmaier G, Henze G, Kreyenberg H, Lang P, von Stackelberg A. Complete remission after blinatumomab-induced donor T-cell activation in three pediatric patients with post-transplant relapsed acute lymphoblastic leukemia. Leukemia. 2011;25(1):181–4. doi:10.1038/leu.2010.239. Official Journal of the Leukemia Society of America, Leukemia Research Fund, UK.
25. Topp M, Gokbuget N, Zugmaier G, et al. Anti-CD19 bite blinatumomab induces high complete remission rate in adult patients with relapsed B-Precursor ALL: updated results of an ongoing phase II trial. 53rd annual meeting society of Hematology, San Diego,USA. Blood. 2011;118(21):252. Abstract.
26. Blanc V, Bousseau A, Caron A, Carrez C, Lutz RJ, Lambert JM. SAR3419: an anti-CD19-Maytansinoid Immunoconjugate for the treatment of B-cell malignancies. Clin Cancer Res. 2011;17(20):6448–58. doi:10.1158/1078-0432.ccr-11-0485. An Official Journal of the American Association for Cancer Research.

27. Watanabe T, Masuyama J, Sohma Y, Inazawa H, Horie K, Kojima K, et al. CD52 is a novel costimulatory molecule for induction of CD4+ regulatory T cells. Clin Immunol (Orlando). 2006;120(3):247–59. doi:10.1016/j.clim.2006.05.006.
28. Angiolillo AL, Yu AL, Reaman G, Ingle AM, Secola R, Adamson PC. A phase II study of Campath-1H in children with relapsed or refractory acute lymphoblastic leukemia: a Children's Oncology Group report. Pediatr Blood Cancer. 2009;53(6):978–83. doi:10.1002/pbc.22209.
29. Parnes A, Bifulco C, Vanasse GJ. A novel regimen incorporating the concomitant administration of fludarabine and alemtuzumab for the treatment of refractory adult acute lymphoblastic leukaemia: a report of three cases. Br J Haematol. 2007;139(1):164–5. doi:10.1111/j.1365-2141.2007.06760.x.
30. Stock W, Sanford B, Lozanski G, et al. 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). Proc ASH. 2009;114(838): Abstract.
31. Porter DL, Kalos M, Zheng Z, Levine B, June C. Chimeric antigen receptor therapy for B-cell malignancies. J Cancer. 2011;2:331–2.
32. 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. doi:10.1056/NEJMoa1103849.
33. Scholler J, Brady TL, Binder-Scholl G, Hwang WT, Plesa G, Hege KM, et al. Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells. Sci Translat Med. 2012;4(132):132ra53. doi:10.1126/scitranslmed.3003761.
34. Maillard I, Weng AP, Carpenter AC, Rodriguez CG, Sai H, Xu L, et al. Mastermind critically regulates Notch-mediated lymphoid cell fate decisions. Blood. 2004;104(6):1696–702. doi:10.1182/blood-2004-02-0514.
35. Weng AP, Ferrando AA, Lee W, Morris JP, Silverman LB, Sanchez-Irizarry C, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science (New York, NY). 2004;306(5694):269–71. doi:10.1126/science.1102160.
36. Deangelo D, Stone R, Silverman L, et al. A phase I clinical trial of the Notch inhibitor MK-0752 in patients with T-cell acute lymphoblastic leukemia/lymphoma (T-ALL) and other leukemias. J Clin Oncol. 2006;24(18S):6585. ASCO Annual Meeting Proceeding, June 20 Supplement.
37. Elyaman W, Bradshaw EM, Wang Y, Oukka M, Kivisakk P, Chiba S, et al. JAGGED1 and delta1 differentially regulate the outcome of experimental autoimmune encephalomyelitis. J Immunol. 2007;179(9):5990–8. Baltimore, MD: 1950.
38. Real PJ, Tosello V, Palomero T, Castillo M, Hernando E, de Stanchina E, et al. Gamma-secretase inhibitors reverse glucocorticoid resistance in T cell acute lymphoblastic leukemia. Nat Med. 2009;15(1):50–8. doi:10.1038/nm.1900.
39. Andrews PD, Knatko E, Moore WJ, Swedlow JR. Mitotic mechanics: the auroras come into view. Curr Opin Cell Biol. 2003;15(6):672–83.
40. Scapin SM, Carneiro FR, Alves AC, Medrano FJ, Guimaraes BG, Zanchin NI. The crystal structure of the small GTPase Rab11b reveals critical differences relative to the Rab11a isoform. J Struct Biol. 2006;154(3):260–8. doi:10.1016/j.jsb.2006.01.007.
41. Quintas-Cardama A, Kantarjian H, Cortes J. Flying under the radar: the new wave of BCR-ABL inhibitors. Nat Rev Drug Discov. 2007;6(10):834–48. doi:10.1038/nrd2324.
42. Okabe S, Tauchi T, Ohyashiki K. Efficacy of MK-0457 and in combination with vorinostat against Philadelphia chromosome positive acute lymphoblastic leukemia cells. Ann Hematol. 2010;89(11):1081–7. doi:10.1007/s00277-010-0998-x.
43. Cortes-Franco J, Dombret H, Schafhausen P, Brummendorf TH, Boissel N, Latini FC, et al Danusertib hydrochloride (PHA-739358), a multi-kinase Aurora inhibitor, elicits clinical benefit in advanced chronic myeloid leukemia and Philadelphia chromosome positive acute lymphoblastic leukemia. 51st Annual Meeting American Society of Hematology,New Orleans,USA. Blood. 2009;114(22): Abstract 864.
44. Evangelisti C, Ricci F, Tazzari P, Tabellini G, Battistelli M, Falcieri E, et al. Targeted inhibition of mTORC1 and mTORC2 by active-site mTOR inhibitors has cytotoxic effects in T-cell acute lymphoblastic leukemia. Leukemia. 2011;25(5):781–91. doi:10.1038/leu.2011.20. Official Journal of the Leukemia Society of America, Leukemia Research Fund, UK.
45. Grimaldi C, Chiarini F, Tabellini G, Ricci F, Tazzari PL, Battistelli M, et al. AMP-dependent kinase/mammalian target of rapamycin complex 1 signaling in T-cell acute lymphoblastic

leukemia: therapeutic implications. Leukemia. 2012;26(1):91–100. doi:10.1038/leu.2011.269. Official Journal of the Leukemia Society of America, Leukemia Research Fund, UK.
46. Uckun FM, Ozer Z, Qazi S, Tuel-Ahlgren L, Mao C. Polo-like-kinase 1 (PLK1) as a molecular target to overcome SYK-mediated resistance of B-lineage acute lymphoblastic leukaemia cells to oxidative stress. Br J Haematol. 2010;148(5):714–25. doi:10.1111/j.1365-2141.2009.07983.x.
47. Hasegawa H, Yamada Y, Iha H, Tsukasaki K, Nagai K, Atogami S, et al. Activation of p53 by Nutlin-3a, an antagonist of MDM2, induces apoptosis and cellular senescence in adult T-cell leukemia cells. Leukemia. 2009;23(11):2090–101. doi:10.1038/leu.2009.171. Official Journal of the Leukemia Society of America, Leukemia Research Fund, UK.
48. Rambal AA, Panaguiton ZL, Kramer L, Grant S, Harada H. MEK inhibitors potentiate dexamethasone lethality in acute lymphoblastic leukemia cells through the pro-apoptotic molecule BIM. Leukemia. 2009;23(10):1744–54. doi:10.1038/leu.2009.80. Official Journal of the Leukemia Society of America, Leukemia Research Fund, UK.
49. Hu X, Xu J, Sun A, Shen Y, He G, Guo F. Successful T-cell acute lymphoblastic leukemia treatment with proteasome inhibitor bortezomib based on evaluation of nuclear factor-kappaB activity. Leuk Lymphoma. 2011;52(12):2393–5. doi:10.3109/10428194.2011.593271.
50. Cortes J, Thomas D, Koller C, Giles F, Estey E, Faderl S, et al. Phase I study of bortezomib in refractory or relapsed acute leukemias. Clin Cancer Res. 2004;10(10):3371–6. doi:10.1158/1078-0432.ccr-03-0508. An Official Journal of the American Association for Cancer Research.
51. Horton TM, Pati D, Plon SE, Thompson PA, Bomgaars LR, Adamson PC, et al. A phase 1 study of the proteasome inhibitor bortezomib in pediatric patients with refractory leukemia: a Children's Oncology Group study. Clin Cancer Res. 2007;13(5):1516–22. doi:10.1158/1078-0432.ccr-06-2173. An Official Journal of the American Association for Cancer Research.
52. Mazumder A, Vesole DH, Jagannath S. Vorinostat plus bortezomib for the treatment of relapsed/refractory multiple myeloma: a case series illustrating utility in clinical practice. Clin Lymphoma Myeloma Leuk. 2010;10(2):149–51. doi:10.3816/CLML.2010.n.022.
53. Furman R, Gore L, Ravandi F, et al. Forodesine IV (Bcx-1777) is clinically active in relapsed/refractory T-cell leukemia: results of a phase II study (interim report). Blood. 2006;108:1851.
54. Kurtzberg J, Ernst TJ, Keating MJ, Gandhi V, Hodge JP, Kisor DF, et al. Phase I study of 506U78 administered on a consecutive 5-day schedule in children and adults with refractory hematologic malignancies. J Clin Oncol. 2005;23(15):3396–403. doi:10.1200/jco.2005.03.199. Official Journal of the American Society of Clinical Oncology.
55. Gokbuget N, Basara N, Baurmann H, Beck J, Bruggemann M, Diedrich H, et al. High single-drug activity of nelarabine in relapsed T-lymphoblastic leukemia/lymphoma offers curative option with subsequent stem cell transplantation. Blood. 2011;118(13):3504–11. doi:10.1182/blood-2011-01-329441.
56. Kantarjian H, Gandhi V, Cortes J, Verstovsek S, Du M, Garcia-Manero G, et al. Phase 2 clinical and pharmacologic study of clofarabine in patients with refractory or relapsed acute leukemia. Blood. 2003;102(7):2379–86.
57. Barba P, Sampol A, Calbacho M, Gonzalez J, Serrano J, Martinez-Sanchez P, et al. Clofarabine-based chemotherapy for relapsed/refractory adult acute lymphoblastic leukemia and lymphoblastic lymphoma. The Spanish experience. Am J Hematol. 2012;87(6):631–4. doi:10.1002/ajh.23167.
58. Tong WG, Wierda WG, Lin E, Kuang SQ, Bekele BN, Estrov Z, et al. Genome-wide DNA methylation profiling of chronic lymphocytic leukemia allows identification of epigenetically repressed molecular pathways with clinical impact. Epigenetics. 2010;5(6):499–508. Official Journal of the DNA Methylation Society Official Journal of the DNA Methylation Society.
59. Garcia-Manero G, Thomas D, Rytting M, et al. Final report of a phase I trial of decitabine with or without hyperCVAD in relapsed acute lymphocytic leukemia (ALL). Blood. 2010;116(21):867. Abstract.
60. Marks PA, Xu WS. Histone deacetylase inhibitors: potential in cancer therapy. J Cell Biochem. 2009;107(4):600–8. doi:10.1002/jcb.22185.

Impact of Genetic Targets on Prostate Cancer Therapy

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Abstract Given the global incidence of prostate cancer and its sociological impact, it remains a challenging disease to clinicians and researchers alike. In the last few years several new drugs have been added to the armamentarium of prostate cancer therapy and offers survival benefit to patients with prostate cancer. However, effective drugs are still needed that offer extended survival benefit and alter the natural history of the disease. Recent efforts have focused on better understanding the underlying biology and genetic heterogeneity of the disease and identified novel targets that can be utilized for drug development and therapeutics in the future. In this review we present an overview of the genetic landscape of prostate cancer, novel targets in the prostate cancer therapy and the results of key clinical trials of these novel drugs.

Keywords Prostate cancer • PSA • Digital rectal examination • Ultrasound • MRI • Gleason score • Androgen deprivation • External beam irradiation • Pelvic nodal radiation • Gonadotropin releasing hormone • Androgen blockade • Testosterone • Docetaxel • Sipuleucel-T • Cabazitaxel • Abiraterone acetate • Hyperdiploidy • Androgen receptor • NCOA2 • MYC • NCOR1 • ETS • PTEN • Akt • TMPRSS2-ERG • TP53 • 3p14 • FOXP1 • RYBP • SHQ1 • SLC45A3:ERG • KLK2:ETV1

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W.S. El-Deiry (ed.), *Impact of Genetic Targets on Cancer Therapy*, Advances in Experimental Medicine and Biology 779, DOI 10.1007/978-1-4614-6176-0_17,

• FKBP5:ERG • TOP2B • mTORC1 • MAG12 • NKX3.1 • 8p21 • SPOP • CADM2 • GWAS • UGT2B17 • IL4 • RNASEL • GSTP1 • H3K27 • Circulating tumor cells • Cancer stem cells • EpCAM • Ki67 • miRNA • VEGF • FGF • EMT • MDV3100 • CYP17 • TAK-700 • BMS-641988 • Lenalidomide • Aflibercept • Sunitinib • TKI • EGFR • Lapatinib • Erlotinib • Dasatinib • Clusterin • RANK ligand • Ipilumumab • CTLA4

Epidemiology of Prostate Cancer

In United States, prostate cancer is the most commonly occurring cancer in men. About one in six men will develop prostate cancer over their lifetime. It is estimated that 241,740 new cases will be diagnosed in 2012, accounting for 29% of new cancer cases in men in 2012 while the estimated number of deaths from prostate cancer is approximately 28,170 in 2012 [1]. Age is the most important risk for prostate cancer. Prostate cancer rarely occurs before the age of 40, but the incidence rises rapidly thereafter. About 5–10% of all cases of prostate cancer are estimated to be hereditary in nature with two-fold increase in life-time risk of developing the disease if one first degree relative is diagnosed with prostate cancer. This life-time risk increases to fourfold if more than two relatives are involved. Prostate cancer affects ethnic groups differently with African American men having higher tumor burden within each stage, earlier age at diagnoses, higher incidence of advanced stage at initial presentation and lower survival rates than age-matched white men [2]. Several observational studies have shown increased risk of prostate cancer for dietary substances like high-saturated fats, red meat, low fruits and vegetables and low fish. Obesity is associated with higher-grade prostate cancer however, this relationship is not fully established.

Clinical Approach to Prostate Cancer

Prostate cancer is a heterogeneous group of diseases and can be divided into clinically distinct stages based on a risk adapted approach. This approach identifies the disease as a sequence of events starting from pre-diagnosis to death and incorporates a risk of disease recurrence after definitive therapy. Therapeutic goals are distinct for each stage of the disease. Prevention on the other hand is the goal of care for men at high risk of developing the disease whereas curative modalities aimed at the prostate alone are utilized in men with localized disease. Similarly, men with locally advanced disease and indolent tumor biology can be managed with watchful waiting while those with more aggressive biology require combined multimodality approach directed at eliminating the cancer locally and eradicating the micro-metastatic disease. Finally in men with castration resistant disease the

therapeutic objective is to palliate or eliminate symptoms and prolong life. Clinical (Tumor Node Metastasis; TNM) staging is employed to characterize prostate cancers by determining Gleason score in the biopsy specimen, digital rectal examination (DRE) and serum Prostate Specific Antigen (PSA) level. Imaging studies (ultrasound, MRI) are not currently part of the routine staging process. According to the risk stratification patients are at low risk for recurrence if they have tumors stage T1 to T2a, low Gleason score (≤6), and serum PSA level below 10 ng/mL. The intermediate-risk category is defined by any T2b to T2c cancer, Gleason score of 7, or PSA value of 10–20 ng/mL. The high risk category is defined by prostate cancer that is clinically localized stage T3a, Gleason score 8–10, or PSA level greater than 20 ng/mL.

Current Standard Therapy

Prostate cancer is managed according to the disease stage and risk category. With standard therapy 15-year relative survival rate is about 81% for Stage I–II disease, 57% for stage III disease and 6% for stage IV disease. Ten-year prostate disease free survival for localized disease alone after radical prostatectomy for tumors of Gleason score 2–4 is 96%, Gleason score 5–6 is 82%, Gleason score 7 is 52% and Gleason score 8–9 is 35%. For low risk localized prostate cancer confined to the prostate treatment consists of monotherapy with active surveillance, interstitial prostate brachytherapy, external beam radiotherapy, and radical prostatectomy. Active surveillance involves serial monitoring of PSA and periodic prostate biopsies. Radical prostatectomy with or without pelvic lymph node dissection is recommended for intermediate risk group [3]. Randomized clinical trials favor the use of neoadjuvant or concurrent androgen deprivation therapy for 3–6 months in intermediate risk patients receiving external beam radiation therapy [4–6]. Preferred treatment for high risk localized prostate cancer patients consists of external beam radiation therapy in combination with neoadjuvant and concurrent androgen deprivation therapy followed by long-term adjuvant androgen deprivation therapy lasting for >2 years [7]. The role of pelvic nodal radiation in intermediate and high risk groups is controversial. There is evidence that favors immediate initiation of adjuvant androgen deprivation therapy in node positive patients [8, 9]. In the metastatic setting, prostate cancer patients are classified as noncastrate and castrate resistant. Noncastrate metastatic prostate cancer is treated with hormonal therapy alone which consists of testosterone lowering agents such as gonadotrophin releasing hormone (GnRH) agonists/antagonists or antiandrogens which block androgen receptors. Surgical castration is also an option although not usually preferred by the patients. Combining antiandrogens with GnRH agonist/antagonist known as combined androgen blockade has a modest benefit in terms of antitumor effects [10, 11]. Data also supports the early use of hormone therapy

in locally advanced, or high risk or non-castrate asymptomatic metastatic prostate cancer patients as it showed improvement in disease-specific survival, local and distant disease control rate when compared to deferred hormonal therapy at relapse or when indicated [9, 12–14]. The treatment of castrate resistant prostate cancer first requires the establishment of castrate levels of serum testosterone (< 50 ng/ml) and then an assessment of disease extent. Metastatic castrate resistant prostate cancer (mCRPC) can present as biochemical recurrence with a rise in serum PSA alone, as presence of bone metastasis or as soft tissue involvement. Patients with mCRPC can be initially given a trial of "withdrawal" of anti-androgen therapy that can induce tumor responses which however are not usually durable. Alternatively, second line hormonal therapy such as Ketoconazole plus hydrocortisone can be considered. Invariably most patients with mCRPC will require chemotherapy agents. Currently, four chemotherapeutic agents namely docetaxel, [15–17] sipuleucel-T, [18] cabazitaxel, [19] and abiraterone acetate [20] have demonstrated improvements in overall survival in large Phase III trials in this setting (Tables 1 and 2). Sipuleucel-T is an FDA approved agent for patients with mCRPC who have good performance level (Eastern Cooperative Oncology Group, ECOG 0-1), estimated life expectancy greater than 6 months, no visceral disease, and no or minimal symptoms. For mCRPC patients with symptomatic, rapidly progressive, or visceral disease, docetaxel-based regimens are recommended based on an overall survival benefit shown in two Phase III studies. (Southwest Oncology Group [SWOG] 9916 and TAX 327) [15–17]. There is a role for systemic radiotherapy with either strontium-89 or samarium-153 in a select group of patients with painful widely metastatic skeletal involvement not responding to palliative chemotherapy or systemic analgesia and who are not candidates for localized external beam radiation therapy (EBRT). Options for second line systemic therapy following failure of docetaxel in mCRPC include abiraterone acetate [20], cabazitaxel [19] and salvage chemotherapy. Abiraterone acetate, an androgen synthesis inhibitor of CYP17 demonstrated clinical benefit in a Phase III, randomized, placebo-controlled trial in men with mCRPC previously treated with docetaxel-containing regimens and is recommended after failure of docetaxel chemotherapy for mCRPC [20]. Cabazitaxel, a semi-synthetic taxane derivative and anti-mitotic chemotherapeutic agent is also FDA approved for second-line therapy after docetaxel failure for patients with symptomatic mCRPC based on the results of an international randomized Phase III trial that showed an overall survival benefit [19]. Previous randomized studies have shown palliative responses and benefit with the use of Mitoxantrone in men who are not candidates for taxane-based therapy and thus is a palliative option in mCRPC patients [21]. For the prevention or delaying of skeletal related events (SREs) as defined by pathological fractures, spinal cord compression, surgery or radiation therapy to bone, the use of zoledronic acid every 3–4 weeks or denosumab 120 mg every 4 weeks is recommended in men with CRPC and bone metastases [22–24]. The optimal duration of zoledronic acid or denosumab in men with CRPC and bone metastases needs to be standardized.

Table 1 Current therapeutic drugs for mCRPC

Drug	Dose	Phase	FDA approval	Target/function	Clinical trial	Survival benefit	References
Docetaxel	75 mg/m^2 IV, every 3 weeks	III	Yes	Anti-tubule	TAX327	2.5 months, p=0.009	[16]
Sipuleucel-T	Three IV infusions, every 2 weeks	III	Yes	PAP/GM-CSF	IMPACT	4.1 months, p=0.032	[18]
Cabazitaxel	25 mg/m^2 IV, every 3 weeks	III	Yes	Anti-tubule	TROPIC	2.4 months, p<0.0001	[19]
Abiraterone	1,000 mg PO daily	III	Yes	CYP17 inhibitor	COU 301	3.9 months, p<0.0001	[20]
MDV3100	160 mg PO daily	III	Pending	AR antagonist	AFFIRM	4.8 months, p<0.0001	[120]
Radium- 223	Six IV infusions every 4 weeks	III	Pending	Alpha-particle emitting pharmaceutical	ALSYMPCA	3.6 month, p=0.00007	[121]
Denosumab	120 mg SC every 4 weeks	III	Pending	RANKL mAb	20050103NCT00321620	N/A	[22]

Table 2 Novel emerging agents for prostate cancer therapy

Clinical trial	Mode of action	Trial design	Indication	Primary endpoint	Status
COU-AA-302 NCT00887198	CYP 17	Abiraterone versus placebo	Chemotherapy naïve mCRPC	OS, PFS	Ongoing
CALGB 90401 NCT00110214	VEGFA	Bevacizumab+Docetaxel versus Docetaxel	Chemotherapynaïve mCRPC	OS	Failure
SUN1120 NCT00676650	Multitargeted small-molecule VEGFR TKI	Sunitinibversus placebo	Second-line mCRPC	OS	Failure
SWOG0421 NCT00134056	Small-molecule ETA receptor antagonist	Atrasentan+Docetaxel Versus Docetaxel	Chemotherapynaïve mCRPC	OS, PFS	Failure
147TRIAL NCT00286091	RANKL mAb	Denosumabversus placebo	CRPC without bone mets	Bone mets free survival	Positive [97]
C21005 NCT01193257	CYP 17, 17.20 lyase activity	TAK-700 versus placebo	Docetaxel pre-treated mCRPC	OS	Ongoing
C21004 NCT01193244	CYP 17, 17.20 lyase activity	TAK-700 versus placebo	Chemotherapy naïve mCRPC	OS, rPFS	Ongoing
PREVAIL NCT01212991	Androgen receptor	MDV3100 versus placebo	Chemotherapy naïve mCRPC	OS, PFS	Ongoing
SATURN NCT01083615	Clusterin mRNA	Custirsen +/− docetaxel	Docetaxel pre-treated mCRPC	Pain, palliation	Ongoing
SYNERGY NCT01188187	Clusterin mRNA	Custirsen +/- docetaxel	Chemotherapy naïve mCRPC	OS	Ongoing
‘306 TRIAL	c-MET and VEGFR2	Cabozantinib versus mitoxantrone	Docetaxel-abiraterone pre-treated mCRPC	Bone pain alleviation	Planned
‘307 TRIAL	c-MET and VEGFR2	Cabozantinib versus Placebo	Docetaxel-abiraterone pre-treated mCRPC	OS	Planned
VENICE NCT00519285	VEGFA, VEGFB, PIGF	Aflibercept +/− Docetaxel	Chemotherapy naïve mCRPC	OS	Ongoing
NCT0123431	Immune-modulatory protein S100A9	Tasquinimod versus placebo	Asymptomatic or minimally symptomatic Docetaxel pre-treated mCRPC	PFS	Ongoing
READY NCT00744497	Src and Src-family kinases	Dasatinib +/−Docetaxel	Chemotherapy naïve mCRPC	OS	Ongoing

ENTHUSE M1C (33) NCT00617669	Endothelin A receptor	Zibotentan +/− Docetaxel	Chemotherapy naïve mCRPC	OS	Failure
PROSPECT NCT01322490	Anti-tumor immune response	PROSTVAC+/-GM-CSF versus placebo	Asymptomatic or minimally symptomatic chemotherapy naïve mCRPC	OS	Ongoing
CA-184-043 NCT00861614	CTLA-4	Ipilimumab versus placebo, following a single dose of radiotherapy	Docetaxel pre-treated mCRPC	OS	Ongoing
CA-184-095 NCT01057810	CTLA-4	Ipilimumab versus placebo	Asymptomatic or minimally symptomatic chemotherapy naïve mCRPC	OS	Ongoing
CC-5013-PC-002 (NCT00988208)	Immune-modulatory	Docetaxel +/− lenalidomide	Chemotherapy naïve mCRPC	OS	Ongoing

PFS progression free survival, *rPFS* radiographic progression-free survival, *OS* overall survival

Genetic Landscape of Prostate Cancer

The clinical heterogeneity of prostate cancer is only surpassed by an even greater heterogeneity of the prostate cancer genome and epigenome. Large-scale cancer genome characterization projects currently underway have identified a large number of genetic and epigenetic alterations found in the prostate cancer that are associated with the hallmark properties of oncogenesis. The most commonly reported abnormalities are gains of 2p, 3q, 7q, 8q, 9q, 17q, 20q and Xq, deletions of 2q, 5q, 6q, 8p, 10q, 12p, 13q, 16q, 17p, 17q, 18q, 21q and 22q, hyperdiploidy and aneusomy of chromosome 7 and 17 [25].

Four pathways are identified which carry the genetic alterations found in one third of primary prostate cancers and majority of all metastatic prostate cancers. We will discuss the salient genetic alteration in each of these pathways below:

Common Genetic Alteration in Androgen Receptor Signaling Pathway

Androgen receptor (AR) is a 110 kb steroid receptor transcription factor situated on Xq12 which mediates the transcription of several genes involved in the differentiation and proliferation of normal epithelial prostate cells upon androgen binding [26]. AR is involved in the development of both normal and malignant prostate cells. Both animal and human models have shown induction of neoplastic process in primary prostate cells over-expressing AR [27]. Results from a recent large scale prostate cancer genome project showed that 60% of primary prostate cancers had alterations in the AR pathway with the most common abnormality noted in *NOCA2* on 8q13.3, an AR co-transcriptional factor that augments its transcriptional output. About 20% of primary tumors carried broad gain of region spanning *NCOA2* on 8q [28]. The project proposed both *NCOA2* on 8q13 and *MYC* on 8q24 amplicon as driver oncogenes in the pathogenesis of primary prostate cancer. Many other AR co activators, including *NCOA1*, *TNK2*, and *EP300* were also found to be unregulated in the metastatic disease whereas AR corepressors, including *NRIP1*, *NCOR1* and *NCOR2* were found to be downregulated [28]. Mutations in the AR itself are found in about 10% of CRPC with AR gene amplification and/or overexpression in about 30% of cases most exclusively for metastatic tumors [29].

Genetic Translocations of ETS Transcription Factors

E26 transformation-specific (ETS) transcription factors are atypical oncogenes as they can be overexpressed in some normal tissues without development of neoplasia. However in the presence of specific conditions such as loss of *PTEN* or *AKT* activation, such as in prostate cancer cells, they are highly oncogenic [30, 31]. In prostate

cancer, *TMPRSS2-ERG* fusion is the single most common genetic lesion with modest oncogenic potential as evidenced by mouse models [32, 33]. Results from a large copy-number alterations analysis of prostate cancer project showed that *TMPRSS2-ERG* fusion was associated with a loss of copy-number in three significant regions. Two of these regions involved tumor suppressor genes *PTEN* and *TP53* and identified the third region at 3p14 as novel tumor suppressor gene carrying locus. The loss of genes identified at 3p14 included *FOXP1*, *RYBP* and *SHQ1* [28]. Other gene fusions involving androgen regulated partners and ETS transcription factors include *SLC45A3:ERG*, *KLK2:ETV1* and *FKBP5:ERG* [32]. Recent studies have shown that there might a role of *TOP2B* gene in recurrent ETS rearrangements as they are co-expressed in *TMPRSS2:ERG* rearrangements [34, 35]. This observation suggests that transcription factors are associated with DNA double-strand breaks and therefore predispose transcribed regions to genomic rearrangements. Gene sequencing studies have also shown that rearrangement breakpoints are enriched near open chromatin, androgen receptor, and *ERG* DNA binding sites in the setting of *ETS* gene fusion *TMPRSS2-ERG* and inversely correlated with these regions in the tumors lacking *ETS* fusions suggesting a novel link between chromatin or transcriptional regulation and origin of genetic alterations [36].

Genetic Alteration in PTEN/PI3K/AKT Pathway

The loss of tumor suppressor *PTEN* either through deletion, mutation or silencing has been found in a significant proportion of prostate cancer cells [37]. Mouse models have previously shown that homozygous deletion of *PTEN* leads to the development and progression of prostate cancer from an early stage to metastatic disease [38–40]. It is somewhat unclear at what stage of tumorigenesis the loss of *PTEN* occurs as about 50% of metastatic lesion harbor loss of *PTEN* as opposed to only 20% of the primary lesions. Furthermore, the loss of *PTEN* may not be involved in the initiation of tumorigenesis as some prostate tumors show loss of *PTEN* only as a later event in the advanced stage of the disease [41]. PTEN is a significant repressor of PI3K/AKT pathway and loss of *PTEN* activates *AKT* which upregulates mTORC1 involved in cell proliferation and survival. It has also been shown that mTORC2 can lead to the development of prostate cancer in *PTEN* −/− mice [42]. Similarly *AKT1* deficiency can suppress the development of prostate cancer in *PTEN* +/− mice [43].

Gene rearrangements disrupting *MAGI2* gene are also found along with the rearrangements in *PTEN* gene. *MAGI2* encodes a *PTEN* interacting protein and its inactivation is thought to subvert *PTEN* function indirectly which was previously unknown [36].

Loss of 8p21 and NKX3.1

The heterozygous loss of 8p21 is found in about 85% of prostate cancer cells including prostatic intraepithelial neoplasia [44]. It is associated with the loss of single allele

of the AR regulated transcription factor NKX3.1 and therefore presents as a putative tumor suppressive gene involved in the tumorigenesis. In PTEN +/− mice loss of NKX3.1 led to the accelerated prostatic neoplasia [45, 46]. On the other hand, despite the monoallelic loss of NKX3.1, mRNA expression levels are noted to be quite adequate in prostate cancer cells which suggest the presence of other tumor suppressors in the 8p21 region whose loss mediates the development of prostate cancer.

Gain of 8q and MYC Amplification

MYC oncogene located on the 8q24 is the most common oncogene that is amplified with the gain of 8q24.21 region along with the novel *NCOA2* amplification on 8q13.3 as discussed above. MYC over-expression is shown to induce tumorigenesis in murine models of prostate cancer and is involved in development of dysplasia in prostate epithelial cells [27, 47].

Other Genetic Alterations in Prostate Cancer

Sequencing studies have shown the presence of recurrent non-synonymous mutations in about 6–13% of prostate cancer involving *SPOP* gene [48]. Loss of *SPOP* gene leads to altered genetic expression. This mutation was exclusively noted in tumors negative for *ERG* rearrangements. Recurrent gene rearrangements involving *CADM2* were also noted in sequencing studies of prostate cancer cell lines. *CADM2* is a nectin-like member of the immunoglobulin like cell adhesion molecule which displays tumor suppressor like properties [36].

In addition to the somatic mutations certain inherited genetic variants associated with prostate cancer risk are also recognized in recent copy-number variant (CNV) studies of the germ line. UGT2B17 gene which plays a role in the catabolism of testosterone was noted to be deleted on 4q13.2 in about 10% of Caucasian individuals [49, 50]. This confers a high risk for developing prostate cancer and more aggressive disease. Similarly, Genome wide association studies (GWAS) and family linkage analysis have found multiple independent single nucleotide polymorphisms (SNPs) as prostate cancer risk markers. SNPs involving *ARVCF*, *LEPR, CRY1*, *RNASEL* and *IL4* genes were recently reported to be associated independently with prostate cancer specific mortality [51].

Epigenetic Alterations in Prostate Cancer

Epigenetic changes such as hypermethylation of the gene promoters have been associated with tumorigenesis and persist through the disease progression. The most common

gene silenced by the hypermethylation of the promoter region in prostate cancer is GSTP1. GSTP1 encodes for π class of GST enzymes which catalyze key intracellular detoxification reactions including inactivation of electrophilic carcinogens. It has been shown in human prostate cancer cells that inactivation of GSTP1 by promoter hypermethylation leads to lack of expression of GST enzymes which results in prostatic neoplasia. [52] Hypermethylation of GSTP1 promoter is known to be present in a significant majority of prostate cancers [53, 54]. Overexpression of EZH2, a polycomb group protein that methylates H3K27 histone, has been associated with high risk localized and castrate resistant prostate cancer [55]. Additionally there are 40 other genes which are shown to be hypermethylated in prostate cancer cells [53].

Circulating Tumor Cells and Cancer Stem Cells

Circulating tumor cells (CTCs), although rare, are identified in the peripheral blood of patients with primary and metastatic cancers. They are thought to be involved in the hematogenous spread of primary cancers to their metastatic sites. Recent technological advances have made it possible to isolate and characterize CTCs and use this information to prognosticate and use the information in the clinical management of cancer. Multiple techniques have been employed to detect CTCs ranging from cell size based separation to the use of immunomagnetic beads conjugated with antibody to EpCAM, an epithelial cell marker. More recently a micro fluidic device has been shown to improve yield and purity of PSA positive CTC in prostate cancer patients [56, 57]. FDA has approved the assessment of CTCs using CellSearch as a prognostic indicator for metastatic prostate cancer. Studies have shown that CTCs are detected in high frequency in CRPC and correlate with clinical outcome [58–60]. A multicenter prospective study specifically showed that CTC number at different time points after treatment was the strongest independent predictor of survival in mCRPC [61]. These data clinically qualify the prognostic significance of baseline CTC and show that post-treatment CTC number predicts survival. In this study CTC number was more predictive than post-therapy changes in PSA, raising the likelihood that CTC number may be an intermediate end point of efficacy. Recently it was also shown that CTCs analysis could also be employed to gauge the proliferative and quiescent components of either primary or metastatic tumor deposit [57]. Patients with metastatic prostate cancer who were highly responsive to androgen withdrawal had a low Ki67 positive fraction, whereas those with progressive mCRPC had significantly higher Ki67 index [57].

Thus the study of CTCs is important to understand the hematagenous spread of tumor to distant sites, for making use of these cells for real-time, noninvasive tumor monitoring and in predicting prognosis both pre- and post- treatment in prostate cancer patients.

The cancer stem cell model (CSC) model proposes that cells within a tumor are organized in hierarchical lineage and display considerably different tumorigenic potential [62]. Prostate cancer stem cells (PCSCs) have increased tumor-initiating

and metastatic potential are enriched in the side population [63]. $CD133^{+}CD44^{+}\alpha2\beta1+$ prostate cancer cells show increased clonogenic potential in vitro [64, 65]. CSCs lead to therapy resistance, progression and relapses and therefore understanding the molecular mechanisms of the CSCs is crucial. MicroRNAs (miRNA) are small non-coding RNAs that are important in normal stem cells during development and may have crucial role in the regulation of CSCs as well. Recently the microRNAs, miR-34a together with let-7b was identified to completely underexpress in all marker-positive cell populations [66]. Most of the work on PCSCs has focused on the isolation, the importance of in vivo structures and tumor microenvironment has not been properly studied. Mouse models would be critical for the investigation of putative prostate cancer cells.

Tumor Microenvironment and Angiogenesis

Tumorigenesis involves not only the genetic and epigenetic changes but also other supportive structures that make up the tumor microenvironment. The tumor microenvironment comprises numerous signaling pathways that influence the angiogenic response of a tumor. Angiogenesis is essential for tumor growth and metastasis and inhibiting this process is crucial in limiting cancer progression. Tumor angiogenesis is initiated by enhanced tumor/tumor-stroma cell-specific pro-angiogenic molecules as well as switching off anti-angiogenic factors as well as tumor-associated hypoxia. VEGF and FGF have been correlated with prostate disease progression [67]. This has led to the development of a number of novel angiogenesis drugs including thalidomide, bevacizumab, sorafenib and others; some of which are in the pipeline for the treatment of prostate cancer.

Epithelial to mesenchymal transition (EMT) is documented in prostate cancer with increased expression of mesenchymal genes and is associated with increased cell motility, invasion and migration. Loss of E-cadherin and increased expression of vimentin is associated with EMT transition in prostate cancer cells [68].

Novel Targeted Therapies in Prostate Cancer

Several targeted agents are available and many more are in clinical trials and are being tested. We will discuss some of the promising ones below:

Inhibitors of Androgens and Androgen Receptor Signaling

Androgens play a central role in the pathogenesis and progression of prostate cancer. They exert their effect through the androgen receptor signaling which activates transcription of genes involved in the growth and survival of the cell. Therefore,

therapies targeting the androgen receptor and androgen blockade are central to the treatment of prostate cancer. Despite standard hormone therapy it has been shown that majority of castrate resistant prostate cancer cells still express AR [69] and the intra-tumoral levels of androgens remain very high along with persistent transcriptional activity [70]. Novel anti-androgen therapies are therefore needed.

MDV3100 is small molecule oral androgen receptor antagonist that directly inhibits AR by binding irreversibly thereby impairing the AR nuclear translocation, DNA binding and recruitment of coactivators [71]. MDV3100 shows a much stronger affinity to AR than bicalutamide in preclinical studies leading to more potent blockade [72]. Unlike bicalutamide, MDV3100 does not possess agonist activity when AR is overexpressed [72]. In Phase I/II studies involving mCRPC patients, MDV3100 showed promising antitumor activity and led to 56% response in the serum PSA and stabilization of bone metastasis [73]. Currently it is being evaluated for effect on overall survival in two Phase III clinical trials (NCT00974311 and NCT01212991) in patients with mCRPC with or without prior chemotherapy exposure respectively [74].

Abiraterone is an inhibitor of 17α-hydroxylase/CYP17 enzyme which catalyzes the conversion of sex hormones into precursors of testosterone in testes, adrenals and prostate tissues. Inhibiting CYP17 decreases the circulating levels of the testosterone [75]. Phase I trials of abiraterone in chemotherapy naïve CRPC patients showed promising activity by decreasing serum PSA levels by >50% in 55–57% of patients [76, 77]. In a Phase II study abiraterone showed significant reductions in PSA level, regression of radiological lesions and symptomatic improvements [78]. This led to a larger Phase III trial in which Abirateone plus prednisone showed an overall survival advantage of 14.8 months vs 10.9 months ($HR = 0.64$; $p < 0.001$) in the placebo plus prednisone control group among 1,195 patients with mCRPC who had disease progression after docetaxel-based chemotherapy [20]. A second Phase III clinical trial [NCT00887198] is comparing abiraterone and prednisone with placebo and prednisone in patients with mCRPC who are chemotherapy naïve.

TAK-700 is a selective non-steroidal potent inhibitor of CYP17 that is currently in Phase III trials. It inhibits CYP17 less potently but more selectively than abiretarone and has an improved safety and tolerability profile. In a Phase I study TAK-700 showed significant PSA level reduction in 80% of patients. Based on these results there are two Phase III randomized trials ongoing evaluating the effect of TAK-700 on overall survival as the primary endpoint vs placebo in post docetaxel and chemotherapy naïve mCRPC patients respectively (NCT01193244 and NCT01193257).

BMS-641988 is a novel antiandrogen with 20-fold greater affinity to AR than bicalutamide. It showed good efficacy in xenograft models that were refractory to bicalutamide and is in Phase I clinical trial for CRPC patients [79].

Inhibitors Targeting Angiogenesis

Several preclinical studies have reported benefit of blocking angiogenesis to inhibit prostate cancer bone metastases [80]. Immunomodulatory agents such as thalidomide and lenalidomide are thought to inhibit the secretion of proangiogenic cytokines from

both epithelial and stromal compartments although their exact mechanism of action is unknown. Phase II studies in combination with docetaxel for thalidomide produced promising results [81, 82]. Lenalidomide due to its improved tolerability is being evaluated in two clinical trials [83]. The first trial is a randomized placebo controlled Phase III study of lenalidomide in combination with docetaxel evaluating the effect on overall survival in mCRPC patients (NCT00988208). In the other Phase II trial for mCRPC patients, lenalidomide is combined with bevacizumab and prednisone to evaluate its safety and efficacy (NCT00942578). Aflibercept, a recombinant fusion protein that acts as "VEGF trap" or decoy receptor by binding to the free ligand and preventing it from interacting with and activating membrane bound VEGF-R1 and VEGF-R2 also showed safety and tolerability in Phase I study in combination with docetaxel [84]. It is currently in Phase III clinical trial in combination with docetaxel in mCRPC patients looking at overall survival as its primary endpoint. However, the efficacy of angiogenic therapy come into question after two recent Phase III clinical trials employing anti-angiogenic agents failed to show any benefit in mCRPC patients. The first trial CALGB 90401 involved the testing of bevacizumab, a monoclonal antibody inhibiting VEGF receptor signaling by binding and neutralizing the ligand, in combination with docetaxel and prednisone. The combination arm failed to show any survival benefit and was associated with higher morbidity. The other study NCT00676650 tested the ability of sunitinib, a multi-tyrosine inhibitor against VEGF and PDGF receptor, in combination with prednisone after failure of docetaxel-based chemotherapy in mCRPC patients. This study was however, terminated earlier at the interim analysis due to futility. These trials have led to a deeper understanding of tumor biology and the underlying angiogenic mechanisms driving these tumors such as the possibility of "tumor escape" from anti-angiogenic therapies resulting from the multiplicity of angiogenic pathways creating "redundancy" in these tumors. This has also led to a better selection of targeting agents and their combinations with other agents such as TKI258 which blocks VEGF, PDGF and βFGF receptors and combinations such as bevacizumab plus lenalidomide. Phase I/II study of sunitinib plus docetaxel in mCRPC patients in the frontline setting has showed substantial reductions in serum PSA and tumor burden.

Kinase Inhibitors

It has been reported that EGFR is overexpressed in 18–37% of prostate cancers and HER2 is associated with AR activation and PSA expression [85]. These studies indicate that there may be a therapeutic utility of targeting growth factors and growth factor receptors such as EGFR and HER2. However agents targeting these EGFR and HER2 including small molecule tyrosine kinase inhibitors (gefitinib, erlotinib, and lapatinib) and monoclonal antibodies (trastuzumab and pertuzamab) have shown disappointing results in clinical trials in CRPC patients despite some preclinical activity [86–89]. One possible explanation is that since EGFR or HER2 are not amplified in CRPC this is not a relevant target. Alternatively loss of PTEN

commonly found in CRPC is also thought to confer resistance to EGFR/HER2 targeted therapies.

Dasatinib is an oral tyrosine kinase inhibitor that targets SRC family kinases, BCR-ABL, c-KIT and PDGFR-B. Results of Dasatinib Phase II clinical trial for chemotherapy naïve mCRPC patients as single agent showed promising results [90]. Dasatinib was also tested in combination with docetaxel in a Phase I/II study of patients with mCRPC and durable PSA decline in more than 50% and partial responses in about 60% of patients [91]. Correlative studies showed an inversely proportional association between dasatinib peak levels and IL-8 and monocyte chemotactic protein-1 (MCP-1) levels. Patients who responded to the combination therapy when continued on dasatinib as maintenance monotherapy showed prolonged periods of disease stabilization [91]. Based on this promising Phase II data, a randomized double blind Phase III trial is currently underway comparing docetaxel plus dasatinib vs docetaxel plus placebo in mCRPC patients. (NCT00744497).

Agents Targeting Chaperone Proteins

Molecular chaperones such as heat shock protein-90 (HSP90) and clusterin (CLU) bind to client proteins and stabilize their quaternary structure. Over-expression of molecular chaperones strengthens the cell damage response and provides stability in oncogenic proliferation. As such HSP-90 is implicated in resistance to treatment in prostate cancer [92]. 17-AAG is an HSP90 inhibitor which showed safety in Phase I trials but reported only minimal activity in Phase II studies in mCRPC patients [93]. Clusterin expression has been associated with progression in CRPC patients. OGX-011 is an antisense oligonucleotide which has shown to decrease clusterin expression in preclinical models. In a recent Phase II randomized trial, OGX-011 was given with or without standard docetaxel plus prednisone chemotherapy in mCRPC patients. Experimental arm was associated with an overall survival advantage although the PSA responses were same in the two groups. Based on these data, OGX-011 is being tested in a large Phase III clinical trial with or without docetaxel plus prednisone in mCRPC patients in the frontline setting (NCT01188187).

Agents Targeting the Tumor Microenvironment

Stromal cells namely; endothelial cells, osteoclasts and osteoblasts constitute the bone tumor microenvironment and contribute to the disease progression. Agents targeting the molecular pathways that inhibit the ability of the stromal cells to support the cancer cells provide evidence that targeting the stromal cells can modulate the tumor microenvironment that can impact tumor progression. Endothelin type A (ETA) receptors are expressed on prostate cancer cells and osteoblasts and their activation leads to survival and proliferation of tumor cells. ET-1 is a signaling peptide

that is overexpressed in prostate cancer cells and its binding activates ETA receptor. Atrasentan is a highly selective ETA receptor antagonist that inhibits the osteoblast-dependent formation of new bone-induced by metastatic cancer cells. Atrasentan has provided "proof of principle" for monotherapy by modulating tumor microenvironment [94, 95] in Phase II and III clinical trials of mCRPC patients. Atrasentan is being tested for an overall survival benefit in a randomized Phase III clinical trial in combination with docetaxel plus prednisone compared to docetaxel plus prednisone alone in the frontline setting for mCRPC patients with bone metastasis (NCT00134056). Zibotentan is another highly selective inhibitor of ETA receptor. It showed favorable results in the Phase II study of patients with mCRPC and bony metastasis [96]. However two large Phase III trials in metastatic prostate cancer patients with bone metastasis testing zibotentan in combination with standard chemotherapy and monotherapy respectively failed to show any benefit [96].

Prostate cancer cells metastasizing to bone overexpress RANK ligand in comparison to the primary tumor. Interactions between the RANK ligand and its receptor are important in regulating osteoclastogenesis and bone remodeling involved in the development of prostate cancer bone metastasis. Denosumab is a human monoclonal antibody against the RANK ligand. It has been tested for efficacy in evaluating the prevention of metastatic bone disease progression in a large multicenter Phase III clinical trial. Denosumab significantly delayed the time to first skeletal related event and improvement in bone turnover markers however, there was no overall survival benefit [22]. Another large multicenter Phase III clinical trial of denosumab versus placebo showed an overall survival advantage of 4 months in delaying bone metastasis in men with non-metastatic CRPC at high risk for developing metastasis [97].

Agents Targeting the Immune System

Prostate cancers generate a host immune response within the tumor microenvironment [98]. However, prostate cancer cells are able to evade such immune responses through several mechanisms. Modulating tumor responses can thus be an effective strategy in inhibiting tumor growth. Sipuleucel-T is a cellular immunotherapy designed to enhance the cytotoxic T lymphocyte response against prostate acid phosphatase (PAP) expressed on prostate cancer cells. Two large Phase III clinical trials evaluated the efficacy of Sipuleucel-T in men with minimally symptomatic advanced prostate cancer [99, 100]. There was a significant improvement in median overall survival in patients treated with immunotherapy versus placebo. The third large Phase III clinical trial (IMPACT) confirmed these findings in men who received Sipuleucel-T [18]. Interestingly, no significant differences in PSA response, tumor regression, time to progression or quality of life were observed in both the arms.

Other agents have exploited the T-effector cell responses by regulating co-stimulatory molecules. Ipilimumab is a monoclonal antibody that blocks the activity of T-cell inhibitory receptor cytotoxic T lymphocyte-associated 4 (CTLA4).

CTLA4 is expressed on the surface of helper T cells and inhibits the response to self-antigens. Ipilimumab therefore inhibits immune tolerance to tumor cells. In a Phase I trials, Ipilimumab has shown safety and efficacy when given to mCRPC patients with GM-CSF [101, 102]. In a Phase II study of ipilimamab with or without docetaxel in mCRPC patients, safety and efficacy was evaluated. Currently there are two ongoing Phase III trials evaluating the overall survival in mCRPC patients treated with or without ipilimamab in pre- and post- docetaxel settings (NCT00861614 and NCT01057810).

PI3K-AKT-mTOR Signaling Pathway

There is evidence to suggest that PI3K/Akt/mTOR pathway contributes to the progression of castrate refractory prostate cancer and as such provides a rationale of targeting this pathway. There are several PI3K, AKt and mTOR inhibitors with preclinical activity and are being tested in ongoing Phase I clinical trials (NCT00110188, NCT00919035 and NCT00629525).

Other Targets in Treatment for Prostate Cancer

Recently, Serine protease inhibitor Kazal type 1 (SPINK1) was found to be increased in an aggressive subset of prostate cancer that do not harbor TMPRSS2-ERG fusion and is associated with a high rate of recurrences [103]. In vivo studies have shown that SPINK1 has similarities with EGF and binds to EGFR and inhibiting SPINK1 diminishes downstream signaling of EGFR pathway. The results provide evidence for the use of EGFR inhibitors in SPINK1 positive prostate cancers and SPINK1+ mice models have shown tumor regression with cetuximab [104, 105].

Survivin is a protein that inhibits caspase activation thereby preventing apoptosis [105, 106]. It is overexpressed in several cancers including that of prostate and can serve as a useful therapeutic target [107]. YM155 is a small molecule that inhibits survivin and has shown pro-apoptotic activity in vitro and in xenograft mice models [108]. YM155 has shown promising activity in Phase I and II trials for safety and efficacy and as a single agent induces PSA responses in prostate cancer patients [105, 108].

MET protooncogene (c-MET) is known to be abnormally activated in mCRPC via hepatocyte growth factor (HGF) signaling and therefore promotes tumor growth through effects on prostate cancers cells and osteoblasts [109, 110]. It has further been shown that androgen ablation leads to an increased expression of c-MET suggesting a possible role of c-MET in therapeutic resistance [111]. XL-184, an oral novel tyrosine kinase inhibitor of c-MET and VEGF-R2, has shown some exceptional results in Phase I clinical studies [112]. In mCRPC patient with measurable bone disease, it led to tumor shrinkage in 84% of patients and 86% had a complete

or partial resolution of bone lesions. A Phase II nonrandomized trial is currently evaluating XL-184 in patients with mCRPC previously treated with docetaxel-based chemotherapy (NCT00940225).

N-terminal domain (NTD) of the androgen receptor contributes towards most of the transcriptional activity of the androgen receptor, with AF-1 being essential for AR activity regardless of androgen [113, 114]. Recent efforts to develop drugs to the AR NTD have yielded EPI-001, a small molecule, sintokamide peptides and decoys to the AR NTD with EPI-001. Each of these has shown significant inhibition of AR with antitumor activity [115–117]. Developing inhibitors to the intrinsically disordered NTD provides a novel concept in the field of steroid hormone receptor therapy, which has previously concentrated on targeting the C-terminus ligand-binding domain [118, 119].

Conclusions and Future Perspectives

Recent advances in understanding the genetic basis of the disease in prostate cancer have provided clues for the development of new drug targets. Several promising agents such as androgen receptor antagonist MDV3100, CYP17 inhibitor TAK-700, SRC kinase inhibitor dasatinib, anti-sense oligonucleotide against clusterin OGX-011 and dual c-MET/VEGFR2 inhibitor cabozantinib are currently being tested in large randomized Phase III clinical trials. Similarly, several agents for new targets are currently being explored in initial Phase I studies and expected to provide new therapeutic options in a very challenging disease. Better understanding of the tumor microenvirnmont and angiogenesis in prostate cancer development has further provided new avenues for targeted therapy and shown promise in drug development. The role of CTCs has been elucidated and offers novel information for prognostication and monitoring of prostate cancer therapy. The advances in the understanding of genetic alterations in prostate cancer pathogenesis and clinical trials of novel agents utilizing novel targets hold the key to the future breakthrough in improving survival in prostate cancer therapy.

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin. 2012;62(1):10–29.
2. Sanchez-Ortiz RF, Troncoso P, Babaian RJ, Lloreta J, Johnston DA, Pettaway CA. African-American men with nonpalpable prostate cancer exhibit greater tumor volume than matched white men. Cancer. 2006;107(1):75–82.
3. Bill-Axelson A, Holmberg L, Filen F, Ruutu M, Garmo H, Busch C, et al. Radical prostatectomy versus watchful waiting in localized prostate cancer: the Scandinavian prostate cancer group-4 randomized trial. J Natl Cancer Instrum. 2008;100(16):1144–54.
4. Horwitz EM, Bae K, Hanks GE, Porter A, Grignon DJ, Brereton HD, et al. Ten-year follow-up of radiation therapy oncology group protocol 92–02: a phase III trial of the duration of

elective androgen deprivation in locally advanced prostate cancer. J Clin Oncol. 2008;26(15):2497–504.
5. Speight JL, Roach 3rd M. Radiotherapy in the management of clinically localized prostate cancer: evolving standards, consensus, controversies and new directions. J Clin Oncol. 2005;23(32):8176–85.
6. Roach 3rd M, Bae K, Speight J, Wolkov HB, Rubin P, Lee RJ, et al. Short-term neoadjuvant androgen deprivation therapy and external-beam radiotherapy for locally advanced prostate cancer: long-term results of RTOG 8610. J Clin Oncol. 2008;26(4):585–91.
7. Widmark A, Klepp O, Solberg A, Damber JE, Angelsen A, Fransson P, et al. Endocrine treatment, with or without radiotherapy, in locally advanced prostate cancer (SPCG-7/SFUO-3): an open randomised phase III trial. Lancet. 2009;373(9660):301–8.
8. Messing EM, Manola J, Yao J, Kiernan M, Crawford D, Wilding G, et al. Immediate versus deferred androgen deprivation treatment in patients with node-positive prostate cancer after radical prostatectomy and pelvic lymphadenectomy. Lancet Oncol. 2006;7(6):472–9.
9. Lawton CA, Winter K, Grignon D, Pilepich MV. Androgen suppression plus radiation versus radiation alone for patients with stage D1/pathologic node-positive adenocarcinoma of the prostate: updated results based on national prospective randomized trial Radiation Therapy Oncology Group 85–31. J Clin Oncol. 2005;23(4):800–7.
10. Prostate Cancer Trialists' Collaborative Group. Maximum androgen blockade in advanced prostate cancer: an overview of the randomised trials. Lancet. 2000;355(9214):1491–8.
11. Samson DJ, Seidenfeld J, Schmitt B, Hasselblad V, Albertsen PC, Bennett CL, et al. Systematic review and meta-analysis of monotherapy compared with combined androgen blockade for patients with advanced prostate carcinoma. Cancer. 2002;95(2):361–76.
12. The Medical Research Council Prostate Cancer Working Party Investigators Group. Immediate versus deferred treatment for advanced prostatic cancer: initial results of the Medical Research Council Trial. Br J Urol. 1997;79(2):235–46.
13. Bolla M, Collette L, Blank L, Warde P, Dubois JB, Mirimanoff RO, et al. Long-term results with immediate androgen suppression and external irradiation in patients with locally advanced prostate cancer (an EORTC study): a phase III randomised trial. Lancet. 2002;360(9327):103–6.
14. Ryan CJ, Small EJ. Early versus delayed androgen deprivation for prostate cancer: new fuel for an old debate. J Clin Oncol. 2005;23(32):8225–31.
15. Petrylak DP, Tangen CM, Hussain MH, Lara Jr PN, Jones JA, Taplin ME, et al. Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. N Engl J Med. 2004;351(15):1513–20.
16. Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. N Engl J Med. 2004;351(15):1502–12.
17. Berthold DR, Pond GR, Soban F, de Wit R, Eisenberger M, Tannock IF. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer: updated survival in the TAX 327 study. J Clin Oncol. 2008;26(2):242–5.
18. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010;363(5):411–22.
19. de Bono JS, Oudard S, Ozguroglu M, Hansen S, Machiels JP, Kocak I, et al. Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. Lancet. 2010;376(9747):1147–54.
20. de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, et al. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med. 2011;364(21):1995–2005.
21. Tannock IF, Osoba D, Stockler MR, Ernst DS, Neville AJ, Moore MJ, et al. Chemotherapy with mitoxantrone plus prednisone or prednisone alone for symptomatic hormone-resistant prostate cancer: a Canadian randomized trial with palliative end points. J Clin Oncol. 1996;14(6):1756–64.
22. Fizazi K, Carducci M, Smith M, Damiao R, Brown J, Karsh L, et al. Denosumab versus zoledronic acid for treatment of bone metastases in men with castration-resistant prostate cancer: a randomised, double-blind study. Lancet. 2011;377(9768):813–22.

23. Saad F, Gleason DM, Murray R, Tchekmedyian S, Venner P, Lacombe L, et al. A randomized, placebo-controlled trial of zoledronic acid in patients with hormone-refractory metastatic prostate carcinoma. J Natl Cancer Inst. 2002;94(19):1458–68.
24. Saad F, Gleason DM, Murray R, Tchekmedyian S, Venner P, Lacombe L, et al. Long-term efficacy of zoledronic acid for the prevention of skeletal complications in patients with metastatic hormone-refractory prostate cancer. J Natl Cancer Inst. 2004;96(11):879–82.
25. Gu G, Brothman AR. Cytogenomic aberrations associated with prostate cancer. Cancer Genet. 2011;204(2):57–67.
26. Chen Y, Sawyers CL, Scher HI. Targeting the androgen receptor pathway in prostate cancer. Curr Opin Pharmacol. 2008;8(4):440–8.
27. Berger R, Febbo PG, Majumder PK, Zhao JJ, Mukherjee S, Signoretti S, et al. Androgen-induced differentiation and tumorigenicity of human prostate epithelial cells. Cancer Res. 2004;64(24):8867–75.
28. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell. 2010;18(1):11–22.
29. Visakorpi T, Hyytinen E, Koivisto P, Tanner M, Keinanen R, Palmberg C, et al. In vivo amplification of the androgen receptor gene and progression of human prostate cancer. Nat Genet. 1995;9(4):401–6.
30. Carver BS, Tran J, Gopalan A, Chen Z, Shaikh S, Carracedo A, et al. Aberrant ERG expression cooperates with loss of PTEN to promote cancer progression in the prostate. Nat Genet. 2009;41(5):619–24.
31. Zong Y, Xin L, Goldstein AS, Lawson DA, Teitell MA, Witte ON. ETS family transcription factors collaborate with alternative signaling pathways to induce carcinoma from adult murine prostate cells. Proc Natl Acad Sci U S A. 2009;106(30):12465–70.
32. 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(5748):644–8.
33. Tomlins SA, Laxman B, Varambally S, Cao X, Yu J, Helgeson BE, et al. Role of the TMPRSS2-ERG gene fusion in prostate cancer. Neoplasia. 2008;10(2):177–88.
34. Ju BG, Lunyak VV, Perissi V, Garcia-Bassets I, Rose DW, Glass CK, et al. A topoisomerase IIbeta-mediated dsDNA break required for regulated transcription. Science. 2006;312(5781):1798–802.
35. Haffner MC, Aryee MJ, Toubaji A, Esopi DM, Albadine R, Gurel B, et al. Androgen-induced TOP2B-mediated double-strand breaks and prostate cancer gene rearrangements. Nat Genet. 2010;42(8):668–75.
36. Berger MF, Lawrence MS, Demichelis F, Drier Y, Cibulskis K, Sivachenko AY, et al. The genomic complexity of primary human prostate cancer. Nature. 2011;470(7333):214–20.
37. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, et al. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science. 1997;275(5308):1943–7.
38. Wang S, Gao J, Lei Q, Rozengurt N, Pritchard C, Jiao J, et al. Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. Cancer Cell. 2003;4(3):209–21.
39. Ma X, der Made AC Z-v, Autar B, van der Korput HA, Vermeij M, van Duijn P, et al. Targeted biallelic inactivation of Pten in the mouse prostate leads to prostate cancer accompanied by increased epithelial cell proliferation but not by reduced apoptosis. Cancer Res. 2005;65(13):5730–9.
40. Backman SA, Ghazarian D, So K, Sanchez O, Wagner KU, Hennighausen L, et al. Early onset of neoplasia in the prostate and skin of mice with tissue-specific deletion of Pten. Proc Natl Acad Sci USA. 2004;101(6):1725–30.
41. Han B, Mehra R, Lonigro RJ, Wang L, Suleman K, Menon A, et al. Fluorescence in situ hybridization study shows association of PTEN deletion with ERG rearrangement during prostate cancer progression. Mod Pathol. 2009;22(8):1083–93.

42. Guertin DA, Stevens DM, Saitoh M, Kinkel S, Crosby K, Sheen JH, et al. mTOR complex 2 is required for the development of prostate cancer induced by Pten loss in mice. Cancer Cell. 2009;15(2):148–59.
43. Chen ML, Xu PZ, Peng XD, Chen WS, Guzman G, Yang X, et al. The deficiency of Akt1 is sufficient to suppress tumor development in Pten+/− mice. Genes Dev. 2006;20(12):1569–74.
44. Abate-Shen C. Deregulated homeobox gene expression in cancer: cause or consequence? Nat Rev Cancer. 2002;2(10):777–85.
45. Kim MJ, Bhatia-Gaur R, Banach-Petrosky WA, Desai N, Wang Y, Hayward SW, et al. Nkx3.1 mutant mice recapitulate early stages of prostate carcinogenesis. Cancer Res. 2002;62(11):2999–3004.
46. Kim MJ, Cardiff RD, Desai N, Banach-Petrosky WA, Parsons R, Shen MM, et al. Cooperativity of Nkx3.1 and Pten loss of function in a mouse model of prostate carcinogenesis. Proc Natl Acad Sci U S A. 2002;99(5):2884–9.
47. Ellwood-Yen K, Graeber TG, Wongvipat J, Iruela-Arispe ML, Zhang J, Matusik R, et al. Myc-driven murine prostate cancer shares molecular features with human prostate tumors. Cancer Cell. 2003;4(3):223–38.
48. Nagai Y, Kojima T, Muro Y, Hachiya T, Nishizawa Y, Wakabayashi T, et al. Identification of a novel nuclear speckle-type protein, SPOP. FEBS Lett. 1997;418(1–2):23–6.
49. Gallagher CJ, Kadlubar FF, Muscat JE, Ambrosone CB, Lang NP, Lazarus P. The UGT2B17 gene deletion polymorphism and risk of prostate cancer. A case-control study in Caucasians. Cancer Detect Prev. 2007;31(4):310–5.
50. Karypidis AH, Olsson M, Andersson SO, Rane A, Ekstrom L. Deletion polymorphism of the UGT2B17 gene is associated with increased risk for prostate cancer and correlated to gene expression in the prostate. T P J. 2008;8(2):147–51.
51. Lin DW, FitzGerald LM, Fu R, Kwon EM, Zheng SL, Kolb S, et al. Genetic variants in the LEPR, CRY1, RNASEL, IL4, and ARVCF genes are prognostic markers of prostate cancer-specific mortality. Cancer Epidemiol Biomarkers Prev. 2011;20(9):1928–36.
52. Lee WH, Morton RA, Epstein JI, Brooks JD, Campbell PA, Bova GS, et al. Cytidine methylation of regulatory sequences near the pi-class glutathione S-transferase gene accompanies human prostatic carcinogenesis. Proc Natl Acad Sci USA. 1994;91(24):11733–7.
53. Bastian PJ, Yegnasubramanian S, Palapattu GS, Rogers CG, Lin X, De Marzo AM, et al. Molecular biomarker in prostate cancer: the role of CpG island hypermethylation. Eur Urol. 2004;46(6):698–708.
54. Nelson WG, De Marzo AM, Yegnasubramanian S. Epigenetic alterations in human prostate cancers. Endocrinology. 2009;150(9):3991–4002.
55. Varambally S, Dhanasekaran SM, Zhou M, Barrette TR, Kumar-Sinha C, Sanda MG, et al. The polycomb group protein EZH2 is involved in progression of prostate cancer. Nature. 2002;419(6907):624–9.
56. Nagrath S, Sequist LV, Maheswaran S, Bell DW, Irimia D, Ulkus L, et al. Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature. 2007;450(7173):1235–9.
57. Stott SL, Lee RJ, Nagrath S, Yu M, Miyamoto DT, Ulkus L, et al. Isolation and characterization of circulating tumor cells from patients with localized and metastatic prostate cancer. Sci Translat Med. 2010;2(25):25ra3.
58. Shaffer DR, Leversha MA, Danila DC, Lin O, Gonzalez-Espinoza R, Gu B, et al. Circulating tumor cell analysis in patients with progressive castration-resistant prostate cancer. Clin Cancer Res. 2007;13(7):2023–9.
59. Danila DC, Heller G, Gignac GA, Gonzalez-Espinoza R, Anand A, Tanaka E, et al. Circulating tumor cell number and prognosis in progressive castration-resistant prostate cancer. Clin Cancer Res. 2007;13(23):7053–8.
60. Moreno JG, Miller MC, Gross S, Allard WJ, Gomella LG, Terstappen LW. Circulating tumor cells predict survival in patients with metastatic prostate cancer. Urology. 2005;65(4):713–8.

61. de Bono JS, Scher HI, Montgomery RB, Parker C, Miller MC, Tissing H, et al. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. Clin Cancer Res. 2008;14(19):6302–9.
62. Wang ZA, Shen MM. Revisiting the concept of cancer stem cells in prostate cancer. Oncogene. 2011;30(11):1261–71.
63. Patrawala L, Calhoun T, Schneider-Broussard R, Zhou J, Claypool K, Tang DG. Side population is enriched in tumorigenic, stem-like cancer cells, whereas ABCG2+ and ABCG2- cancer cells are similarly tumorigenic. Cancer Res. 2005;65(14):6207–19.
64. Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. Cancer Res. 2005;65(23):10946–51.
65. Patrawala L, Calhoun-Davis T, Schneider-Broussard R, Tang DG. Hierarchical organization of prostate cancer cells in xenograft tumors: the CD44+alpha2beta1+ cell population is enriched in tumor-initiating cells. Cancer Res. 2007;67(14):6796–805.
66. Liu C, Kelnar K, Liu B, Chen X, Calhoun-Davis T, Li H, et al. The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. Nat Med. 2011;17(2):211–5.
67. Chung LW, Baseman A, Assikis V, Zhau HE. Molecular insights into prostate cancer progression: the missing link of tumor microenvironment. J Urol. 2005;173(1):10–20.
68. Veveris-Lowe TL, Lawrence MG, Collard RL, Bui L, Herington AC, Nicol DL, et al. Kallikrein 4 (hK4) and prostate-specific antigen (PSA) are associated with the loss of E-cadherin and an epithelial-mesenchymal transition (EMT)-like effect in prostate cancer cells. Endocr Relat Cancer. 2005;12(3):631–43.
69. Agoulnik IU, Weigel NL. Androgen receptor action in hormone-dependent and recurrent prostate cancer. J Cell Biochem. 2006;99(2):362–72.
70. Locke JA, Guns ES, Lubik AA, Adomat HH, Hendy SC, Wood CA, et al. Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer. Cancer Res. 2008;68(15):6407–15.
71. Jung ME, Ouk S, Yoo D, Sawyers CL, Chen C, Tran C, et al. Structure-activity relationship for thiohydantoin androgen receptor antagonists for castration-resistant prostate cancer (CRPC). J Med Chem. 2010;53(7):2779–96.
72. Tran C, Ouk S, Clegg NJ, Chen Y, Watson PA, Arora V, et al. Development of a second-generation antiandrogen for treatment of advanced prostate cancer. Science. 2009;324(5928):787–90.
73. Scher HI, Beer TM, Higano CS, Anand A, Taplin ME, Efstathiou E, et al. Antitumour activity of MDV3100 in castration-resistant prostate cancer: a phase 1–2 study. Lancet. 2010;375(9724):1437–46.
74. Wu Y, Rosenberg JE, Taplin ME. Novel agents and new therapeutics in castration-resistant prostate cancer. Curr Opin Oncol. 2011;23(3):290–6.
75. Haidar S, Ehmer PB, Barassin S, Batzl-Hartmann C, Hartmann RW. Effects of novel 17alpha-hydroxylase/C17, 20-lyase (P450 17, CYP 17) inhibitors on androgen biosynthesis in vitro and in vivo. J Steroid Biochem. 2003;84(5):555–62.
76. Ryan CJ, Smith MR, Fong L, Rosenberg JE, Kantoff P, Raynaud F, et al. Phase I clinical trial of the CYP17 inhibitor abiraterone acetate demonstrating clinical activity in patients with castration-resistant prostate cancer who received prior ketoconazole therapy. J Clin Oncol. 2010;28(9):1481–8.
77. Attard G, Reid AH, Yap TA, Raynaud F, Dowsett M, Settatree S, et al. Phase I clinical trial of a selective inhibitor of CYP17, abiraterone acetate, confirms that castration-resistant prostate cancer commonly remains hormone driven. J Clin Oncol. 2008;26(28):4563–71.
78. Attard G, Reid AH, A'Hern R, Parker C, Oommen NB, Folkerd E, et al. Selective inhibition of CYP17 with abiraterone acetate is highly active in the treatment of castration-resistant prostate cancer. J Clin Oncol. 2009;27(23):3742–8.
79. Attar RM, Jure-Kunkel M, Balog A, Cvijic ME, Dell-John J, Rizzo CA, et al. Discovery of BMS-641988, a novel and potent inhibitor of androgen receptor signaling for the treatment of prostate cancer. Cancer Res. 2009;69(16):6522–30.

80. Madan RA, Dahut WL. Angiogenesis inhibition in the treatment of prostate cancer. Anticancer Agents Med Chem. 2009;9(10):1070–8.
81. Figg WD, Arlen P, Gulley J, Fernandez P, Noone M, Fedenko K, et al. A randomized phase II trial of docetaxel (taxotere) plus thalidomide in androgen-independent prostate cancer. Semin Oncol. 2001;28(4 Suppl 15):62–6.
82. Figg WD, Dahut W, Duray P, Hamilton M, Tompkins A, Steinberg SM, et al. A randomized phase II trial of thalidomide, an angiogenesis inhibitor, in patients with androgen-independent prostate cancer. Clin Cancer Res. 2001;7(7):1888–93.
83. Dahut WL, Aragon-Ching JB, Woo S, Tohnya TM, Gulley JL, Arlen PM, et al. Phase I study of oral lenalidomide in patients with refractory metastatic cancer. J Clin Pharmacol. 2009;49(6):650–60.
84. Lockhart AC, Rothenberg ML, Dupont J, Cooper W, Chevalier P, Sternas L, et al. Phase I study of intravenous vascular endothelial growth factor trap, aflibercept, in patients with advanced solid tumors. J Clin Oncol. 2010;28(2):207–14.
85. Schlomm T, Kirstein P, Iwers L, Daniel B, Steuber T, Walz J, et al. Clinical significance of epidermal growth factor receptor protein overexpression and gene copy number gains in prostate cancer. Clin Cancer Res. 2007;13(22 Pt 1):6579–84.
86. Gross M, Higano C, Pantuck A, Castellanos O, Green E, Nguyen K, et al. A phase II trial of docetaxel and erlotinib as first-line therapy for elderly patients with androgen-independent prostate cancer. BMC Cancer. 2007;7:142.
87. Curigliano G, De Braud F, Teresa Sandri M, Renne G, Zorzino L, Scardino E, et al. Gefitinib combined with endocrine manipulation in patients with hormone-refractory prostate cancer: quality of life and surrogate markers of activity. Anti-Cancer Drugs. 2007;18(8):949–54.
88. Ziada A, Barqawi A, Glode LM, Varella-Garcia M, Crighton F, Majeski S, et al. The use of trastuzumab in the treatment of hormone refractory prostate cancer; phase II trial. Prostate. 2004;60(4):332–7.
89. Agus DB, Sweeney CJ, Morris MJ, Mendelson DS, McNeel DG, Ahmann FR, et al. Efficacy and safety of single-agent pertuzumab (rhuMAb 2C4), a human epidermal growth factor receptor dimerization inhibitor, in castration-resistant prostate cancer after progression from taxane-based therapy. J Clin Oncol. 2007;25(6):675–81.
90. Yu EY, Wilding G, Posadas E, Gross M, Culine S, Massard C, et al. Phase II study of dasatinib in patients with metastatic castration-resistant prostate cancer. Clin Cancer Res. 2009;15(23):7421–8.
91. Araujo JC, Mathew P, Armstrong AJ, Braud EL, Posadas E, Lonberg M, et al. Dasatinib combined with docetaxel for castration-resistant prostate cancer: Results from a phase 1–2 study. Cancer. 2012;118(1):63–71.
92. Whitesell L, Lindquist SL. HSP90 and the chaperoning of cancer. Nat Rev Cancer. 2005;5(10):761–72.
93. Heath EI, Hillman DW, Vaishampayan U, Sheng S, Sarkar F, Harper F, et al. A phase II trial of 17-allylamino-17-demethoxygeldanamycin in patients with hormone-refractory metastatic prostate cancer. Clin Cancer Res. 2008;14(23):7940–6.
94. Carducci MA, Saad F, Abrahamsson PA, Dearnaley DP, Schulman CC, North SA, et al. A phase 3 randomized controlled trial of the efficacy and safety of atrasentan in men with metastatic hormone-refractory prostate cancer. Cancer. 2007;110(9):1959–66.
95. Michaelson MD, Kaufman DS, Kantoff P, Oh WK, Smith MR. Randomized phase II study of atrasentan alone or in combination with zoledronic acid in men with metastatic prostate cancer. Cancer. 2006;107(3):530–5.
96. James ND, Caty A, Payne H, Borre M, Zonnenberg BA, Beuzeboc P, et al. Final safety and efficacy analysis of the specific endothelin a receptor antagonist zibotentan (ZD4054) in patients with metastatic castration-resistant prostate cancer and bone metastases who were pain-free or mildly symptomatic for pain: a double-blind, placebo-controlled, randomized Phase II trial. BJU Int. 2010;106(7):966–73.
97. Smith MR, Saad F, Coleman R, Shore N, Fizazi K, Tombal B, 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.

98. Whiteside TL. The tumor microenvironment and its role in promoting tumor growth. Oncogene. 2008;27(45):5904–12.
99. Small EJ, Schellhammer PF, Higano CS, Redfern CH, Nemunaitis JJ, Valone FH, 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.
100. Higano CS, Schellhammer PF, Small EJ, Burch PA, Nemunaitis J, Yuh L, 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.
101. Small EJ, Tchekmedyian NS, Rini BI, Fong L, Lowy I, Allison JP. A pilot trial of CTLA-4 blockade with human anti-CTLA-4 in patients with hormone-refractory prostate cancer. Clin Cancer Res. 2007;13(6):1810–5.
102. Fong L, Kwek SS, O'Brien S, Kavanagh B, McNeel DG, Weinberg V, et al. Potentiating endogenous antitumor immunity to prostate cancer through combination immunotherapy with CTLA4 blockade and GM-CSF. Cancer Res. 2009;69(2):609–15.
103. Tomlins SA, Rhodes DR, Yu J, Varambally S, Mehra R, Perner S, et al. The role of SPINK1 in ETS rearrangement-negative prostate cancers. Cancer Cell. 2008;13(6):519–28.
104. Ozaki N, Ohmuraya M, Hirota M, Ida S, Wang J, Takamori H, et al. Serine protease inhibitor Kazal type 1 promotes proliferation of pancreatic cancer cells through the epidermal growth factor receptor. Mol Cancer Res. 2009;7(9):1572–81.
105. Fu W, Madan E, Yee M, Zhang H. Progress of molecular targeted therapies for prostate cancers. Biochim Biophys Acta. 2011;1825(2):140–52.
106. Li F, Ling X. Survivin study: an update of "what is the next wave"? J Cell Physiol. 2006;208(3):476–86.
107. Sah NK, Khan Z, Khan GJ, Bisen PS. Structural, functional and therapeutic biology of survivin. Cancer Lett. 2006;244(2):164–71.
108. Nakahara T, Takeuchi M, Kinoyama I, Minematsu T, Shirasuna K, Matsuhisa A, et al. YM155, a novel small-molecule survivin suppressant, induces regression of established human hormone-refractory prostate tumor xenografts. Cancer Res. 2007;67(17):8014–21.
109. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. Nat Rev Mol Cell Biol. 2003;4(12):915–25.
110. Knudsen BS, Edlund M. Prostate cancer and the met hepatocyte growth factor receptor. Adv Cancer Res. 2004;91:31–67.
111. Tu WH, Zhu C, Clark C, Christensen JG, Sun Z. Efficacy of c-Met inhibitor for advanced prostate cancer. BMC Cancer. 2010;10:556.
112. You WK, Sennino B, Williamson CW, Falcon B, Hashizume H, Yao LC, et al. VEGF and c-Met blockade amplify angiogenesis inhibition in pancreatic islet cancer. Cancer Res. 2011;71(14):4758–68.
113. Jenster G, van der Korput HA, van Vroonhoven C, van der Kwast TH, Trapman J, Brinkmann AO. Domains of the human androgen receptor involved in steroid binding, transcriptional activation, and subcellular localization. Mol Endocrinol. 1991;5(10):1396–404.
114. Simental JA, Sar M, Lane MV, French FS, Wilson EM. Transcriptional activation and nuclear targeting signals of the human androgen receptor. J Biol Chem. 1991;266(1):510–8.
115. Sadar MD, Williams DE, Mawji NR, Patrick BO, Wikanta T, Chasanah E, et al. Sintokamides A to E, chlorinated peptides from the sponge Dysidea sp. that inhibit transactivation of the N-terminus of the androgen receptor in prostate cancer cells. Org Lett. 2008;10(21): 4947–50.
116. Andersen RJ, Mawji NR, Wang J, Wang G, Haile S, Myung JK, et al. Regression of castrate-recurrent prostate cancer by a small-molecule inhibitor of the amino-terminus domain of the androgen receptor. Cancer Cell. 2010;17(6):535–46.
117. Quayle SN, Mawji NR, Wang J, Sadar MD. Androgen receptor decoy molecules block the growth of prostate cancer. Proc Natl Acad Sci USA. 2007;104(4):1331–6.

118. Sadar MD. Small molecule inhibitors targeting the “achilles’ heel” of androgen receptor activity. Cancer Res. 2011;71(4):1208–13.
119. Sadar MD. Advances in small molecule inhibitors of androgen receptor for the treatment of advanced prostate cancer. World J Urol. 2012;30(3):311–8.
120. Scher HI, et al. Effect of MDV3100, an androgen receptor signaling inhibitor (ARSI), on overall survival in patients with prostate cancer postdocetaxel: results from the phase III AFFIRM study. J Clin Oncol. 2012;30(suppl 5; abstr LBA1).
121. Parker C, et al. Updated analysis of the phase III, double-blind, randomized, multinational study of radium-223 chloride in castration-resistant prostate cancer (CRPC) patients with bone metastases (ALSYMPCA). J Clin Oncol. 2012;30(suppl; abstr LBA4512).

Small-Cell Lung Cancer: An Update on Targeted Therapies

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Abstract Lung cancer is the leading cause of cancer-related deaths world-wide and small-cell lung cancer (SCLC) accounts for up to 25% of lung cancer deaths. There has been a considerable amount of research in the understanding of the depth of biology of SCLC and utilizing this knowledge to develop targeted approaches. The treatment of SCLC remains a challenge, despite remarkable initial efficacy to combination chemotherapy and radiation therapy. The response is usually short-lived and the prognosis of SCLC has not changed over the past few decades, necessitating the critical need for evaluating novel agents/therapies. Several signaling pathways have been found to be activated in SCLC tumor cells, forming a rationale for blocking some of the drugable targets. Molecular changes and biological markers have been identified but remain to be validated. Novel and targeted agents have been evaluated but without much success. Increasing understanding of the biology and potential clinical evaluation of biomarkers will pave the way for more effective treatments.

Keywords Small cell lung cancer • Keratin • Etoposide • Topotecan • Irinotecan • Docetaxel • Gemcitabine • 3p21.3 • RASSFIA • myc • NFkB • c-Met • HGF • VEGF • IGF-1 • GRP • FRAP • Thalidomide • Carboplatin • Bevacizumab • Cisplatin • Sorafenib • Cediranib • Vandetanib • Aflibercept • c-kit • EGFR • TKI • bcr-abl • Imatinib • Gefitinib • mTOR • Everolimus • Ganitumab • Dasatinib • Obatoclax • HDAC inhibitor • Hedgehog inhibitor • Bendamustine • Ifosfamide • Circulating tumor cells

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W.S. El-Deiry (ed.), *Impact of Genetic Targets on Cancer Therapy*, Advances in Experimental Medicine and Biology 779, DOI 10.1007/978-1-4614-6176-0_18,

Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide with more than a million deaths per year [1]. It is estimated to account for about 160,340 estimated deaths in the United States in 2012 [2]. Small cell lung cancer (SCLC) accounts for about 15% of all lung cancer diagnosed annually and up to 25% of lung cancer deaths [3]. There has been a decline in both the incidence and mortality of SCLC in the United States with the changing patterns in smoking [4]. Histologically, the malignant cells are small, round or spindle shaped with minimal cytoplasm. These cells are immunoreactive for keratin, epithelial membrane antigen and thyroid transcription factor 1. There is also a spectrum of neuroendocrine and neural differentiation.

Clinically, SCLC can be divided into limited disease (LD-SCLC) and extensive disease (ED-SCLC). The former is defined as tumors confined to the hemithorax of origin, the mediastinum, or the supraclavicular lymph nodes that can be encompassed in a radiation port or field, when the malignancy has spread beyond the aforementioned area. The current treatment approach to SCLC is concurrent chemotherapy (cisplatin or carboplatin with etoposide) and radiation therapy in patients with LD and chemotherapy alone in patients with ED. Prophylactic cranial irradiation has been shown to improve survival in patients who responded to initial treatment regimen in both LD and ED. However the treatment of SCLC remains a challenge. Most patients are diagnosed with ED-SCLC and majority of these patients will relapse despite initial response to combination chemotherapy. The overall prognosis of SCLC has not changed much over the past few decades and this area is ripe for early phase development. Without treatment, patients with SCLC have a very aggressive course with the median survival of about 2–4 months from the time of diagnosis [4]. The median survival of LD-SCLC treated with chemotherapy and concurrent radiation is around 16–24 months and the median survival of ED-SCLC, treated with chemotherapy alone is around 7–12 months [4].

Following relapse, at present there are limited options for treatment of SCLC in second-line setting. Chemotherapy drugs such as etoposide, topotecan, irinotecan, docetaxel, vinorelbine, gemcitabine have been evaluated for efficacy in relapsed/recurrent SCLC [5–7]. Topotecan is the only approved Food and Drug Administration (FDA) agent for second-line treatment for SCLC. However, the basis for its approval was symptom control without survival benefit [8]. There has been a considerable amount of effort in understanding the depth of molecular biology of SCLC and utilizing this knowledge to develop targeted therapies for this disease. There is an emergent need for development of maintenance strategies that could keep the responsive disease quiescent and ultimately impact overall survival.

Genetics in Small Cell Lung Cancer

There are several genetic abnormalities in SCLC, some at a higher frequency than others. These aberrations involve both oncogenes and tumor suppressor genes and are complex and heterogenous. One of the earliest changes to occur in smoking-induced

lung epithelium is the loss of heterozygosity at the 3p21.3 region and may be a necessary and key event in lung cancer development [9]. The p53 gene is reported to maintain the integrity of the human genome and acts as a transcription factor, inducing expression of downstream genes in response to DNA damage by carcinogens and is found in 75–100% of SCLC [10]. Therefore, aberration of p53 by tumor cells lead to unregulated progression and survival of these damaged cells [11]. Most of the mutations are missense, deletions, insertions or splicing error [12]. Another tumor suppressor gene implicated in majority of SCLC is deletion of gene RASSFIA which is epigenetically inactivated leading to lung cancer development [13]. The myc (myelocytomatosis) family members are also implicated as growth-regulatory system in lung cancer. These oncogenes are activated by gene amplification or transcriptional dysregulation [14]. The Bcl2 (B-cell lymphoma 2) functions in programmed cell death and is regulated by the inhibitor of NF-kB (IkB) but its dysregulation may confer resistance to treatment with cytotoxic chemotherapy [15]. Another tumor suppressor gene implicated in SCLC is RB (retinoblastoma) which is located on chromosome 13q14.11 and its role in the regulation of cell cycle is well documented [16]. Inactivation of this gene has been associated with the development of SCLC and it accounts for about 90% of SCLC [17].

The c-MET receptor tyrosine kinase is the receptor for HGF/scatter factor (SF) and this may be over-expressed, mutated, or amplified in a number of solid tumors including SCLC [18]. Activation of c-MET, a tyrosine kinase receptor is also found to be highly expressed in SCLC and this activation leads to proliferation, angiogenesis, and mutations [19]. It has also been reported that activating mutations in the juxtamembrane domain of this receptor eventually results in an aggressive phenotype [20] and the level of its ligand, hepatic growth factor (HGF), is higher in patients with SCLC compared to control subjects [21].

The vascular endothelial growth factor (VEGF) is a well-known promoter of angiogenesis and its presence connotes poor prognosis in many cancers and may be a target for many malignancies [22]. Researchers found that patients with ED-SCLC had higher VEGF concentration when compared to the patients with limited stage disease. Epidermal growth factor receptor (EGFR) is also expressed in SCLC but at a low level and this is also believed to confer more invasiveness than cells without EGFR expression [23]. Multiple neuropeptides and polypeptides including gastrin releasing peptide (GRP), stem cell factor (c-kit) and insulin-like growth factor-1 (IGF-1) are also reported to promote the growth of SCLC via autocrine growth loops. IGF-1 promotes anti-apoptotic effects via activation of the PIK3-AKT1-FRAP (mammalian target of rapamycin pathway). GRP is expressed in 20–60% of SCLC and forms a potential target for developing a targeted treatment strategy.

Targeted Therapies in Small Cell Lung Cancer

Several signaling pathways are involved in tumorigenesis that are thought to have a potential for development of targeted therapies in SCLC (please see Fig. 1). The different agents for inhibition of cancer cell growth can be divided into at least

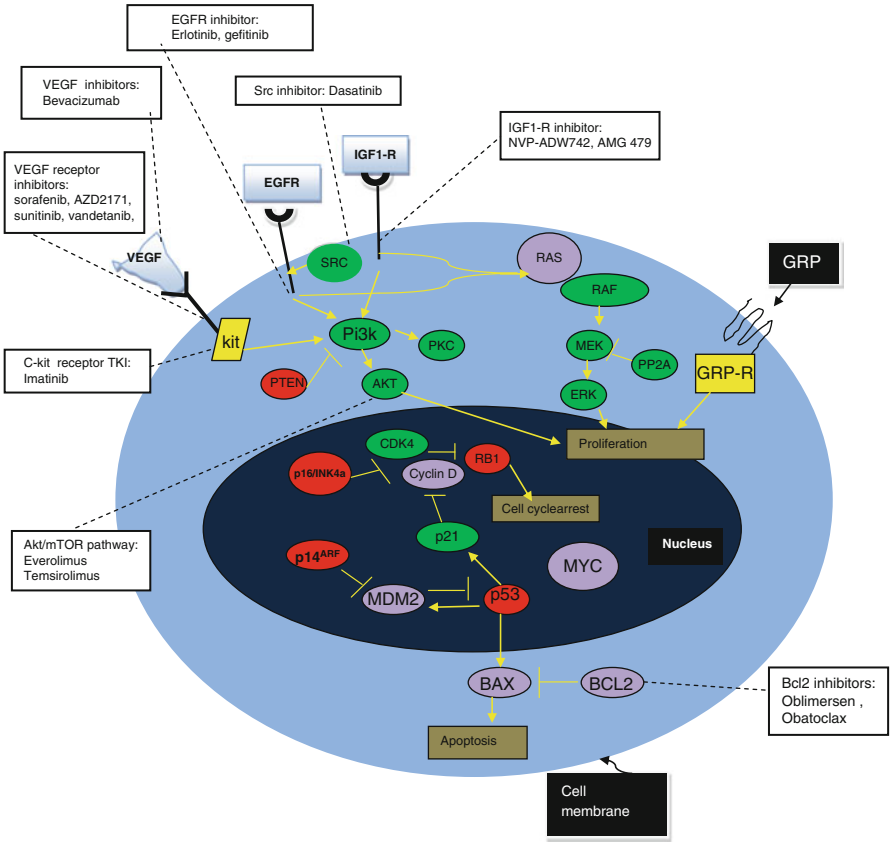

Fig. 1 *VEGF* vascular endothelial growth factor, *EGFR* epidermal growth factor receptor, *IGF1-R* insulin growth factor 1 receptor, *PI3K* phosphatidylinositol 3-kinase, *PTEN* phosphatase and tensin homolog, *P53* protein 53, *RB1* retinoblastoma 1, *GRP* gastrin releasing peptide, *CDK4* cyclin dependant kinase 4, *p16/INK4a* cyclin dependant kinase inhibitor p16, *p21* cyclin dependant kinase inhibitor 1, *MDM2* murine double minute 2, *BCL2* B-cell lymphoma 2, *RAS* rat sarcoma, *PP2A* protein phosphatase 2A, *MEK* mitogen activated protein kinase, *PKC* protein kinase C, *ERK* extracellular-signal-regulated kinase, *MYC* myelocytomatosis

four sub-types (for discussion purposes) depending upon their mechanism of action: (a) Antiangiogenic agents (b) Signaling pathways inhibitors, (c) Apoptotic agents, (d) Novel agents with miscellaneous mechanisms.

(a) Antiangiogenic agents:

- **Thalidomide**: Thalidomide has been around for decades and its effect in myeloma is well known. Thalidomide has anti-angiogenic properties and there is pre-clinical data suggesting that its action is regulated by the balance between ceramide and sphingosine -1-phosphatase signal [24]. There are at least two significant clinical trials that did not seem to show any significant

effect on overall survival in SCLC patients. The French Intergroup conducted a phase III randomized double blind, clinical trial to determine the efficacy of thalidomide in ED-SCLC [25]. Patients, who responded to 2 cycles of initial four chemotherapy drug regimens (etoposide, cisplatin, cyclophosphamide, and 4′-epidoxorubicin), were then randomized to receive four additional cycles of the similar initial chemotherapy plus or minus thalidomide. There was a trend towards longer median survival amongst patients receiving thalidomide but it did not reach any statistical significance (11.7 months in thalidomide group vs. 8.7 months in placebo group, hazard ratio [HR] 0.74, 95% confidence interval [CI] 0.49–1.12; P=0.16). In addition there was a higher incidence of neuropathy and thrombosis in the thalidomide group compared to placebo, necessitating withdrawal or dose reduction of thalidomide in about half of the patients in the treatment group. The other study conducted by Lee et al. was based on a similar approach [26]. Seven hundred and twenty four patients were randomized to thalidomide versus placebo (51% with LD-SCLC and 49% with ED-SCLC) in addition to chemotherapy. The patients were assigned to receive etoposide and carboplatin every 3 weeks for up to 6 cycles along with placebo or thalidomide (100–200 mg daily) for up to 2 years. The median overall survival (OS) was similar in both groups (10.5 months in placebo vs. 10.1 months in thalidomide; HR 1.09, 95% CI 0.93–1.27; P=0.28). There was no difference in progression free survival (PFS) and quality of life. There was a higher risk of thrombotic events (19% in thalidomide vs. 10% in placebo) in the treatment group. Thalidomide is probably not worthy of further evaluation in SCLC but we need to further decipher the vascular biology of the tumor cells in SCLC.

- **Bevacizumab**: It is a humanized monoclonal antibody that inhibits VEGF factor A, and has been studied in SCLC. Patton and his colleagues assessed the efficacy of bevacizumab in the maintenance setting in LD-SCLC [27]. The patients received the initial treatment with platinum based chemotherapy (carboplatin and irinotecan) along with concurrent radiotherapy followed by bevacizumab if they have no progressive disease. The dose of bevacizumab was 10 mg/kg, every 14 days for 10 doses. The overall response rate (ORR) was 80% with complete response (CR) of 26% and partial response (PR) of 54%. The 1-year and 2 year PFS was 63 and 54% respectively with the median OS of 15 months. Amongst the initial 29 patients that were enrolled on this trial, 2 patients had a serious adverse event of tracheoesophageal (TE) fistula and the other patient had a fatal aerodigestive tract hemorrhage and death of unknown cause, where TE fistula was suspected but not confirmed [28]. There were two single arm phase II trials evaluating the effect of addition of bevacizumab to initial chemotherapy in ED-SCLC patients, the Cancer and Leukemia Group B (CALGB)-3036 trial and the Eastern Cooperative Oncology Group (ECOG), ECOG-E3501 trial. CALGB-3036 clinical trial enrolled 72 ED-SCLC chemotherapy naïve patients, who were treated with the combination of cisplatin, irinotecan on day 1, 8 in combination with bevacizumab on day 1, every 21 days for a total of 6 cycles

[29]. There were 3 CR (5%), 45 PR (70%) and 11 with stable disease (SD), 17%. The ORR in evaluable patients was 75%, median OS was 11.6 months (95% CI, 10.5–15.1 months) and median PFS was 7.0 months (95%CI, 6.4–8.4 months). There was no grade 3 or more hemoptysis or any hemorrhagic episodes. Hypertension grade 1 or more was associated with improved survival when adjusted for age and performance status (PS) (HR 0.55; 95% CI 0.31–0.97; P=0.04). Lower VEGF levels correlated with worse PFS when adjusted for age and PS (HR 0.90; 95% CI 0.83–0.99; P=0.03). However this trial failed to meet its primary end point, which was median survival of ≥15 months. ECOG-E3501 was also a phase II clinical trial, where patients with untreated ED-SCLC (n=64) were treated with standard chemotherapy, cisplatin and etoposide combination plus bevacizumab [30]. A median of 6 cycles of bevacizumab treatment was administered. This study met its primary end point (increase in PFS rate at 6 months, from 16% to 33%). Of the 39 evaluable responses, the response rate was 69% with 33% of patients alive at 6 months; PFS was 4.7 months with OS of 11.1 months. None of the patients had hemoptysis greater than grade 2 but two patients experienced grade 5 toxicities (hypotension and infection with grade 3/4 neutropenia). There was another phase II clinical trial of bevacizumab in chemotherapy naïve ED-SCLC patients by Spigel et al., reported at the same time [31]. This was a randomized study, where patients with SCLC were assigned to receive either bevacizumab or placebo, with chemotherapy (cisplatin or carboplatin plus etoposide), for four cycles followed by single-agent bevacizumab or placebo until progression or unacceptable toxicity. There were a total of 52 patients in the bevacizumab arm and 50 patients in the control arm. There was a 1-month improvement in the PFS with the addition of bevacizumab compared to the control group (5.5 months vs. 4.4 months, HR 0.53; 95% CI 0.32–0.86), but without any impact in OS.

- **Other VEGF inhibitors**: Sorafenib, cediranib (AZD2171), sunitinib, vandetanib, and aflibercept are other anti-VEGF agents that are being tested in SCLC.

  Sorafenib is a multiple kinase inhibitor that has been shown to target raf kinase, VEGF receptor (VEGFR)-2, VEGFR-3, platelet derived growth factor receptor (PDGFR)-beta [32]. The Southwest oncology group trial (SWOG), S0435, conducted a phase II clinical trial on previously platinum based-treated, ED-SCLC patients with sorafenib [33]. Eighty-nine patients were enrolled, 79 were evaluated for responses and none of the patients had CR. Three patients (4%) had PR and 25 (32%) had SD. The median survival was 7 months in platinum sensitive (progression >90 days after platinum based therapy) and 5 months in platinum refractory group (progression during or ≤90 days after platinum based therapy). This median survival was comparable to previous historical based controls receiving salvage chemotherapy. The main toxicities were dermatological (grade 3, 25%), flu-like illness (grade 3/4, 14%) and metabolic (grade 3/4, 11%).

Cediranib, is an oral multi-kinase inhibitor that targets VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-alpha, PDGFR-beta, c-KIT [34]. Ramalingam et al. conducted a phase II study evaluating the efficacy of this drug as second-line therapy in SCLC [35]. Twenty five patients with prior platinum based therapy only, PS- 0–2, adequate bone marrow reserve, hepatic and renal functions were enrolled in this study. Treatment was given on a daily basis schedule and tolerability was found to be with 30 mg dose once a day. Only nine patients showed SD but none had partial response. The median PFS was 2 months with median OS of 4 months. In this study cediranib failed to demonstrate any objective response in recurrent or refractory SCLC.

Vandetanib is another multi-kinase inhibitor which targets VEGFR-2, and EGFR. National Cancer Institute of Canada study- CAN-NCIC-BR20, tested vandetanib in maintenance setting in SCLC patients, in a phase-II setting who achieved CR/PR after initial therapy (chemotherapy, plus or minus radiation) [36]. Patients who had CR or PR after initial therapy were randomly assigned to receive chemotherapy with or without vandetanib until disease progression. A total of 107 patients were enrolled (46 with LD-SCLC, and 61 with ED-SCLC). There was no significant difference in either PFS (2.7 months in vandetanib arm vs. 2.8 months in placebo arm) or OS (10.6 months for vandetanib vs. 11.9 months for placebo). This study failed to show any efficacy of vandetanib in the maintenance therapy in SCLC.

Aflibercept (AVE0005), also known as VEGF-trap, is a fully humanized recombinant fusion protein that contains immunoglobulin (Ig) domains from VEGFR-1 and VEGFR-2 fused to the constant region (Fc) of IgG1 [37]. This uses decoy soluble receptors to bind VEGF thereby preventing binding of VEGF to its usual receptors, thus preventing angiogenesis. SWOG- SO82, is a phase II clinical trial that is currently testing aflibercept with or without topotecan in patients with ED-SCLC, who have been previously treated with platinum-based chemotherapy.

(b) Signaling pathway inhibitors:

Targeting the signaling pathways involved in cell proliferation has shown to be very effective in some tumor types. An example would be the use of EGFR tyrosine kinase (TK) inhibitor like erlotinib in NSCLC. Similarly there have been clinical trials targeting c-KIT TK, insulin like growth factor TK, c-MET receptor TK, EGFR TK, Src kinase and mammalian target of rapamycin (mTOR) pathways in SCLC. Unfortunately the story of blocking these pathways has not been that successful in SCLC.

- **c-KIT receptor tyrosine kinase inhibitors**: Imatinib, inhibitor of c-KIT, bcr-abl tyrosine kinase and PDGFR, has been studied in treatment of SCLC. Potti et al. showed that over two-thirds of the SCLC cell lines expressed c-KIT by immunohistochemistry (IHC) and would be a potential target for treatment [38]. Johnson et al. conducted a small phase II study where 19 patients with SCLC (ED-SCLC n = 9, sensitive relapse [relapse or progression ≥90 days after completing first-line therapy] n = 10) were treated with 600 mg of

imatinib daily [39]. The median time to progression was 0.8 months in previously untreated patients and 1.2 months in sensitive relapsed patients. This study failed to demonstrate any anti-tumor activity. However only 4 (21%) patients had positive KIT receptor (CD117). Another study also tested imatinib in SCLC. Twelve patients with progressive SCLC, whose tumors expressed c-KIT, were enrolled in the treatment portion of the study and they received higher dose of imatinib, 400 mg bid [40]. Of the 36 tumor samples assessable, 28 (78%) were positive for c-KIT protein by IHC. There was no CR or PR and all patients progressed on treatment showing that imatinib did not have anti-tumor activity in SCLC, even in c-KIT positive tumors, confirming the initial study by Johnson et al.

Schneider and his colleagues conducted a phase II clinical trial of maintenance imatinib after irinotecan and cisplatin in patients with c-KIT positive ED-SCLC [41]. This study had a very small number of patients (n = 14 and only 8/14 who did not have disease progression went on to receive imatinib) and it failed to show any benefit from imatinib despite c-KIT tumor positivity. Another multicenter phase II clinical study by Spigel et al. evaluated the role of imatinib in combination with chemotherapy in untreated ED-SCLC [42]. A total of 68 patients were enrolled in this trial and 48 of 56 available tumor specimens were found to be c-KIT positive. Untreated ED-SCLC patients were treated with irinotecan, carboplatin in combination with imatinib 600 mg/day. Chemotherapy was given for 4–6 cycles and patients who achieved remission or SD then continued 600 mg of imatinib daily until disease progression. The objective response rate was 66%; median overall survival was 8.4 months (95% CI: 6.3–10.5 months); 35% of patients were alive at 1 year. The grade 3/4 hematologic toxicity included neutropenia (43%), anemia (16%), and thrombocytopenia (9%) and grade 3 non-hematologic toxicity included diarrhea (19%), fatigue (24%), and nausea (26%). Overall this therapy was reasonably well tolerated but the combination therapy failed to show any improvement when compared to the results expected from chemotherapy alone. Different theories have been postulated as to why imatinib failed to make an impact in the treatment of SCLC. There may be a possibility that this c-KIT pathway is not so important in the survival of cancer cells in SCLC or imatinib does not effectively inhibits the wild type KIT kinase [43].

- **EGFR inhibitors**: The drugs causing inhibition of EGFR has been very successful in treating non-small cell lung cancer, especially those expressing EGFR Mutation. EGFR is over-expressed in tumors with adenocarcinoma but the previous clinical trials have shown benefit in other histologies as well [44]. The SCLC does not express EGFR as commonly as non-small cell lung cancer. In one of the studies, only 4% of the SCLC patients (n = 5 out of 122) were found to have EGFR mutation [45]. Moore et al. conducted a phase II clinical trial with gefitinib (Iressa) in patients with chemo-sensitive and chemo-refractory relapses SCLC [46]. SCLC accounted for 18 out of total of 19 patients, and 1 patient had metastatic merkel cell carcinoma (12 were

chemo-sensitive and 7 were chemo-resistant). Only two patients had SD and 17 patients progressed on gefitinib. The grade 3 toxicities observed were: fatigue in three pts (15.8%), pulmonary toxicities in three pts (15.8%), and one pt (5.3%) each with hyperglycemia or pain. Grade 4 toxicities seen were: one pt (5.3%) with fatigue and three pts (15.8%) with dyspnea. Median time to tumor progression (TTP) was 50 days (95% CI 21–58 days) and 1 year OS was 21% (95% CI = 6–45.6%). This trial failed to show any efficacy of gefitinib in SCLC.

- **c-MET receptor tyrosine kinase inhibitor**: The c-MET/HGF pathway seems to play an important role in the tumorigenesis. Maulik et al. showed in their in-vitro study that c-Met/HGF pathway is functional in SCLC, and may be a target for development of newer agents [47]. The same group of researchers also found that through phosphatidylinositol 3-kinase (PI3K) pathway, HGF also stimulates activation of the cytoskeletal focal adhesion proteins paxillin, focal adhesion kinase (FAK) and proline rich kinase (PYK2) in SCLC. AMG 102, is an investigational drug that binds to HGF/SF, inhibiting binding to its c-MET receptor, thus inhibiting the growth of tumor [48]. This drug is currently under phase 1b/2 clinical trial in combination with etoposide plus carboplatin or cisplatin as a first-line in the treatment of ED-SCLC [49].
- **Akt/mTOR pathway inhibitors**: This pathway has been known to play an important role in the tumor biology of SCLC. Activation of this signaling pathway has been found to be involved in the survival and proliferation of SCLC [50].

  Everolimus, inhibits this pathway, and has been tested in SCLC patients. Marinov et al. showed in their pre-clinical study that blocking mTOR pathway by everolimus can potentially disrupt growth and survival signaling in SCLC [51]. It was tested in the maintenance setting in a phase II clinical trial in previously treated, relapsed SCLC patients [52]. The disease control rate (primary end point) was found to be 26% (95% CI 11–40). The median survival was 6.7 months and high phosphorylated AKT expression was modestly associated with OS (HR = 2.07; 95% CI 0.97–4.43). This study showed that although it was reasonably well tolerated, it had limited activity in SCLC patients. Owonikoko et al. conducted another phase II clinical trial, with everolimus in previously treated SCLC patients [53]. In 16 of 17 evaluable responses, none of the patients had an objective response. Only three patients (19%) had stable disease, with duration of stable disease being 69–117+ days. However 13 patients (81%) had progressive disease. This again failed to show any efficacy of mTOR inhibitor as a single agent in SCLC.

  Temsirolimus is another drug that blocks the mTOR pathway and was tested in a phase II clinical trial amongst patients with ED-SCLC, who either had stable or responsive disease to induction chemotherapy [54]. A total of 44 out of 85 patients who had SD or responsive disease, were randomized to receive either 25 mg (arm A) or 250 mg (arm B) of temsirolimus IV weekly until disease progression. The median PFS was 2.5 months for the arm B and

1.9 months for arm A, but the difference was not significant, p=0.24. This design reflected the concept of maintenance therapy in SCLC but unfortunately the patients did not do that well as expected and it failed to show any increase in PFS in this patient population.

- **Insulin-like growth factor-1 receptor inhibitor**: IGF-1 receptor is overexpressed in various cancer cell lines including SCLC. This signaling pathway seems to play a crucial role in cell survival and apoptosis and its action is produced by activation of PI3K-Akt signaling pathway and its downstream effect [55]. A pre-clinical study, showed that a novel agent, NVP-ADW742, is a potent and selective IGF-I receptor kinase inhibitor that can efficiently inhibit the growth of cells that are highly dependent on IGF-I signaling in SCLC cell lines [56]. AMG 479 (ganitumab), is an another investigational fully monoclonal antibody directed against the IGF-1 receptor, and is currently being tested in the phase 1B/II clinical trial in combination with etoposide plus carboplatin or cisplatin as a first-line in the treatment of ED SCLC [48, 49].
- **c-Src kinase inhibitor**: c-Src kinase is a tyrosine kinase that plays a role in the invasiveness and metastasis of SCLC [57]. Blockade of c-Src kinase in tissue in liquid culture and semisolid medium decreased basal and neuropeptide- induced survival of SCLC cells [58]and this kinase activity was detected in SCLC cells and it was found to be low in normal lung tissues [59]. Miller et al. showed in their phase II study that use of dasatinib to block c-Src protein kinase in SCLC patients did not show efficacy in SCLC [57]. This study was conducted to determine the efficacy of second-line dasatinib in patients with chemosensitive small cell lung cancer. However dasatinib failed to meet its pre-specified efficacy criteria (the study was to be deemed negative if one or less objective response and 14 or fewer instances of PFS ≥6 weeks were observed among the initial 27 patients). However the accrual had continued while the initial responses were evaluated. Among the initial 27 patients, only 13 instances of PFS ≥6 weeks were observed. The median follow up time was 7.1 months, with median estimated overall survival of 17.0 weeks with PFS of 5.9 weeks amongst 43 patients who were treated on the study. Disease progression was the most common reason for discontinuation of the protocol (65%).

(c) Apoptotic agents:

There is an emerging role for agents that cause promotion of apoptosis and it has been of considerable interest in the newer treatment approaches in SCLC including inhibitions of Bcl-2 and histone deacetylation.

- **Bcl-2 inhibitor**: B cell lymphoma -2 (Bcl-2) is a protein involved in cell survival and is expressed in about 80% of SCLC [60]. Bcl-2 family of proteins are considered as key regulators in the mitochondrial apoptosis and seemed to play a role in resistance to anticancer therapies [61]. This family of proteins share one or more of Bcl-2 homology (BH) domains and its anti-apoptotic members have all four domains (e.g., Bcl-2, Bcl-xL, Bcl-w, Mcl-1, A1) [61]. The pro-apoptotic members of this family containing BH domains

1–3, Bax and Bak are direct mediators of apoptosis and cause initiation of mitochondrial apoptotic pathway [62]. Other pro-apoptotic members have only the BH domain 3 and are therefore termed BH3-only proteins (e.g., Bid, Bim, Bad, Noxa, Puma) and act as sensors of molecular stress or damage [63]. Oblimersen is an antisense oligonucleotide compound designed to specifically bind to human *bcl-2* mRNA, and it is one of the first Bcl-2 inhibitor therapy that was tested in SCLC. Rudin et al. conducted a phase II, randomized study to assess the efficacy and toxicity of this drug in ED-SCLC as initial therapy in combination with carboplatin and etoposide [64]. This was a randomized clinical trial (3:1) involving chemotherapy naïve patients with ED-SCLC. They were randomized to carboplatin and etoposide plus oblimersen arm versus carboplatin and etoposide alone arm. Unfortunately the arm with oblimersen combination resulted in worst outcome, with percentage of patients alive at 1 year being higher in the non-treatment arm (24% with oblimersen, and 47% without oblimersen). The failure free survival (FFS) was better in the arm without treatment (6.0 months in oblimersen vs. 7.6 months in arm without oblimersen; HR 1.8; 95% CI, 1.0–3.4; $p=0.07$). The median survival was 8.6 months for treatment arm and 10.6 months for arm without treatment (HR 2.1; 95% CI, 1.1–4.1; $p=0.02$). This drug failed to show any efficacy in SCLC and the authors suggested that perhaps this drug did not suppress the intratumoral Bcl-2 levels to affect the chemotherapeutic sensitivity in SCLC. Thereafter came obatoclax mesylate, a pan Bcl-2 inhibitor, mimetics of BH3 family of proteins and was tested in a phase II clinical trial by Langer and his colleagues [65]. Patients with chemotherapy naïve SCLC were randomized to receive carboplatin and etoposide (CE) or carboplatin and etoposide plus obatoclax (CEOb) and the primary end point was ORR. Even though the study did not meet its primary end point, the CEOb arm demonstrated a trend towards improved ORR, PFS and OS. The ORR was 64.9% in CEOb versus 54.8% in CE arm, $p=0.11$; PFS 6.0 months in CEOb versus 5.4 in CE, $p=0.08$; 12 months survival 42.5% in CEOb versus 37.2 in CE, $p=0.19$; OS 10.6 months in CEOb versus 9.9 months in CE, $p=0.0506$; OS in PS 0–1, 11.9 months in CEOb versus 10.1 months in CE, $p=0.052$. Somnolence (46%) and euphoric mood (31%) occurred during and shortly after the infusion of Obatoclax. Grade 3/4 non-hematological toxicity, somnolence, occurred in >5% absolute increase in frequency in CEOb arm. Obatoclax failed to meet its primary end-point, but this did provide some hope in the treatment of SCLC, where most of the other newer agents have not shown any efficacy.

- **Histone deacetylase inhibitors (HDACi)**: These are a newer class of anticancer agents that enhance the acetylation of core histones and weaken the histone-DNA interaction, causing increase in DNA accessibility [66]. They increase the transcription of tumor suppressor genes causing inhibition of cancer cell growth. Vorinostat (suberoylanilide hydroxamic acid) is one such promising drug that has shown activity in solid tumors including SCLC in pre-clinical studies. HDACi combination with topoisomerase I inhibitor has

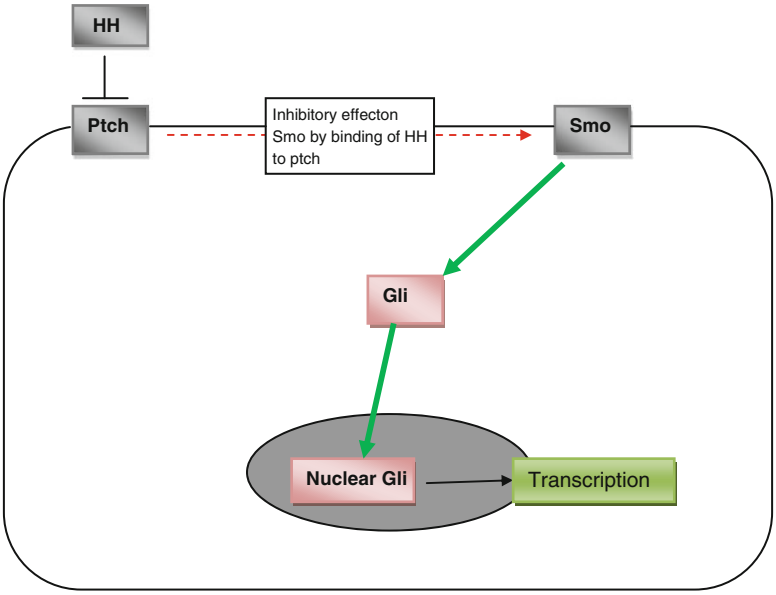

Fig. 2 Hedgehog (HH) pathway. HH signaling is typically initiated by the binding of hedgehog ligands (Sonic, Indian and Desert hedgehog) to a 12-transmembrane protein receptor patched (*Ptch*). Binding of HH to Ptch relieves Smoothened (*Smo*), a 7-transmembrane protein, from the inhibitory effect of Ptch and activated Smo in turn triggers a series of intracellular events, resulting in the regulation of downstream target genes through the *Gli* transcriptional factors and causes transcription of HH responsive genes [49]

shown enhanced cytotoxic activity in SCLC cell lines [67]. There is an ongoing phase I/II study of vorinostat with carboplatin and etoposide in ED-SCLC [68]. There is another similar HDACi, belinostat that is also being tested in and early clinical trial for SCLC [69].

(d) Novel agents with miscellaneous mechanisms:

- **Hedgehog inhibitor**: The Hedgehog (HH) pathway seems to be an important signaling mechanism in cancer cell growth and its inhibition is a potential therapeutic strategy in treatment of SCLC (Fig. 2) [70]. Park et al. found in their pre-clinical in-vivo study that the hedgehog signaling pathway is activated in SCLC cells independent of the lung microenvironment and pharmacological inhibition of this pathway causes inhibition of SCLC [71]. We are conducting a randomized phase II study in ED-SCLC where by patients will receive cisplatin and etoposide with or without a hedgehog inhibitor, GDC-0449 [72]. This trial has completed accrual and the results are eagerly awaited. This will allow us to understand how well this drug would work in combination with chemotherapy in SCLC patients.

- **Bendamustine**: It is a cytotoxic agent combining a purine-like benzimidazole and a bifunctional alkylating nitrogen mustard group. Like other alkylating agents, this drug causes DNA breaks. However, DNA single- and double-strand breaks caused by bendamustine are more extensive and significantly more durable [73]. It has already proven to be beneficial in hematological malignancies like non-hodgkin's lymphoma and multiple myeloma. It has also been tested in SCLC. Koster et al. conducted a phase II study to evaluate the efficacy of combination therapy with bendamustine and carboplatin in treatment of untreated ED-SCLC patients [74]. Fifty-five patients were assessable for response and the ORR was 72.7% (95% CI, 59–84%), with one CR (1.8%). At the time of evaluation 71% of patients had died. The median time to progression was 5.2 months (95% CI, 4.2–5.6) and the median survival time reached was 8.3 months (95% CI, 6.6–9.9). The major toxicity of this regimen was myelosuppression, including grade 3 or 4 neutropenia (46%), thrombocytopenia (26%), anemia (15%), and infections (11%) with toxic death being recorded in two patients (3.6%). The authors concluded that this regimen had comparable efficacy to platinum-based treatment. Soon after came another study where the efficacy of bendamustine was assessed as a single agent in second and third line setting in relapsed/refractory SCLC patients [75]. Patients with stable treated brain metastasis were allowed to participate. Bendamustine was given at a dose of 120 mg/m^2 IV on days 1 and 2 every 3 weeks for up to 6 cycles. A total of 28 patients were enrolled (target accrual 60) and 19 were evaluable for responses. Preliminary results showed seven PR, seven SD and five PD and median TTP was 2.5 months. Median number of cycles given at the time of evaluation was 2. Common grade 3/4 adverse events included neutropenia 14.3%, fatigue 10.7%, anemia and diarrhea 7.1%. Final results of this study are still awaited and it will throw more light on efficacy of bendamustine as a single agent in SCLC.

Circulating Tumor Cells and Other Biomarkers in SCLC

Circulating tumor cells (CTCs) has prognostic implications in metastatic breast, prostate, colorectal and non-small cell lung cancer [76–79]. This concept has also been tested in SCLC. One of the initial studies to detect CTCs in SCLC was done by Kularatne et al. in 11 SCLC patients [80]. The fall in CTCs to baseline levels set by normal controls was achieved by 12 weeks in the accelerated treatment arm (two weekly chemotherapy with ifosfamide, carboplatin and etoposide, with granulocyte colony stimulating factor and autologous stem cell transplant) versus 24 weeks in the standard arm (four weekly chemotherapy with ifosfamide, carboplatin, and etoposide). Hou JM et al. did two studies on CTCs in SCLC. In their first study, they evaluated the clinical significance of CTCs in patients with SCLC [81]. CTCs were detected in 86% of the patients (43/50 CTCs cohort) and the median CTCs number was 28. In the same study they evaluated three cell death biomarkers, M30, M65

and nucleosome DNA (nDNA) and all three were found to be high in SCLC cohort (n = 78) .compared to normal control (n = 85). The median values for M30 was 268 U/L in SCLC cohort versus 198 U/L in control (P = 0.02), M65 was 609 in SCLC cohort versus 245 U/L in control (P < 0.0001) and nDNA was found to be 1.40 in SCLC cohort versus 0.3 in control (p < 0.0001). M30, M65, nDNA and CTC correlated with stage of the disease and PS of patients. Median survival for patients with ≥300 CTCs was 134 days versus 443 days in patients who had CTCs <2, with p < 0.005. The median survival for patients with M65 >1,061 U/L was 151 days versus 388 days for patients with M65 <309 U/L (P < 0.0001). More than 78% of SCLC patients in this study had ≥2 CTC, which would correlate with the aggressiveness of the disease. The second study examined the clinical significance and molecular characteristics of CTCs and CTCs clusters (CTC microemboli [CTM]) in patients with SCLC [82]. CTCs were present in 85% of patients and were abundant (mean ± standard deviation = 1,589 ± 5,565). The OS was 5.4 months in patients who have ≥50 CTCs per 7.5 ml of blood versus 11.5 months in patients who have <50 CTCs, prior to the start of chemotherapy (HR 2.45, 95% CI 1.39–4.30; P = 0.02). This was one of the first initial studies which showed an association between presence of CTM and prognosis in SCLC. Twenty four percent of patients had CTM detected at baseline and those who had presence of CTM at baseline showed poor PFS of 4.6 months versus 8.2 months in patients with absence of baseline CTM (P < 0.001). Similar was true for median OS of 4.3 months in patients with presence of baseline CTM versus 10.4 months in patients who had absence of baseline CTM (P < 0.001). Pretreatment CTCs, changes in CTC number after one cycle of chemotherapy, CTM were found to be independent prognostic factors. Naito et al. also studied the impact of CTCs in SCLC patients and a total of 51 patients were enrolled with newly diagnosed SCLC. CTCs level was checked at baseline, after chemotherapy and at relapse [83]. Thirty-five patients (68.6%) had ≥2 CTC at baseline, and the HR signifying the difference between favorable (less than threshold) and unfavorable (more than or equal to threshold) groups was maximum at CTC of eight (HR 3.5, 95% CI 1.45–8.6). Patients with ≥8 CTCs at baseline had worse survival than patients with <8 CTCs at baseline, with P = 0.0014. Patients who had ≥8 CTCs after treatment and at the time of relapse also showed worse survival than patients with <8 CTCs (P = 0.0096 for post treatment; P < 0.0001 for relapse). This study again reflected the fact that CTCs may play a significant role in determining the prognosis of patients in SCLC and it may help develop a rationale for treating favorable prognosis patients with more intensive therapy to maximize the benefit.

Few other biomarkers have been looked into to determine their efficacy in predicting prognosis amongst patients with SCLC such as VEGFR-3 positive peripheral blood circulating lymphatic/vascular endothelial progenitor cells (LVEPC), CYRFA21-1, a fragment of cytokeratin subunit 19, the neuroendocrine marker neuron-specific enolase (NSE), and carcinoembryonic antigen (CEA) [84–86]. Bogos K et al. did do a study to determine this and they evaluated VEGFR-3-positive LVEPC in 88 patients with LD-SCLC and 32 tumor free-control subjects [84]. CD34-positive VEGFR-3-positive LVEPC levels are significantly higher in SCLC patients versus control, with P < 0.01. High circulating LVEPC numbers correlated with lymphatic spread and poor OS (11.5 vs. 20 months with P < 0.01).

Yang X et al. evaluated the efficacy of 12 tumor markers including CEA, NSE, CA 19-9, CA 125, CA 15-3 in determining the prognosis in SCLC [85]. A total of 116 SCLC patient samples of blood at the time of diagnosis were analyzed and patients were followed for a maximum of 54 weeks. CEA was the most frequently detected tumor marker, 32.8% and was the only tumor marker that showed correlation with OS. The average survival time was higher for patients who had CEA less than 5 ng/ml than those who had above 5 ng/ml (16.78 months for patients with CEA <5 ng/ml versus 11.4 months for patients with CEA ≥5 ng/ml; $P<0.001$). However despite positive results from CTCs study and CEA none of these markers have been adapted in the everyday clinical practice. More studies to validate these results are needed.

Conclusion

The treatment of SCLC remains a challenge. Combination chemotherapy with or without radiation therapy remains the only effective therapy in the front-line setting. However the effect is not usually durable and there is paucity of impressive salvage chemotherapy strategies in relapsed disease. Topotecan, even though the only FDA approved drug in second-line setting fails to show an OS benefit and thus the façade of SCLC remains dark.

There are many ongoing studies in SCLC that may change the overall treatment landscape. The intriguing feature about SCLC is the difference in behavior at initial presentation as compared to that seen at relapse or recurrence. Understanding the mechanism of resistance in SCLC along with indicators for relapse will allow us to use appropriate targeted approaches. There are a number of cytogenetic alterations that could explain the pugnaciousness of SCLC. Unfortunately, in spite of this extensive research, we are still lagging behind when it comes to developing an improved treatment strategy for SCLC patients. The work with Bcl-2 directed therapy appears encouraging and deserves further evaluation. The role of HH pathway in the pathogenesis of SCLC looks promising but the results are preliminary. Currently the prognosis of SCLC appears dismal but a greater understanding of mediators of resistance and sensitivity will allow us to take the therapeutic strategies to the next level.

References

1. Society AC. Cancer facts and figures 2007. Atlanta: American Cancer Society; 2007.
2. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin. 2012;62(1):10–29.
3. Navada S, Lai P, Schwartz AG, Kalemkerian GP. Temporal trends in small cell lung cancer: analysis of the national Surveillance, Epidemiology, and End-Results (SEER) database. J Clin Oncol. 2006;24:7082. 2006 ASCO Annual Meeting Proceedings Part I. 2006, (June 20 Supplement).
4. Govindan R, Page N, Morgensztern D, Read W, Tierney R, Vlahiotis A, et al. Changing epidemiology of small-cell lung cancer in the United States over the last 30 years: analysis of the surveillance, epidemiologic, and end results database. J Clin Oncol. 2006;24(28):4539–44.

5. Kelly K. Treatment of extensive stage small cell lung cancer. Cancer Treat Res. 2001;105:253–76.
6. Thatcher N, Eckardt J, Green M. Options for first- and second-line therapy in small cell lung cancer—a workshop discussion. Lung Cancer. 2003;41(Suppl 4):S37–41.
7. Chiappori AA, Rocha-Lima CM. New agents in the treatment of small-cell lung cancer: focus on gemcitabine. Clin Lung Cancer. 2003;4(Suppl 2):S56–63.
8. Ardizzoni A, Hansen H, Dombernowsky P, Gamucci T, Kaplan S, Postmus P, et al. Topotecan, a new active drug in the second-line treatment of small-cell lung cancer: a phase II study in patients with refractory and sensitive disease. The European Organization for Research and Treatment of Cancer Early Clinical Studies Group and New Drug Development Office, and the Lung Cancer Cooperative Group. J Clin Oncol. 1997;15(5):2090–6.
9. Wistuba II, Behrens C, Virmani AK, Mele G, Milchgrub S, Girard L, et al. High resolution chromosome 3p allelotyping of human lung cancer and preneoplastic/preinvasive bronchial epithelium reveals multiple, discontinuous sites of 3p allele loss and three regions of frequent breakpoints. Cancer Res. 2000;60(7):1949–60.
10. Tammemagi MC, McLaughlin JR, Bull SB. Meta-analyses of p53 tumor suppressor gene alterations and clinicopathological features in resected lung cancers. Cancer Epidemiol Biomarkers Prev. 1999;8(7):625–34.
11. Hainaut P, Hernandez T, Robinson A, Rodriguez-Tome P, Flores T, Hollstein M, et al. IARC Database of p53 gene mutations in human tumors and cell lines: updated compilation, revised formats and new visualisation tools. Nucleic Acids Res. 1998;26(1):205–13.
12. Casey G, Lopez ME, Ramos JC, Plummer SJ, Arboleda MJ, Shaughnessy M, et al. DNA sequence analysis of exons 2 through 11 and immunohistochemical staining are required to detect all known p53 alterations in human malignancies. Oncogene. 1996;13(9):1971–81.
13. Dammann R, Li C, Yoon JH, Chin PL, Bates S, Pfeifer GP. Epigenetic inactivation of a RAS association domain family protein from the lung tumour suppressor locus 3p21.3. Nat Genet. 2000;25(3):315–9.
14. Richardson GE, Johnson BE. The biology of lung cancer. Semin Oncol. 1993;20(2):105–27.
15. Kitada S, Takayama S, De Riel K, Tanaka S, Reed JC. Reversal of chemoresistance of lymphoma cells by antisense-mediated reduction of bcl-2 gene expression. Antisense Res Dev. 1994;4(2):71–9.
16. Ewen ME. The cell cycle and the retinoblastoma protein family. Cancer Metastasis Rev. 1994;13(1):45–66.
17. Wistuba II, Gazdar AF, Minna JD. Molecular genetics of small cell lung carcinoma. Semin Oncol. 2001;28(2 Suppl 4):3–13.
18. Ma PC, Tretiakova MS, Nallasura V, Jagadeeswaran R, Husain AN, Salgia R. Downstream signalling and specific inhibition of c-MET/HGF pathway in small cell lung cancer: implications for tumour invasion. Br J Cancer. 2007;97(3):368–77.
19. Rygaard K, Nakamura T, Spang-Thomsen M. Expression of the proto-oncogenes c-met and c-kit and their ligands, hepatocyte growth factor/scatter factor and stem cell factor, in SCLC cell lines and xenografts. Br J Cancer. 1993;67(1):37–46.
20. Ma PC, Kijima T, Maulik G, Fox EA, Sattler M, Griffin JD, et al. c-MET mutational analysis in small cell lung cancer: novel juxtamembrane domain mutations regulating cytoskeletal functions. Cancer Res. 2003;63(19):6272–81.
21. Bharti A, Ma PC, Maulik G, Singh R, Khan E, Skarin AT, et al. Haptoglobin alpha-subunit and hepatocyte growth factor can potentially serve as serum tumor biomarkers in small cell lung cancer. Anticancer Res. 2004;24(2C):1031–8.
22. Wójcik E, Jakubowicz J, Skotnicki P, Sas-Korczyńska B, Kulpa JK. IL-6 and VEGF in small cell lung cancer patients. Anticancer Res. 2010;30(5):1773–8.
23. Damstrup L, Rygaard K, Spang-Thomsen M, Poulsen HS. Expression of the epidermal growth factor receptor in human small cell lung cancer cell lines. Cancer Res. 1992;52(11):3089–93.
24. Yabu T, Tomimoto H, Taguchi Y, Yamaoka S, Igarashi Y, Okazaki T. Thalidomide-induced antiangiogenic action is mediated by ceramide through depletion of VEGF receptors, and is antagonized by sphingosine-1-phosphate. Blood. 2005;106(1):125–34.
25. Pujol JL, Breton JL, Gervais R, Tanguy M-L, Quoix E, David P, et al. Phase III double-blind, placebo-controlled study of thalidomide in extensive-disease small-cell lung cancer after

response to chemotherapy: an intergroup study FNCLCC cleo04 IFCT 00-01. J Clin Oncol. 2007;25(25):3945–51.
26. Lee SM, Woll PJ, Rudd R, Ferry D, O'Brien M, Middleton G, et al. Anti-angiogenic therapy using thalidomide combined with chemotherapy in small cell lung cancer: a randomized, double-blind, placebo-controlled trial. J Natl Cancer Inst. 2009;101(15):1049–57.
27. Patton JF, Spigel DR, Greco FA, Liggett WH, Zubkus JD, Baskette M, et al. Irinotecan (I), carboplatin (C), and radiotherapy (RT) followed by maintenance bevacizumab (B) in the treatment (tx) of limited-stage small cell lung cancer (LS-SCLC): update of a phase II trial of the Minnie Pearl Cancer Research Network. J Clin Oncol. 2006;24(18S):7085. 2006 ASCO Annual Meeting Proceedings Part I. 2006, (June 20 Supplement).
28. Genentech Letter: Important drug warning regarding Avastin (bevacizumab). http://www.gene.com/gene/products/information/pdf/avastin_te_letter.pdf, April, 2007.
29. Ready NE, Dudek AZ, Pang HH, Hodgson LD, Graziano SL, Green MR, et al. Cisplatin, irinotecan, and bevacizumab for untreated extensive-stage small-cell lung cancer: CALGB 30306, a phase II study. J Clin Oncol. 2011;29(33):4436–41.
30. Sandler A, Szwaric S, Dowlati A, Moore DF, Schiller JH. A phase II study of cisplatin (P) plus etoposide (E) plus bevacizumab (B) for previously untreated extensive stage small cell lung cancer (SCLC) (E3501): A trial of the Eastern Cooperative Oncology Group. J Clin Oncol (Meeting Abstracts) June 2007, 25(18_suppl), 7564
31. Spigel DR, Townley PM, Waterhouse DM, Fang L, Adiguzel I, Huang JE, et al. Randomized phase II study of bevacizumab in combination with chemotherapy in previously untreated extensive-stage small-cell lung cancer: results from the SALUTE trial. J Clin Oncol. 2011;29(16):2215–22.
32. Herbst RS, Onn A, Sandler A. Angiogenesis and lung cancer: prognostic and therapeutic implications. J Clin Oncol. 2005;23(14):3243–56.
33. Gitlitz BJ, Glisson BS, Moon J, Reimers H, Gandara DR. Sorafenib in patients with platinum (plat) treated extensive stage small cell lung cancer (E-SCLC): A SWOG (S0435) phase II trial. ASCO annual meeting 2008. J Clin Oncol 26: 2008 (May 20 suppl; abstr 8039).
34. Wedge SR, Kendrew J, Hennequin LF, Valentine PJ, Barry ST, Brave SR, et al. AZD2171: a highly potent, orally bioavailable, vascular endothelial growth factor receptor-2 tyrosine kinase inhibitor for the treatment of cancer. Cancer Res. 2005;65(10):4389–400.
35. Ramalingam SS, Belani CP, Mack PC, Vokes EE, Longmate J, Govindan R, et al. Phase II study of Cediranib (AZD 2171), an inhibitor of the vascular endothelial growth factor receptor, for second-line therapy of small cell lung cancer (National Cancer Institute #7097). J Thorac Oncol. 2010;5(8):1279–84.
36. Arnold AM, Seymour L, Smylie M, Ding K, Ung Y, Findlay B, et al. Phase II study of vandetanib or placebo in small-cell lung cancer patients after complete or partial response to induction chemotherapy with or without radiation therapy: National Cancer Institute of Canada clinical trials group study BR.20. J Clin Oncol. 2007;25(27):4278–84.
37. Eichholz A, Merchant S, Gaya AM. Anti-angiogenesis therapies: their potential in cancer management. Onco Targets Ther. 2010;3:69–82.
38. Potti A, Moazzam N, Ramar K, Hanekom DS, Kargas S, Koch M. CD117 (c-KIT) overexpression in patients with extensive-stage small-cell lung carcinoma. Ann Oncol. 2003;14(6):894–7.
39. Johnson BE, Fischer T, Fischer B, Dunlop D, Rischin D, Silberman S, et al. Phase II study of imatinib in patients with small cell lung cancer. Clin Cancer Res. 2003;9(16 Pt 1):5880–7.
40. Krug LM, Crapanzano JP, Azzoli CG, Miller VA, Rizvi N, Gomez J, et al. Imatinib mesylate lacks activity in small cell lung carcinoma expressing c-kit protein: a phase II clinical trial. Cancer. 2005;103(10):2128–31.
41. Schneider BJ, Gadgeel S, Ramnath N, Worden FP, Wozniak A, Ruckdeschel J, et al. Phase II trial of imatinib maintenance therapy after irinotecan and cisplatin in patients with c-kit positive extensive-stage small cell lung cancer J Clin Oncol (Meeting Abstracts) June 2006 24 (18_suppl 17089). 2006.
42. Spigel DR, Hainsworth JD, Simons L, Meng C, Burris 3rd HA, Yardley DA, et al. Irinotecan, carboplatin, and imatinib in untreated extensive-stage small-cell lung cancer: a phase II trial of the Minnie Pearl Cancer Research Network. J Thorac Oncol. 2007;2(9):854–61.

43. Glisson BS. Targeting an autocrine loop in small-cell lung cancer: irrelevant target or ineffective drug? Clin Lung Cancer. 2010;11(4):222. © 2010 other.
44. Cappuzzo F, Ciuleanu T, Stelmakh L, Cicenas S, Szczesna A, Juhasz E, et al. SATURN: A double-blind, randomized, phase III study of maintenance erlotinib versus placebo following nonprogression with first-line platinum-based chemotherapy in patients with advanced NSCLC. J Clin Oncol. 2009;27(15 s,):suppl; abstr 8001.
45. Tatematsu A, Shimizu J, Murakami Y, Horio Y, Nakamura S, Hida T, et al. Epidermal growth factor receptor mutations in small cell lung cancer. Clin Cancer Res. 2008;14(19):6092–6.
46. Moore AM, Einhorn LH, Estes D, Govindan R, Axelson J, Vinson J, et al. Gefitinib in patients with chemo-sensitive and chemo-refractory relapsed small cell cancers: a Hoosier Oncology Group phase II trial. Lung Cancer. 2006;52(1):93–7.
47. Maulik G, Kijima T, Ma PC, Ghosh SK, Lin J, Shapiro GI, et al. Modulation of the c-Met/hepatocyte growth factor pathway in small cell lung cancer. Clin Cancer Res. 2002;8(2):620–7.
48. Bonnie S, Glisson M. First-line therapy of small-cell lung cancer with platinum-based chemotherapy plus AMG 479 or AMG 102. Commun Oncol. 2009;6(suppl 1):1–4. © 2009 Elsevier Inc.
49. NCT00791154-A Phase 1b/2 Trial of AMG 479 or AMG 102 in combination with platinum-based chemotherapy as first-line treatment for extensive stage small cell lung cancer. www.clinicaltrials.gov.
50. Arcaro A, Khanzada UK, Vanhaesebroeck B, Tetley TD, Waterfield MD, Seckl MJ. Two distinct phosphoinositide 3-kinases mediate polypeptide growth factor-stimulated PKB activation. EMBO J. 2002;21(19):5097–108. doi:10.1093/emboj/cdf512.
51. Marin M, Algirdas Z, Pardo OE, et al. AKT/mTOR pathway activation and BCL-2 family proteins modulate the sensitivity of human small cell lung cancer cells to RAD001. Clin Cancer Res. 2009;15(4):1227–86.
52. Tarhini A, Kotsakis A, Gooding W, Shuai Y, Petro D, Friedland D, et al. Phase II study of everolimus (RAD001) in previously treated small cell lung cancer. Clin Cancer Res. 2010;16(23):5900–7.
53. Owonikoko TK, Stoller RG, Petro D, Flaugh R, Hershberger PA, Belani CP, et al. Phase II study of RAD001 (Everolimus) in previously treated small cell lung cancer (SCLC). J Clin Oncol (Meeting Abstracts). May 2008;26(15_suppl 19017).
54. Pandya KJ, Dahlberg S, Hidalgo M, Cohen RB, Lee MW, Schiller JH, et al. A randomized, phase II trial of two dose levels of temsirolimus (CCI-779) in patients with extensive-stage small-cell lung cancer who have responding or stable disease after induction chemotherapy: a trial of the Eastern Cooperative Oncology Group (E1500). J Thorac Oncol. 2007;2(11):1036–41.
55. Kulik G, Klippel A, Weber MJ. Antiapoptotic signalling by the insulin-like growth factor I receptor, phosphatidylinositol 3-kinase, and Akt. Mol Cell Biol. 1997;17(3):1595–606.
56. Warshamana-Greene GS, Litz J, Buchdunger E, Hofmann F, Garcia-Echeverria C, Krystal GW. The insulin-like growth factor-I (IGF-I) receptor kinase inhibitor NVP-ADW742, in combination with STI571, delineates a spectrum of dependence of small cell lung cancer on IGF-I and stem cell factor signaling. Mol Cancer Ther. 2004;3(5):527–35.
57. Miller AA, Pang H, Hodgson L, Ramnath N, Otterson GA, Kelley MJ, et al. A phase II study of dasatinib in patients with chemosensitive relapsed small cell lung cancer (cancer and leukemia group B 30602). J Thorac Oncol. 2010;5(3):380–4.
58. Roelle S, Grosse R, Buech T, Chubanov V, Gudermann T. Essential role of Pyk2 and Src kinase activation in neuropeptide-induced proliferation of small cell lung cancer cells. Oncogene. 2008;27(12):1737–48.
59. Rosen N, Bolen JB, Schwartz AM, Cohen P, DeSeau V, Israel MA. Analysis of pp 60c-src protein kinase activity in human tumor cell lines and tissues. J Biol Chem. 1986;261(29):13754–9.
60. Jiang SX, Sato Y, Kuwao S, Kameya T. Expression of bcl-2 oncogene protein is prevalent in small cell lung carcinomas. J Pathol. 1995;177(2):135–8.
61. Hurwitz JL, McCoy F, Scullin P, Fennell DA. New advances in the second-line treatment of small cell lung cancer. Oncologist. 2009;14(10):986–94.
62. Wolter KG, Hsu YT, Smith CL, Nechushtan A, Xi XG, Youle RJ. Movement of Bax from the cytosol to mitochondria during apoptosis. J Cell Biol. 1997;139(5):1281–92.

63. Huang DC, Strasser A. BH3-Only proteins-essential initiators of apoptotic cell death. Cell. 2000;103(6):839–42.
64. Rudin CM, Salgia R, Wang X, Hodgson LD, Masters GA, Green M, et al. Randomized phase II Study of carboplatin and etoposide with or without the bcl-2 antisense oligonucleotide oblimersen for extensive-stage small-cell lung cancer: CALGB 30103. J Clin Oncol. 2008;26(6):870–6.
65. Langer CJ, Albert PKI, Blakely LJ, Pajkos G, Petrov P, Somfay A, et al. A randomized phase II study of carboplatin (C) and etoposide (E) with or without pan-BCL-2 antagonist obatoclax (Ob) in extensive-stage small cell lung cancer (ES-SCLC). J Clin Oncol. 2011; 29(suppl; abstr 7001). 2011 ASCO Annual Meeting.
66. Bruzzese F, Rocco M, Castelli S, Di Gennaro E, Desideri A, Budillon A. Synergistic antitumor effect between vorinostat and topotecan in small cell lung cancer cells is mediated by generation of reactive oxygen species and DNA damage-induced apoptosis. Mol Cancer Ther. 2009;8(11):3075–87.
67. Gray J, Cubitt CL, Zhang S, Chiappori A. Combination of HDAC and topoisomerase inhibitors in small cell lung cancer. Cancer Biol Ther. 2012;13(8):614–22.
68. NCT00702962-Carboplatin and etoposide in combination with vorinostat for patients with extensive stage small cell lung cancer. www.clinicaltrials.gov.
69. NCT00926640-A phase I study of belinostat in combination with cisplatin and etoposide in adults with small cell lung carcinoma and other advanced cancers. www.clinicaltrials.gov.
70. Watkins DN, Berman DM, Baylin SB. Hedgehog signaling: progenitor phenotype in small-cell lung cancer. Cell Cycle. 2003;2(3):196–8.
71. Park KS, Martelotto LG, Peifer M, Sos ML, Karnezis AN, Mahjoub MR, et al. A crucial requirement for Hedgehog signaling in small cell lung cancer. Nat Med. 2011;17(11):1504–8.
72. NCT00887159-Cisplatin and etoposide phosphate with or without gdc-0449 or cixutumumab in treating patients with extensive-stage small cell lung cancer. www.clinicaltrials.gov.
73. Leoni LM, Bailey B, Reifert J, Bendall HH, Zeller RW, Corbeil J, et al. Bendamustine (Treanda) displays a distinct pattern of cytotoxicity and unique mechanistic features compared with other alkylating agents. Clin Cancer Res. 2008;14(1):309–17.
74. Koster W, Heider A, Niederle N, Wilke H, Stamatis G, Fischer JR, et al. Phase II trial with carboplatin and bendamustine in patients with extensive stage small-cell lung cancer. J Thorac Oncol. 2007;2(4):312–6.
75. Venepalli NK, Hutchison AS, Carbone DP, Johnson DH, Keedy VL, Pao W, et al. A phase II study of second-line bendamustine in relapsed or refractory small cell lung cancer (SCLC). J Clin Oncol. 2011; 29: (suppl; abstr e17505) [serial on the Internet].
76. Cohen SJ, Punt CJ, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, et al. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. J Clin Oncol. 2008;26(19):3213–21.
77. Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med. 2004;351(8):781–91.
78. de Bono JS, Scher HI, Montgomery RB, Parker C, Miller MC, Tissing H, et al. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. Clin Cancer Res. 2008;14(19):6302–9.
79. Krebs MG, Sloane R, Priest L, Lancashire L, Hou JM, Greystoke A, et al. Evaluation and prognostic significance of circulating tumor cells in patients with non-small-cell lung cancer. J Clin Oncol. 2011;29(12):1556–63.
80. Kularatne BY, Lorigan P, Browne S, Suvarna SK, Smith MO, Lawry J. Monitoring tumour cells in the peripheral blood of small cell lung cancer patients. Cytometry. 2002;50(3):160–7.
81. Hou JM, Greystoke A, Lancashire L, Cummings J, Ward T, Board R, et al. Evaluation of circulating tumor cells and serological cell death biomarkers in small cell lung cancer patients undergoing chemotherapy. Am J Pathol. 2009;175(2):808–16.
82. Hou JM, Krebs MG, Lancashire L, Sloane R, Backen A, Swain RK, et al. Clinical significance and molecular characteristics of circulating tumor cells and circulating tumor microemboli in patients with small-cell lung cancer. J Clin Oncol. 2012;30(5):525–32.

83. Naito T, Tanaka F, Ono A, Yoneda K, Takahashi T, Murakami H, et al. Prognostic impact of circulating tumor cells in patients with small cell lung cancer. J Thorac Oncol. 2012;7(3):512–9.
84. Bogos K, Renyi-Vamos F, Dobos J, Kenessey I, Tovari J, Timar J, et al. High VEGFR-3-positive circulating lymphatic/vascular endothelial progenitor cell level is associated with poor prognosis in human small cell lung cancer. Clin Cancer Res. 2009;15(5):1741–6.
85. Yang X, Wang D, Yang Z, Qing Y, Zhang Z, Wang G, et al. CEA is an independent prognostic indicator that is associated with reduced survival and liver metastases in SCLC. Cell Biochem Biophys. 2011;59(2):113–9.
86. Pujol JL, Quantin X, Jacot W, Boher JM, Grenier J, Lamy PJ. Neuroendocrine and cytokeratin serum markers as prognostic determinants of small cell lung cancer. Lung Cancer. 2003;39(2):131–8.

Impact of Genetic Targets on Cancer Therapy in Acute Myelogenous Leukemia

Mithun Vinod Shah, Amit Barochia, and Thomas P. Loughran Jr.

Abstract Acute myelogenous leukemia (AML) is characterized by uncontrolled proliferation of the cells of myeloid origin. It can present at all ages, but is more common in adults. It is one of the most common leukemias in adults and continues to pose significant challenge in diagnosis and long-term management.

AML is a disease at the forefront of genetic and genomic approaches to medicine. It is a disease that has witnessed rapid advances in terms of diagnosis, classification, prognosis and ultimately individualized therapy. Newly diagnosed AML patients are now routinely stratified according to cytogenetics and molecular markers which guides long-term prognosis and treatment. On the other hand, with few exceptions, the initial treatment (also known as induction treatment) of AML has been 'one-size-fits-all'. It remains a great challenge for patients and physicians to consolidate and translate these advances into eventual success in clinic [1, 2].

Keywords AML • MDS • Bone marrow failure • APL • Induction chemotherapy • Consolidation chemotherapy • Hematopoietic stem cell transplant • GVHD • Retinoic acid • ATRA • RAR-alpha • PML-RAR • Prognostic factors • Cytogenetics • FLT3-ITD • NPM1 • IDH1 • IDH2 • ASXL1 • MLL-PTD • TET2 • DNMT3A • FLT3 • CD117 • JAK-STAT • DNMT3A • MDR1 • CBF • CXCR4 • miRNA • Anti-CD33 antibody • Cediranib • CD33 • CD52 • Vorinostat • Lintuzumab • Midostaurin • Lestaurtinib • Sunitinib • KW-2449 • Tandutinib • Sorafenib • mTOR • Demethylating agents • Panobinostat • KX2-391 • TL32711 • Bortezomib • MLN4924 • Bevacizumab • Aflibercept • WT1

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W.S. El-Deiry (ed.), *Impact of Genetic Targets on Cancer Therapy*, Advances in Experimental Medicine and Biology 779, DOI 10.1007/978-1-4614-6176-0_19,

Clinical Features of Acute Myelogenous Leukemia

AML is one of the most common leukemias in adults with an age-adjusted rate of 3.4 per 100,000 in USA. The incidence of AML increases drastically with increasing age. It accounts for approximately 10,000 deaths every year in the US. Risk factors for developing AML include male gender, smokers especially above the age of 60, prior chemotherapy or radiation, and exposure to certain dyes such as benzene. Congenital genetic disorders such as Down syndrome, Li-Fraumeni syndrome and Fanconi anemia are also associated with higher incidence of AML. Other hematological conditions such as myelodysplastic syndrome (MDS) or rarely paroxysmal nocturnal hemoglobinuria (PNH) have also been associated with increased incidence of AML.

Clinically, the presentation of AML is a consequence of either BM failure or over proliferation of leukemic cells. Symptoms of BM failure are due to replacement of the normal marrow cells by leukemic cells resulting in anemia, thrombocytopenia, or neutropenia. In its most extreme form, over proliferation of leukemic cells manifests as leukostasis and hyperviscosity resulting in multiorgan dysfunction. It may also manifest as accumulation of leukemic cells in skin (known as Sweet syndrome) or in gums causing gingival hypertrophy.

Acute Promyelocytic Leukemia (APL): A Unique Subtype of AML

Acute promyelocytic leukemia (APL) corresponds to M3 subtype of the French-American-British (FAB) classification. APL accounts for about 10% AML cases occurring in approximately 1 in 250,000 people or 600–800 cases per year in the United States. APL, once regarded as the most fatal subtype of AML, is now considered the most curable form of AML largely due to dramatic advances in targeted molecular therapy.

The diagnosis of APL is suspected by the characteristic morphology of the leukemic cells, immunophenotype, or the presence of disseminated intravascular coagulation (DIC). Morphologically, APL is characterized by the presence of atypical promyelocytes in the BM as well as periphery. Promyelocytes are larger myeloid cells with distinguishing presence of Auer rods. DIC is a unique feature of APL and is either present at diagnosis or ensues soon after the initiation of chemotherapy. It is important to identify APL at the time of diagnosis, since if not treated promptly APL carries a very high mortality. DIC in APL patients is a medical emergency, with mortality reaching up to 20% in untreated patients [3–6].

Current Standards of Therapy for Acute Myelogenous Leukemia

It has long been known that AML is a heterogeneous disease with differing prognostic subgroups. Despite this recognition, with an exception of APL, AML is treated with an umbrella approach.

Chemotherapy is the standard of care for newly diagnosed AML patients. Chemotherapeutic regimen is further divided into 'induction' chemotherapy and 'consolidation' chemotherapy. The goal of the treatment is to achieve complete remission (CR) defined as (1) less than 5% blasts in BM in a marrow with at least 20% cellularity, (2) absolute neutrophil count of 1,000/μl or more, (3) platelet count of 100,000/μl or more and, (4) absence of extramedullary leukemia. Induction chemotherapy is given with the goal to achieve remission, while consolidation chemotherapy is given with the hope to maintain permanent remission. An initial chemotherapy regimen usually comprises an anthracycline in combination with cytarabine (3 + 7). Consolidation regimens differ both in the choice of chemotherapeutic agents and duration.

(1) **Induction chemotherapy**: For more than three decades, standardized initial therapy for AML, except APL, is 3 + 7 (also known as 7 + 3)—a continuous infusion of cytarabine in combination with an anthracycline such as doxorubicin, daunorubincin or idarubicin. The response rate with this initial regimen varies between 60 and 80% in patients younger than 60 years. Typically, a BM biopsy is performed on day 14–21 of chemotherapy to evaluate the response. If BM biopsy shows persistent leukemia, a second similar or intensified dose of chemotherapy is given.

Several modifications have been proposed for induction chemotherapy. In younger patients, addition of etoposide or topotecan can be considered. In younger patients, idarubicin is considered the drug of choice among anthracyclines, as patients treated with idarubicin have higher CR rates and fewer patients require the second dose of induction chemotherapy to achieve CR. Most importantly, using idarubicin is shown to prolong event-free survival. Recently, it has been shown that in younger patients (age <60 years) induction chemotherapy with intermediate dose of cytarabine (200 mg/m^2) achieves similar results to high-dose cytarabine (1 g/m^2) with fewer toxicities [1, 2, 7]. A recent study showed that in the presence of mutant *DNMT3A* or *NPM1* or *MLL*-PTD, high-dose induction chemotherapy, compared to the standard-dose induction chemotherapy, resulted in improved overall survival (3-year OS, 44 vs. 25%) [8].

(2) **Consolidation chemotherapy**: Once a person is deemed to be in CR, consolidation chemotherapy is considered to maximize the duration of CR. It is widely accepted that without consolidation chemotherapy, nearly all patients in CR would relapse sooner or later. The strategy for consolidation is not as standardized as that for induction phase. The regimen depends on multiple factors including the patient's age, cytogenetics, and molecular genetics as discussed in greater details below.

Consolidation chemotherapy usually involves high dose of cytarabine (high dose Ara-C or HiDAC). The optimal number of cycles, the most appropriate dose and schedule, and the role of combining HiDAC with other agents are not standardized. Cancer and Leukemia Group B (CALGB) showed that four cycles of HiDAC are superior to four courses of intermediate- or standard-dose cytarabine [9]. This beneficial effect of cytarabine dose intensification, however, is restricted

to patients with core-binding factor (CBF) AML and, to a lesser extent, in patients with AML with normal cytogenetics (cytogenetically normal AML or CN-AML). The outcome of patients with other cytogenetic abnormalities is not affected by cytarabine dose.

(3) **Hematopoietic stem cell transplant (HSCT)**: is an approach where chemotherapy is followed by rescue hematopoietic stem cell (HSC) infusion. The chemotherapy can be non-myeloablative (reduced intensity chemotherapy; RIC) or high-dose myeloabletive. The source of the HSC can be from previously harvested HSC from the patient (autologous transplant) or from another human (allogeneic transplant). HSC donors are usually matched according to recipient's human leukocyte antigen (HLA)-type to maximize the chances of grafting and minimize the changes of rejection (graft-versus-host-disease, GvHD). In high-risk patients (for example those with adverse-risk cytogenetics, vide infra) allogeneic stem cell is the only potentially curative therapy and an allogeneic stem cell transplant is considered as soon as remission is achieved. Meta analysis of patients with AML in the first remission showed that allogeneic SCT results in significant relapse-free survival and OS compared to nonallogeneic SCT therapies in both intermediate- and adverse-risk AML but not in good-risk AML [10].

Current Standards of Therapy for Acute Promyelocytic Leukemia

Prior to use of all-trans retinoic acid (ATRA, tretinoin), APL was considered the most fatal subtype of AML with median survival of less than a month. Advent of ATRA is the first and one of the most dramatic examples of molecularly targeted therapy for cancer. APL patients with *PML-RARα* genetic mutation are treated with ATRA that results in prolonged remission in more than 80% of patients. ATRA is unique among chemotherapeutic regimens of acute leukemia in that, unlike most chemotherapeutic agents that results in cell death, ATRA induces differentiation of the malignant promyelocytic cells.

Later, the introduction of arsenic trioxide (ATO) has further added to our efficacy in treating APL. Arsenic has long known to be both a poison and a medicine. In 1992, it was identified as the active ingredient of a Chinese herb that was known to have efficacy against APL [5, 6, 11–14].

1. **Induction Therapy**: The current standard for induction treatment for APL is concurrent ATRA and chemotherapy with anthracycline and cytarabine (7 + 3). This regimen has shown to be better than ATRA alone, chemotherapy alone, or ATRA followed by 7 + 3 regimens. There is an ongoing debate whether ATRA and anthracycline are similar effective to ATRA and 7 + 3. There is some data to suggest that cytarabine can be safely omitted from induction regimen in most patients.

A unique aspect of treatment with ATRA or ATO is possibility of development of differentiation syndrome which is thought to occur due to release of large amounts of cytokines from the differentiating myeloid cells ('cytokine storm'). It is controversial whether cytokine storm is inseparably linked to differentiation of leukemic cells. If suspected, differentiation syndrome is managed by high dose of steroids.

2. **Consolidation Therapy**: It is widely known that although complete remission can be obtained with ATRA alone, without additional treatment, most patients will ultimately relapse. Optimal consolidation regimens for APL are not established. In general, two to three cycles of anthracycline based chemotherapy along with ATRA is considered standard of care in low-risk patients (WBC count $<10\times10^9$/L at presentation). There is a possibility that addition of cytarabine to this regimen provides benefit in high-risk patients (WBC count $>10\times10^9$/L at presentation) [5, 6, 12].
3. **Maintenance Therapy**: There is no consensus whether maintenance therapy is indicated in all patients with APL post-remission. It is known that many patients with no molecular evidence of residual disease will relapse, especially the high-risk patients. These patients can be identified by sequential minimal residual disease (MRD) monitoring using qualitative or quantitative PCR, and treated with preemptive therapy to prevent relapse. There is evidence for both in favor of and against standard maintenance therapy. The majority of trials showing benefit of maintenance therapy took place prior to advent of ATRA, ATO, and Ara-C in consolidation therapy. It is possible that those with no evidence of MRD do not benefit from such treatment. However, given the low toxicity profile many physicians prefer maintenance therapy to observation [12, 13].
4. **HSCT**: Generally speaking, given the high cure rates obtained using induction treatment with ATRA and chemotherapy, HSCT is not considered routinely. However, in patients with persistent minimal residual disease (MRD), allogeneic HSCT is considered [12].

In various studies, ATO appears to be more efficacious than ATRA in reducing the degree of MRD in APL. While remissions induced by ATRA are short-lived, ATO induces molecular remission in almost all patients treated at relapse. This led to further studies using ATO either as a single agent or in combination with chemotherapy in newly diagnosed APL. In a small single-center study using single-agent ATO for newly diagnosed APL patients, complete hematologic remission was achieved in 86% which is comparable to conventional chemotherapy. Three-year EFS, DFS, and OS was 75, 87, and 86% respectively. The authors also observed that patients presented with WBC count $<5\times10^9$/L and platelet count $>20\times10^9$/L had EFS, OS, and DFS of 100% at the end of the study. As expected, ATO was better tolerated than conventional chemotherapy and had an advantage that consolidation therapy could be administered as out-patient basis [15]. This, and multiple other studies showing similar results, has led to a debate if APL can be managed solely with differentiation agents such as ATRA and ATO without any need for conventional chemotherapy.

Molecular Genetics of Acute Promyelocytic Leukemia

Approximately 98% of persons with APL carry reciprocal translocation t(15,17) that results in the fusion between retinoic acid receptor-α (*RARα*) and the promyelocytic leukemia (*PML*) genes. *PML-RARα* is unique and defining molecular characteristic of APL. The resulting fusion protein PML-RARα is shown to inhibit the differentiation of myeloid cells by abnormal recruitment of transcription factors and histone-modifying enzymes (histone deacetylases, histone methyltransferases, and DNA methyltransferases). Retinoic acid is a transcription factor that belongs to steroid hormone receptor superfamily. RARα contains a series of functional domains, including an N-terminal transcriptional activation domain, followed by DNA binding-, dimerization-, and retinoid-binding domains. PML-RARα also activates self-renewal pathways such as Wnt and Notch signaling pathways in leukemic cells.

Variants of APL: As mentioned above, about 98% of patients with APL have *PML-RARα* mutation. In a minority of other patients other translocations are observed. The second most common translocation observed in APL is t(11;17) that results in the fusion of 3′ end of *RARα* to 5′ end of promyelocytic leukemia zinc finger (*PLZF*). Clinically, it is important to identify this mutation at diagnosis, as patients with t(11;17) APL are almost always resistant to treatment with ATRA. Yet another variant of APL is characterized by similar translocation t(11;17), but resulting in the fusion of nuclear matrix-mitotic apparatus protein gene (*NUMA*) with *RARα* instead of *PLZF-RARα*. Patients with this mutation are sensitive to treatment with ATRA. Another rare variant of APL (~0.5% of all APL cases) is characterized by translocation t(5;17) resulting in fusion of *NPM* to *RARα*. Patients with this translocation are responsive to ATRA therapy. A few cases of fusion between signal transducer and activator of transcription 5b (*STAT5b*) with *RARα* have also been described in patient with an interstitial chromosome 17 deletion. These phenotypes were found to result in ATRA-resistant form of APL.

Prognostic Factors

Cytogenetic, morphological, and molecular genetic criteria are routinely used as adjunct to clinical markers while evaluating a patient with new diagnosis of AML. Clinical and molecular markers are critical in discussing and deciding prognostic and therapeutic options. AML is increasingly subclassified based on recurring genetic abnormalities that predict prognosis and response to therapy. These prognostic factors may be subdivided into those related to patient characteristics and general health condition and those related to characteristics particular to the AML clone. The former subset usually predicts treatment-related mortality (TRM) and becomes more important as the patient's age increases. Factors related to AML predict response, or lack thereof, to conventional therapy [16].

(a) **Patient-related factors**: Increasing age is an adverse prognostic factor in AML even after adjusting for other risk factors. Other known factors are pre-existing renal impairment, and Eastern Cooperative Oncology Group (ECOG) performance status 2 or higher [17, 18].

(b) **Disease-related factors**: Disease related poor prognostic factors are high white blood count (WBC), AML arising from pre-existence of MDS (secondary AML), chemotherapy related AML, and cytogenetic and molecular genetic changes. Recently, a large single center prospective trial showed that 97.3% of patients with AML had at least one somatic mutation identified. It was also shown that mutational heterogeneity was greater in patients with intermediate-risk AML than in patients with favorable- or adverse-risk AML patients. The karyotype of the leukemic cells is the strongest prognostic factor for response to induction therapy and survival. Greater details of effect of each mutation on survival are discussed in details in the following sections.

Cytogenetic Abnormalities

Initial karyotype obtained at the time of diagnosis is the most important prognostic factor in AML. Cytogenetic studies are used to classify patients into 'adverse', 'intermediate' and 'favorable' risk categories. A little less than half (~45%) of the patients do not have any demonstrable clonal chromosomal aberration, and are called cytogenetically normal AML or CN-AML. Traditionally CN-AML is categorized as the intermediate-risk group. Traditionally, intermediate-risk category is further divided into Intermediate-I and –II categories according to co-existence of various mutations. Newer studies have focused on the effect of co-existing mutations on survival in patients with CN-AML. These studies agree that in general the presence of *FLT3*-ITD is a poor prognostic factor. Patients without *FLT3*-ITD *and* mutant *NPM1*, *IDH1* or *IDH2* are considered favorable risk. Those without *FLT3*-ITD and with wild-type *ASXL1*, *MLL*-PTD and *TET2* are considered intermediate risk. The presence of mutant *CEBPA* with or without *FLT3*-ITD is also considered as a marker of intermediate-risk disease. Whereas, patients with *FLT3*-ITD mutation in the presence of mutant *TET2*, *MLL*-PTD, *DNMT3A* or trisomy 8 without mutant *CEBPA,* are considered to be adverse-risk patients. The presence of mutant *TET2* or *MLL*-PTD irrespective of *FLT3*-ITD is considered as adverse-risk [8].

While there is emerging consensus about management of favorable- and adverse-risk patients, it is increasingly evident that the intermediate risk CN-AML is a incredibly heterogeneous group [19]. Most of the current research is focused on identifying prognostic markers and tailoring treatment within this group of patients.

Favorable cytogenetics. Traditionally, translocation of chromosome 8 and 21 t(8;21) resulting in Runt-related transcription factor-runt-related transcription factor 1, translocated to 1 (*RUNX1-RUNX1T1*) fusion; inversion of chromosome 16—inv(16) or t(16;16) resulting in cord binding factor β-myosin, heavy chain 11, smooth muscle (*CBFB-MYH11*) fusion; translocation of chromosome 15 and 17 resulting in promyelocytic leukemia retinoic receptor-α (*PML-RARα*)—a defining characteristic of APL, mutated nucleophosmin (*NPM1*) and/or CAAT/enhancer binding protein-α (*CEBPA*) without fms-related kinase-3 (*FLT3*) mutation and otherwise normal karyotype, are all considered favorable cytogenetic aberrations. In the absence of DNA-methyltransferase 3A (*DNMT3A*) mutations, biallelic *CEBPA* mutations or *NPM1* mutations are considered as favorable-risk prognostic markers.

Adverse-risk cytogenetics. Deletion of chromosome 5 (−5,5q-), chromosome 7 (−7,7q-), inversion of chromosome 3—inv(3) or t(3;3) *RPN1-EVI1*, translocation of chromosomes 6 and 9 t(6;9) *DEK-NUP214*, t(v;11)(v;q23), mixed lineage leukemia (*MLL*) rearranged, or complex karyotypes (defined as more than three chromosomal abnormalities) are considered to be high risk cytogenetic features.

Intermediate-risk cytogenetic. In one of the largest cohort of CN-AML patients studied so far interesting patterns have emerged. The highest mutation incidences were observed for the *CEBPA* (in 22% patients). *NPM1* mutations were found in 20.9%, followed by *MLL* mutations that were present in 14% CN-AML patients. Mutation in ten-eleven-2 (*TET2*) was present in 12.7%, while *DNMT3A* mutations were found in 12.3% patients [20].

Generally, in the absence of *FLT3*-internal tandem duplicate (*FLT3*-ITD) *NPM1* mutation carries favorable prognosis conferring higher complete remission (CR) rates, better overall survival (OS), event-free survival (EFS), and disease-free survival (DFS). *CEBPA* mutation is considered to be a favorable mutation again conferring better DFS, EFS, and OS. *FLT3*-ITD is one of the most common mutations found in AML. Prognostic implications of harboring *FLT3* mutations are discussed in detail in the following sections. Multivariate analysis identified that both *DNMT3A* and *MLL* mutations are independent factors predicting inferior OS and EFS [20].

Known Genetic Abnormalities in Pathogenesis of AML

The pathogenesis of AML is complex and essentially involves two overarching processes—class I mutations are the mutations that confer survival advantage by promoting proliferation or enhancing survival of the leukemic cells; and class II mutations are those affecting transcription regulation resulting in impairment of differentiation. The 'two-hit model' describes development of AML as a multistep process. Generally, a combination of class I and II mutations are required for the development of the disease [21]. Recently, gene mutations involved in epigenetic regulation are proposed as a third class, distinct from the first two classes, as they

seem to have a distinct regulatory network, as well as common features of aggressive disease, poor prognosis, and older age onset [20].

FMS-related tyrosine kinase 3 (FLT3, CD135). is a class-III receptor tyrosine kinase (RTK) with key role in hematopoiesis. FLT3 receptor is a typical cell membrane RTK with an extracellular domain, a transmembrane domain, and a cytoplasmic domain. In the BM, higher expression of FLT3 is largely restricted to early progenitor cells such as multipotent progenitor (MPP) and common lymphoid progenitor (CLP) cells. In CD34+ cells, the expression of FLT3 is associated with high levels of expression of CD117 (c-KIT). Its extracellular domain is composed of five immunoglobulin (Ig)-like domains. The cytoplasmic domain possesses kinase activity and is split into two parts by a kinase-insert domain. The receptor is activated by binding of the FLT3 ligand to the extracellular domain, which induces homodimer formation in the plasma membrane leading to autophosphorylation of the receptor. The activated receptor complex then induces a cascade of signaling that regulates differentiation, proliferation and apoptosis of the HSC.

Being a class-III RTK, FLT3 activation leads to engagement of multiple signaling cascades including phosphoinositide-3 kinase/protein kinase B (PI3k-Akt) signaling, Ras/MAPK kinase/extracellular signal-regulated kinase signaling (Ras/Mek/Erk) signaling, and Janus Kinase—signal transducer and activator of transcription (Jak-Stat) signaling pathways. These signaling cascades exert cell proliferation, growth, and anti-apoptotic effects which cumulatively lead to leukemogenesis. STAT5 acts downstream to FLT3 in FLT3-signaling pathway and is constitutively activated by persistent FLT3 activation. STAT5, a transcription factor, in turn activates Ras-related C3 botulinum toxin substrate-1 (RAC1) which is an essential part of NADPH oxidase system resulting in increased generation of reactive oxygen species (ROS). Increased ROS impairs cell's ability to carry out DNA repair resulting in increased DNA damage, decreased end-joining fidelity and ultimately increased chromosomal instability (CIN). CIN is associated with overall worse prognosis, elucidating, at least in part, why *FLT3*-ITD mutations are associated with such distinctly poor prognosis [21–23].

Over the last decade, the role of *FLT3* is well-established in pathogenesis and prognosis of AML. About a third of patients with AML harbor mutant *FLT3*. Two distinct classes of *FLT3* mutations are described in AML. The most common mutation is a 3–400 base pair internal tandem duplication (ITD) found in about a fourth of all AML cases. ITD insertions are seen in the juxtamembrane region of the receptor and may vary in length. They characteristically maintain a head-to-tail orientation, and are always in-frame. The second most common type of *FLT3* mutations in AML are those involving the activation loop of the tyrosine kinase domain (TKD). These mutations account for about 5–7% of all AML. The most common of TKD mutations is substitution of aspartate for tyrosine at the codon 835 [23, 24].

Prognostic Implications of FLT3 Mutations. FLT3-ITD mutation is associated with higher WBC count at presentation. While harboring *FLT3*-ITD does not impact ability to achieve CR, patients with *FLT3*-ITD are at increased risk for relapse (relapse risk, RR), decreased DFS and OS. Multiple studies have found *FLT3*-ITD to be the most significant factor for predicting an adverse outcome [23, 25].

Five-year overall survival OS and DFS rates as low as 15% were observed in patients with *FLT3*-ITD mutation in contrast to OS and DFS rates of approximately 40% in AML patients with wild-type *FLT3* [26].

DNA-methyltransferase 3A (DNMT3A). Aberrant methylation of CpG island is known to contribute to the pathogenesis of various cancers. DNA-methyltransferases (DNMT) are a group of enzymes that facilitate the addition of a methyl group to the cytosine residue of CpG dinucleotides. In a study evaluating 281 AML patients, about a fourth of patients had mutations in *DNMT3A* that were predicted to affect translation. Interestingly, no DNMT3A mutations were found in favorable-risk AML patients, while about a third of patients with intermediate-risk AML had *DNMT3A* mutations. *DNMT3A* mutations are considered 'driver' mutations and are more predominant in leukemic cells of monocytic lineage. Overall, the presence of mutated *DNMT3A* is considered to be an independent adverse risk factor and is associated with poor remission induction with conventional chemotherapy. It is an independent risk factor for OS as well, with median OS among patients with *DNMT3A* mutations (12.3 months) being significantly shorter than that among patients without such mutations (41.1 months) [20, 27].

Multi-drug resistance-1 (MDR1, P-Glycoprotein, P-gp). P-glycoprotein [ATP-binding cassette (ABC)—transporters B1] is a well-characterized ABC transporter with known role in molecular transport across the cellular membrane. P-gp is overexpressed in many cancer cells resulting in efflux of various drugs. This results in decreased concentration of the drug intracellularly, effectively rendering the tumor cells resistant to chemotherapy. Overexpression of MDR1 inhibits intracellular uptake of many chemotherapeutic drugs such as vincristine, vinblastine, doxorubicin, and daunorubicin. The expression of the MDR1 protein was detected by immunohistochemistry in one-third of patients less than 56 years old and in about 60% of patients aged 56 years and older [18]. The presence of P-gp is considered an adverse prognostic factor. A meta-analysis of 74 studies examined a total of 4,069 patients who had both CR and P-gp status documented. The median CR rate was 78% in $P\text{-}gp^-$and 47% in $P\text{-}gp^+$ patients. The overall pooled odds ratio for CR was 0.3 for $P\text{-}gp^+$ status compared to $P\text{-}gp^-$status confirming MDR1 expression as an adverse prognostic factor [28].

Nucleophosmin-1 (NPM1). NPM1 mutations are one of the most common genetic abnormalities in AML. Mutated NPM1 is seen in 50–60% of patients with CN-AML. *NPM1* belongs to a new category that functions both as an oncogene and tumor-suppressor gene, depending on expression levels, interacting partners, and compartmentalization. It is a nucleocytoplasmic protein with multiple functions including interaction with p53 resulting in regulation of proliferation and apoptosis; maintenance of genomic stability; and ribosome biogenesis [16, 20]. AML patients with *NPM1* mutation typically have high CD33 but absent or low CD34 expression [17].

Prognostic Implications of NPM1 Mutation. In patients with CN-AML, *NPM1* mutations are prognostically favorable in the absence of FLT3-ITD. *NPM1* mutations also have favorable prognostic impact in older patients with CN-AML,

especially those of age more than 70 years. *NPM1* mutation in CN-AML has been associated with higher CR rates and better RFS and EFS [16, 17].

Isocitrate dehydrogenase-1 and -2 (IDH1 and IDH2). In a study of 358 patients with CN-AML, one third of patients harbored mutations in *IDH* genes. About 14% had *IDH1* mutations, 19% had *IDH2* mutations, while none had both *IDH1* and *IDH2* mutations. In another recent study of more than 1,000 patients with AML in China, prevalence of mutations *IDH1* and *IDH2* were determined to be just less than ten percent each. Overall, patients with *IDH* mutations have an unfavorable prognosis [20, 29]. The exact role of *IDH* mutations in leukemogenesis is not known, but it might be related to enzymatic property of converting α-ketoglutarate to 2-hydroxyglutarate (2HG). It is hypothesized that 2HG prevents the histone demethylation which in turn blocks differentiation of lineage-specific progenitor cells [30].

Ten-eleven (TET)-2. TET2 converts 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC), which is an intermediate step in DNA demethylation pathway. In a CALG-B study, among the patients classified as favorable-risk (patients with CN-AML who have mutated *CEBPA* and/or mutated *NPM1* without *FLT3*-ITD), those with mutated *TET2* had statistically shorter EFS secondary to lower CR rate, as well as shorter DFS and OS compared to *TET2*-wild type patients. Interestingly, the presence of *TET2* mutation was not associated with any significant difference in outcomes in the intermediate-I-risk group (CN-AML with wild-type *CEBPA* and wild-type *NPM1* and/or *FLT3*-ITD) patients [21, 31].

Core-binding factor (CBF). CBF is a heterodimeric transcription factor composed of α- and β-subunits. The CBF-α subunit is encoded by a one of three members of the *RUNX* family, the *RUNX1* (*AML1*, *CBFA2*, and *PEBP2aB*) gene, whereas the CBF-β subunit is encoded by the *CBFB* (also known as *PEBP2B*) gene. The CBF-α subunit binds directly to the DNA promoter sequences of the target genes while the β-subunit stabilizes the CBF complex and enhances its affinity to DNA.

CBF AML is characterized by the presence of one of the two distinct chromosomal translocation namely t(8;21) or inv(16)/t(16;16). These translocations result in formation of chimeric fusion genes *AML1-ETO* and *CBFB-MYH11* respectively [32]. Overall, the incidence of CBF-AML is around 15%, with both subtypes accounting for approximately half of the cases. CBF AML is a favorable prognostic factor in patients with AML with CR rates approaching 90% or more with conventional chemotherapy. Lower relapse incidences contribute to longer DFS, OS and higher long-term cure rates. It should be noted though that even the CBF-AML is not a homogenous group. Among CBF-AML patients, the presence of a c-KIT mutation is shown to confer adverse OS. KIT mutation testing, therefore, appears to be prognostically important for survival in CBF AML and now is a part of National Comprehensive Cancer Network (NCCN) guidelines.

For CBF AML, retrospective CALGB study showed that high dose Ara-C (HiDAC, cumulative dose: 54–72 g/m^2) is superior to single cycle (18 g/m^2) regimen. While no survival advantage was seen with HSCT after first remission, allogeneic HSCT may be considered in higher risk CBF AML patients such as those with high white count or those with c-KIT mutations [17].

Mixed Lineage Leukemia (MLL). Mutations in *MLL* can either be due to chromosomal translocations or intragenic partial tandem duplications (PTD). *MLL* mutations are more common in patients with therapy-related secondary AML. Mutations in *MLL* were thought to be present in about 5% patients with AML and confer poor prognosis. A recent study showed the prevalence of MLL in 14% patients with CN-AML.

MLL encodes a histone methyltransferase and interestingly shares many common phenotypic similarities with *DNMT3A* mutations. While DNMT3A is a DNA-methyltransferase, MLL is a histone methyltransferase. Mutations in either *MLL* or *DNMT3A* are associated with elder age, poor prognosis, M4–M5 AML, as well as with upregulation of genes such as HOX9 [20]. These similarities are likely due to shared mechanisms of action—epigenetic modulation. The study also found that *MLL* mutations seldom coexisted with other mutations.

CCAAT/enhancer binding protein-α (CEBPA). Familial AML is defined as germline mutation resulting in a phenotype in which multiple individuals in a family have AML. *CEBPA* mutation in the germline has been associated with familial AML. This can be differentiated from somatic *CEBPA* mutation as patients with familial AML carry a *CEBPA* mutation even in non-leukemic cells. The age of onset of familial AML is earlier compared to sporadic AML; with disease onset being as early as age of 4 years. The prognosis of individuals with familial AML with mutated *CEBPA* appears to be favorable (~50–65% overall survival) compared to the ~25–40% overall survival of those who have normal karyotype AML but no germline *CEPBA* mutation. Individuals with familial AML with mutated *CEBPA* who have been cured of their initial disease may be at a greater risk of developing additional malignant clones than those with sporadic disease [33].

C-X-C chemokine receptor type 4 (CXCR4, fusin, CD184). CXCR-4 is an alpha-chemokine receptor specific that responds to binding of its ligand stromal-derived-factor-1 (SDF-1 or CXCL12). SDF-1 has a well known role in HSC homing and maintaining quiescence. Recently, a study examining the expression levels of CXCR4 as a prognostic marker was done in patients with AML. CXCR4 expression in AML was found to be an independent prognostic predictor for disease relapse and survival. The study found that low CXCR4 expression on leukemic cells correlated with longer DFS and OS. [34, 35].

Micro RNA (miRNA or microRNA) Expression as a Prognostic Marker in AML

Micro RNAs are regulatory, non-coding RNAs of 22 nucleotides size. They are considered to be regulatory molecules in nature in that higher expression of a given miRNA negatively regulates its corresponding RNA—in turn resulting in decreased expression of its corresponding protein. It is known that there is a global downregulation of microRNA in malignancies, and the microRNA profile also reflects the origin and differentiation state of the tumors [36]. In AML, miRNA signature was

created using leukemic cells from younger high-risk CN-AML patients (i.e. age <60 years, *FLT3*-ITD and/or wild-type *NPM1*). This signature was then validated in a different set of patients with comparable demographics. Of the more than 300 miRNA studied, 12 were found to be associated with difference in outcomes. MicroRNA compound covariate predictor (called a microRNA summary value) was calculated on the basis of weighted levels of the miRNAs forming the outcome signature. Higher microRNA summary value was inversely associated with favorable outcomes including EFS [37].

Whole Genome Sequencing Approach in Acute Myelogenous Leukemia

The first fully sequenced genome of any human malignancy published was that of a patient with AML. In 2008–2009, parallel sequencing technology was used to sequence genome of leukemic cells from a patient with cytogenetically normal AML. With sequencing, more than 30-fold coverage of the tumor genome was achieved. As a control, lower coverage genomic sequencing of the normal skin tissue was obtained. Comparing mutations in non-leukemic cells to those in leukemic cells, 98% of potential variants were identified as inherited. Further analysis identified ten genes with acquired mutations unique to leukemic cells—two of which are well known (*NPM1* and *FLT3*). Eight novel mutations were also identified in virtually all tumor cells at presentation and relapse. This was a landmark study that showed feasibility of whole genome sequencing as a method for identifying mutations in cancer genomes. Further use of this technology also promises the possibility of identifying novel genes that may respond to targeted therapies [38].

Whole genome sequencing approach also allows identifying patterns of clonal evolution in patients with relapsed disease. The two models observed are (1) the founding clone in the primary tumor gains mutations and evolves into the relapse clone; or (2) a subclone of the founding clone survives induction chemotherapy, gains additional mutations and expands at relapse. Interestingly, in all cases of relapsed AML studied, there was an increase in mutations that was attributed to chemotherapy [39].

A recent whole-genome sequencing study in patients with MDS that progress to AML elucidated two key findings. Traditionally, manual counting of myeloblasts in the BM is used to differentiate MDS from AML. A blast count of <20% in BM is considered as MDS while, a blast count of >20% in BM is diagnostic for secondary AML. Deep genomic sequencing of samples before and after transformation showed that percentage of cells harboring founding clone did not differ between MDS and AML stages. Also, relatively few somatic mutations were acquired during transformation. In all the cases studied, the founding clone of MDS persisted following transformation and continued to be the dominant clone in secondary AML. These findings suggest blurring of the boundary between MDS and secondary AML at molecular level [40].

It is widely believed that with wider and more affordable use of genome-sequencing techniques, high-throughput sequencing will become routine in care of patients with variety of cancers including AML. Identifying recurrent mutations in AML may be helpful to identify 'healthy' people who are at higher risk of developing disease. Once the disease is diagnosed, sequencing data may be used to stratify patients accurately, thus predicting course of disease and treatment. The presence or absence of certain mutations may be helpful in identifying patients who would respond to certain medications while also identifying those at potential risk of developing toxicities. In short, we are rapidly ushering into an era of personalized medicine with AML at the forefront of such advance.

Targeted Therapeutics in Acute Myelogenous Leukemia

Multiple new therapeutic agents are being evaluated for patients with AML. A search on FDA Clinical Trials website returns more than 1,400 recently conducted trials suggesting tremendous translational effort in treatment of AML. Table 1 describes some of the notable trials evaluating targeted therapy in AML [41]. The following section will focus on well studied pathways and biological therapies that are being developed.

CD33 Based Targets

1. ***Gemtuzumab ozogamicin***: is a humanized anti-CD33 antibody chemically linked to calicheamicin. CD33 portion of the molecule guides the cytotoxic agent calicheamicin to its targets while calicheamicin then induces apoptosis in target cells by inhibiting DNA synthesis [17]. Recent clinical data suggested that in combination with conventional chemotherapeutic regimens, GO is efficacious in both favorable- and intermediate-risk AML. In a recently published phase III trial 280 patients (age 50–70 years) were treated with conventional chemotherapy with or without GO. There was no difference in patients achieving CR or CRp. However, EFS was estimated at 15.6% in conventional chemotherapy arm compared to 41.4% in the arm treated with additional GO. DFS was 18.1% in chemotherapy only arm while 48.5% achieved DFS when GO was added to chemotherapy regimen. Most importantly, the addition of GO to the chemotherapeutic regimen resulted in improved OS (25.4 months in GO arm compared to15.3 months in chemotherapy only arm). Three liver veno-occlusive diseases (VOD) were observed in GO arm, two of which were fatal [53]. These EFS benefits were still observed after excluding favorable-risk patients (i.e. in intermediate- and adverse-risk patients).

In contrast, another study published at the same meeting studied younger AML patients (age 18–60 years) for 3 years. In the 238 patients studied, difference was

Table 1 Notable clinical trials evaluating targeted therapies in patients with AML.

No	Target molecule/pathway	Description	Identifiers/references
Phase II trials			
1	GRNVAC1 peptide vaccine—Human telomerase reverse transciptase (hTERT)	GRNVAC1 sensitizes CTLs to tumor cells expressing hTERT peptides as surface antigens via the HLA class I pathway causing an expansion of telomerase-specific CD8+ CTLs leading to targeted killing of telomerase positive tumor cells	NCT00510133, [42]
2	WT1 peptide based vaccine	WT-1 specific CTLs eliminate CD34+ CML cells while sparing normal CD34+ stem cells.	NCT01513109, NCT01266083, [42, 43]
3	WT1 and PR1 peptide based vaccines	Combined immunization with WT1 and PR1 peptides lead to measurable CTL response that is associated with decrease in tumor load as measured by WT1 expression levels.	NCT00488592, [42]
4	GTI2040—Antisense to R2 subunit of human ribonucleotide reductase	GTI-2040 is a 20-mer oligonucleotide containing sequence complementary to a coding region of human ribonucleotide reductase. It decreases mRNA and protein levels of R2 may inhibit tumor cell proliferation in human tumors in vivo. GTI-2040 was studied with high-dose Ara-C with successful downregulation of the R2 target. The clinical efficacy of this combination is being tested in ongoing phase II study.	NCT00565058, [44]
5	Anti-CD33 antibodies	Gemtuzumab ozogomicin and Lintuzumab. Both these antibodies have been conjugated with various chemotherapeutic as well as radioimmunotherapeutic agents to obtain targeted cell death. Trials describing these antibodies are discussed in detail in the text.	NCT00006084, NCT00528333, NCT00997243, NCT00283114, NCT00672165, NCT00002800
6	Anti-PD-1 antibody	Humanized mAb to PD1	NCT01096602

(continued)

Table 1 (continued)

No	Target molecule/pathway	Description	Identifiers/references
7	IL-3 protein fused with diphtheria toxin	DT388IL3 (diphtheria toxin/IL-3 fusion protein)	NCT00397579
8	Vascular endothelial growth factor (VEGF) receptor signaling inhibitor—Aflibercept (AVE0005)	Aflibercept slowed disease progression in pre-clinical studies. Combining aflibercept with doxorubicin enhanced antileukemic effects.	NCT00601991, NCT00509249 and [45]
9	Cediranib	Phase II study of AZD2171 for the treatment of patients with AML.	ASCO meeting abstract [46]
10	L-grb-2 antisense	Preliminary results presented suggests that BP-100.1.01, was well tolerated and there were transient benefits noted in 7 AML patients tested. Further studies are underway and results are awaited.	[47]
11	Pazopanib (TKI)	Pazopanib	NCT01361334
12	Topoisomerase II	Pixantrone/BBR 2778 is being evaluated in patients with refractory AML in a single center, open label phase I/II trial. The study is complete and results are expected.	NCT00106600
Phase III/IV diagnostic trials			
1	WT1	WT1 for detection of MRD	NCT00179829
Phase III/IV therapeutic trials			
1	CD33	Gemtuzumab ozogamicin is a chimeric molecule consisting of anti-CD33 antibody chelated with chemotherapeutic agent ozogamicin. It is thought to selectively inhibit apoptosis in CD33+ cells and hence believed to be a targeted therapeutic agent for AML.	NCT 00927498, NCT00860639, NCT00091234, NCT00121303, NCT00038805
3	CD33	Lintuzumab (see details in text)	NCT00006045
4	CD52	Campath1, Alemtuzumab	NCT00226512

5	Oblimersen antisense	Phase 2 study evaluated the safety and efficacy of GO with oblimersen for older patients with AML. Of 48 patients evaluated, 12 achieved either CR (n=5) or CRp (n=7). The median time to remission was 52 days.	NCT00085124, [48]
6	FLT3 signaling	FLT3 ligand	NCT00006223
7	FLT3 signaling	Midostaurin (see details in text)	NCT00651261
8	P-glycoprotein, MDR1	PSC-833 is a second generation P-gp modulator. In a randomized trial in patients with AML evaluating PSC-833, 302 patients were randomized to receive standard induction chemotherapy with or without PSC-833. The incidence of CR, therapy related mortality, DFS, and OS did not differ in two groups while reversible grade 3- and 4- toxicities were significantly more common in group treated with PSC-833 (CALGB 19808).	NCT00003190, NCT00006363, [49]
8	RTK signaling	bortezomib and sorafenib (see details in text)	NCT01371981
9	Farnesylation pathway	Lonafarnib—a farneysyl transferase inhibitor (FTI) is being studied in AML	NCT00109538
Other notable trials			
1	VEGF-R signaling	A trial with AZD4877, a VEGFR signaling inhibitor, was terminated early due to failure to achieve CR in all 8 evaluable patients treated with maximum of 2 doses.	NCT00486265
2	Farnesylation pathway	Tipifarnib—a farnesyl-transferase inhibitor that inhibits mTOR signaling in vivo and in vitro.	NCT00096122,NCT00093990, NCT00093470, [50]
3	P-gp or MDR1	Randomized placebo-controlled double-blind trial in 449 patients with AML (age>60) was carried out with Zosuquidar, an inhibitor of P-gp. Zosuquidar did not improve remission rate or OS. It was concluded that zosuquidar did not improve outcome in older patients with AML.	NCT00046930, [51]

(continued)

Table 1 (continued)

No	Target molecule/pathway	Description	Identifiers/references
4	Vorinostat	Vorinostat is a HDACi that is known to have synergistic activity with conventional chemotherapy in a sequence dependent manner.	NCT00479232, NCT00656617, [52]
5	Intrinsic apoptosis pathway (caspase 9)	Tasisulam (or LY573636) sodium is an acyl-sulfonamide that induces cytochrome C release, caspase -2 and -9 activation eventually culminating in apoptosis by intrinsic apoptotic pathway. It has been evaluated in a phase I trial in patients with AML and MDS.	NCT00718159

NCT number refers to clinical trial identifier number as it appears on http://clinicaltrials.gov

observed neither for EFS, nor for OS. In the subset of patients who could not receive an allogeneic transplant, EFS was doubled in the GO group, while there was no difference for OS. It appeared that the benefit of addition of GO was largely limited to patients classified as intermediate risk-1 or -2 [54].

2. ***Lintuzumab (humanized CD33 antibody, HuM195, SGN-33)*** is a humanized, native anti-CD33 monoclonal antibody that has been tested with or without additional chemotherapy in patients with AML. In a phase II trial, 50 adult patients with relapsed/refractory AML were randomized to receive higher or lower doses of HuM195. Of the 49 patients evaluated after treatment, two patients achieved CR while one achieved PR. Additionally, a decrease in blast counts (range of decrease 30–74%) was seen in nine additional patients. It was concluded that HuM195 as a single agent exerted observable, but minimal, therapeutic activity in these patients. In a phase III randomized multicenter trial, lintuzumab was evaluated in combination with chemotherapy as compared to chemotherapy alone in relapsed/refractory AML. Of the 191 patients studied, 28% of patients treated with chemotherapy alone achieved CR or CRp, while 36% of patients achieved CR or CRp following addition of lintuzumab. Treatment with lintuzumab did not increase OS. It was concluded that lintuzumab was tolerated well but did not improve remission rates or OS [55]. Lintuzumab was evaluated with or without low-dose Ara-C in a randomized, placebo-controlled, double-blinded phase IIb trial. The combination of lintuzumab and low-dose cytarabine did not improve OS compared with placebo and low-dose cytarabine [56]. While the native CD33 antibody (SGN-33) has failed to achieve substantial therapeutic goals, further trials are underway to test if lintuzumab has synergistic activity when combined with traditional chemotherapy or as a radioimmunotherapeutic tool in both AML and MDS [57, 58].

Therapeutic FLT3 Inhibitors. As discussed above a plethora of work has shown the importance of FLT3 signaling in the pathogenesis of AML. It was clear from early on that FLT3 is a promising target for treatment of AML. Semaxinib was one of the first tyrosine kinase inhibitor (TKI) tried. However, further development of semaxinib did not ensue due to modest clinical benefits. Currently, multiple FLT3 inhibitors are in various stages of development.

1. ***Midostaurin (PKC412)*** is a staurosporine derivative that has activity against FLT3 as well as other TK such as VEGFR, PDGFR, and c-KIT. A phase I trial of midostaurin was performed in patients with relapsed/refractory AML. Seven of the 20 patients had transient reduction in peripheral blasts and five experienced reduction in BM blasts. A phase Ib trial that used oral midostaurin in addition to conventional chemotherapy in newly diagnosed AML patients age 60 or less, showed CR in 80% of all patients (74% of wild-type *FLT3* patients, and 92% of mutated *FLT3* patients). OS at one- and 2-years in patients harboring *FLT3* mutation was 85 and 62%, comparable to 81 and 59%, respectively for patients without *FLT3* mutations. These preliminary results are in stark contrast from observational studies that have shown significantly poor prognosis in patients

with *FLT3* mutations. It is hypothesized that the effect of midostaurin and other FLT3 inhibitors in patients with wild-type FLT3 is related to their ability to inhibit tyrosine kinases other than FLT3. Further trials are needed to confirm this hypothesis. Also, this trial did not further stratify patients into FLT3-ITD and FLT3-TKD groups, which are known to have different prognosis [59].

In a phase IIb trial 35 patients with mutated FLT3 were compared with 60 patients with wild-type AML that were randomized to get midostaurin 50 or 100 mg twice daily. The study results showed that midostaurin has hematologic activity in patients with mutant FLT3 as well as those with wild-type FLT3. Currently, a randomized multicenter phase III study of conventional induction chemotherapy with placebo or midostaurin followed by consolidation chemotherapy with either placebo or midostaurin is underway in newly diagnosed patients with *FLT3*-ITD AML (NCT00651261) [26, 60, 61].

2. ***Lestaurtinib (CEP-701)*** is an indolocarbazole derivative that inhibits FLT3 in vitro and in vivo. It is one of the most studied FLT3 inhibitors in clinical trials. It is a non-selective FLT3 inhibitor as it also inhibits other RTKs such as rearranged-during-transfection (RET), KIT, PDGF-R, JAK2, and tropomyosin-related kinase (TRK) at clinically significant concentrations. A phase I/II trial of seventeen patients with relapsed/refractory AML with mutant *FLT3* showed that four patients had significant decrease in peripheral myeloblasts, while one patient had reduction of BM blasts to less than 5%. CEP-701 was tested in phase II trial in both wild type and mutant *FLT3* in older patients who were considered ineligible for conventional chemotherapy. Promising activity was seen in 60% of mutant- and 23% of wild type-*FLT3* patients. It is not clear whether the clinical activity of lestaurtinib in wild-type *FLT3* AML patients is due to possible overexpression of FLT3 or due to the promiscuity of lestaurtinib for other RTK. Data also suggested that in all the patients that responded to lestaurtinib, the phosphorylation of FLT3 was constitutively suppressed to less than 15% of the baseline and the blasts from these patients were sensitive to FLT3 inhibition in vitro.

In a phase II trial 224 patients with *FLT3*-mutant AML in the first relapse were treated with chemotherapy alone or chemotherapy plus lestaurtinib. No statistically significant difference in OS was observed between the two arms. In the lestaurtinib arm, FLT3 inhibition was highly correlated with remission rate, but target inhibition on day 15 was achieved in only 58% of patients receiving lestaurtinib. It was concluded that lestaurtinib treatment after chemotherapy did not increase response rates or PFS in the studied patients. It would be of interest to note that simultaneous pharmacokinetic and molecular studies revealed that unlike the first study, only a minority of patients had achieved greater than 85% inhibition of FLT3 [7, 62, 63]. Based on these results, a Cardiff University sponsored trial (AML17: A programme of treatment development in younger patients with Acute Myeloid Leukaemia and high risk myelodysplastic syndrome) is underway.

3. ***Sunitinib (SU11248)***: is a TKI used to treat gastrointestinal stromal tumors (GIST), advanced renal cell carcinoma (RCC), or pancreatic neuroendocrine

tumors (pNET). It has activity against mutant FLT3 in vitro but also inhibits VEGFR1, VEGFR2, VEGFR3, PDGFR1, PDGFR2, c-KIT as well as RET. In vitro studies showed that sunitinib had synergistic inhibitory effects on FLT3-dependent leukemic cell proliferation combined with enhanced apoptotic effects when combined with cytarabine or daunorubicin. These effects were limited to primary AML myeloblasts expressing mutant *FLT3*-ITD but not in the wild-type cells [64]. These studies led to multiple clinical trials evaluating the role of sunitinib as a monotherapy or in combination with conventional chemotherapy.

In a phase I study with small number of patients, addition of sunitinib was evaluated as a single agent in patients with refractory AML. All the patients with *FLT3* mutations had morphologic or partial responses while only two of ten evaluable patients with wild-type *FLT3* had response to the treatment. Even in those with mutated *FLT3*, responses were of short duration.

In a combined phase I/II trial, sunitinib was evaluated in conjunction with standard chemotherapy for elderly patients (age >60 years). Of the twelve patients evaluated eight had *FLT3*-ITD four patients had *FLT3*-TKD mutation. Monotherapy with sunitinib induced partial remissions of short duration in AML patients. A phase I/II study evaluating addition of sunitinib to induction and consolidation therapy showed a response rate of 70% CR/CRi [65, 66].

4. ***KW-2449*** is an orally bioavailable multikinase inhibitor of FLT3, ABL, ABL-T315I, and Aurora kinase. KW-2449 was investigated in patients with AML. While the drug had promising pre-clinical data, in vivo half-life of the drug was very short requiring frequent dosing. Hence, the trial was terminated earlier due to suboptimal dosing schedule (NCT00779480). In another trial, KW-2449 was evaluated in patients with refractory/relapsed AML. A wide range of KW-2449 dosing was tested (25–500 mg/day) for 2–3 weeks. Of the 31 AML patients tested, eight showed 50% reduction in peripheral and/or BM blasts at the end of the first cycle. An AML patient treated with the maximum dose exhibited >50% decrease in peripheral blasts, increased platelets, and ANC, and decreased WBC count. However, there were no CR or PR observed [67].
5. ***Tandutinib (CT53518, MLN518)*** is a relatively selective inhibitor of FLT3, though it also inhibits other TK including c-KIT and PDGF-R at higher concentrations. A phase I trial in patients with relapsed/refractory AML and high-risk MDS showed that a significant number of patients had dose-limiting toxicities (DLT). Two of the eight evaluated patients with *FLT3*-ITD AML experienced transient decreases in blast percentage in periphery and BM, while no antileukemic effects were noted in patients with wild-type *FLT3*. In vitro studies with *FLT3*-ITD leukemic samples show that tandutinib has synergistic antileukemic activity with daunorubincin and cytarabine. A phase II trial of tandutinib was planned in patients with newly diagnosed AML who were either unfit or unwilling to receive standard chemotherapy; however, this study has been withdrawn (NCT00064584, NCT00274248, NCT00297921). Overall, it appears that tandutinib is a low potency agent with unfavorable pharmacokinetics and hence, is unlikely to be an agent of significant clinical promise [17, 23, 26].

6. ***Sorafenib (BAY 43–9006)*** is a multitarget kinase inhibitor currently approved for treatment of advanced RCC, unresectable hepatocellular carcinoma, and pNET. Given its ability to inhibit multiple RTK including FLT3, it has been extensively studied in AML either as a monotherapy or in combination with conventional regimens. As a monotherapy, sorafenib was studied in phase I trials with brief decline in BM blasts in relapse/refractory AML. In a phase I/II trial with newly diagnosed AML patients, sorafenib was added to conventional induction regimen leading to 75% CR, with especially excellent response in patients with *FLT3*-ITD. However, in a recently presented update by a European group investigating conventional chemotherapy with or without sorafenib in elderly patients, did not observe any benefit with sorafenib in terms of achieving CR, EFS, or OS—even in those with mutant-*FLT3* [68–71]. It is thought that resistance to sorafenib develops in most AML patients with ITD mutations during prolonged therapy and leads to relapse. A recent study showed that acquired point mutations in the TKD1 and TKD2 domains of the *FLT3* play a crucial role in resistance by upregulating levels of activated FLT3. Constitutively activated FLT3 then carries out downstream signaling resulting in resistant phenotype [72].

Mammalian target-of-rapamycin (mTOR) signaling. Tipifarnib, a farnesyl transferase inhibitor (FTI), inhibits mTOR signaling enhancing antiproliferative effects of chemotherapy which is thought to be the basis of its efficacy in treating patients with AML. A multicenter phase II clinical trial was performed to assess the effect of tipifarnib in combination with etoposide (T+E) in elderly AML patients with unfavorable prognostic factors (elderly age, adverse cytogenetics or secondary AML) who were not considered fit for conventional chemotherapy. Of the 84 patients receiving T+E, 25% achieved CR, with a median duration of CR being 9.8 months. Patients achieving CR had a median age of 77 years and their median survival was 22 months, with 14 (67%) surviving more than 1 year. These data are similar to results achievable after conventional chemotherapy [73, 74]

Demethylating Agents and Histone Deacetylase Inhibitors (HDACi)

1. ***Belinostat (PXD101)***: Belinostat is a hydroxymate-type histone deacetylase inhibitor (HDACi) that is being studied in AML either alone or in combination with conventional chemotherapy. In an ongoing open-label, non-randomized, multi-centre, phase I/II trial, 22 patients with AML were evaluated. The combination of idarubicin and belinostat resulted in CRi after one cycle in a patient with secondary AML and after three cycles in another patient with secondary AML. It also resulted in CR after one cycle in one *de novo* AML patient. Clinical efficacy of single agent belinostat was seen in seen a patient with secondary AML who achieved CRi after two cycles [75, 76]. An expanded phase II trial evaluating the role of PXD101 in either elderly patients or in those with relapsed/refractory AML is currently underway (NCT00357032).

2. ***Panobinostat (LBH-5789)***: Panobinostat is a pan-deacetylase inhibitor that causes hyperacetylation of lysine residues on both histone and non-histone targets. Panobinostat is available both in oral and intravenous forms. Panobinostat has potent apoptotic activity against AML cells lines and primary AML cells in vitro. Studies showed that panobinostat also potentiated the action of several chemotherapeutic compounds and induced apoptosis by intrinsic and extrinsic pathways. In addition, panobinostat also inhibits CXCR4 expression. With promising reports as apoptosis inducer in AML cell lines, there are multiple phase I/II studies evaluating the effect of panobinostat either as a single agent or in combination with various chemotherapeutic agents [77, 78].

Inhibitors of Other Kinases

1. ***KX2-391***: KX2-391 is a Src-kinase inhibitor with excellent oral bioavailability that is being evaluated in patients with AML. KX2-391 is unique among small molecule kinase inhibitors in that it binds to peptide substrate-binding site and not the ATP-binding site and hence acts as a non-ATP competitive inhibitor. KX2-391 is the first peptide site targeted tyrosine kinase inhibitor to enter clinical trials. A phase Ib clinical trial is underway to evaluate safety, tolerability and activity of KX2-391 in elderly AML patients who were refractory to or were not suitable for conventional chemotherapy (NCT01397799).
2. ***TL32711***: is a small molecule peptidomimetic of second mitochondria-derived activator of caspase (Smac) that specifically antagonizes multiple inhibitors of apoptosis proteins (IAP), resulting in inhibition of nuclear factor (NF)-κB signaling and caspase-dependent apoptosis of tumor cells. In Phase 1 clinical studies as a single agent and in combination with standard-of-care chemotherapies, TL32711 demonstrated strong correlation between drug exposure, target coverage and apoptosis induction in tumors at well-tolerated doses as well as promising anti-tumor activity in patients [79]. TL32711 is currently being evaluated in phase I/II clinical study for patients with AML (NCT01486784).

Proteosome Inhibitors

1. ***Bortezomib***: In vitro studies have shown synergy between bortezomib (proteasome inhibitor) and belinostat (HDACi). It is likely that the combination works by Akt/NF-κB inhibition; downregulation of Bcl-xL and XIAP; and upregulation of the pro-apoptotic Bcl-2 family member protein Bim. A phase I trial evaluating the combination of bortezomib and belinostat in patients with relapsed/refractory AML showed the combination is well tolerated [76]. In a study published recently, the combination of panobinostat and bortezomib was shown to exert a synergistic effect in AML cells. The combination also enhanced the sensitivity of AML cells to doxorubicin [80].

2. ***MLN4924***: In the ubiquitin proteosome system (UPS), the E3 ligases are multiprotein complexes. Nedd8 conjugation pathway controls the activity of the cullin-dependent E3 ubiquitin ligases, which in turn, are essential in regulating ubiquitination and subsequent degradation of downstream targets that have myriad roles in cell cycle progression, DNA repair and cellular proliferation. The Nedd8 activating enzyme (NAE) acts as a regulator of this Nedd8 conjugation pathway. Anti-leukemic activity of MLN4924—a small molecule inhibitor of the Nedd8 activating enzyme—has been tested in preclinical models of AML. MLN4924 induces DNA damage followed by rapid and selective caspase-dependent apoptosis in AML cells. MLN4924 also synergizes with cytarabine in decreasing cellular viability, inhibiting survival, and inducing mitochondrial-dependent apoptosis in AML cells but not in control cells. These results indicate that MLN4924 is a promising agent in treatment for patients with AML [24, 81, 82].

Vascular Endothelial Growth Factor (VEGF) Inhibitors. At diagnosis, BM biopsies from AML patients have different microvascular densities that correlate with VEGFA expression. Three distinct patterns have been described—(1) 'low vessel count'; (2) 'angiogenic sprouting'; and (3) 'vessel hyperplasia'. The patients with 'angiogenic sprouting' and 'low vessel count' have decreased EFS as compared to those with 'vessel hyperplasia'. Multivariate analysis has shown that vessel morphology is an independent prognostic indicator. Patients with 'angiogenic sprouting' profile are known to have unfavorable prognosis. It is thought that inefficient drug delivery by leukemia-associated vasculature may mediate resistance to chemotherapy and the addition of VEGF inhibitors may be result in better delivery and decreased clearance of chemotherapeutic agents leading to enhanced antileukemic effect [45, 83].

1. ***Cediranib (AZD2171)***: is an orally bioavailable inhibitor of VEGFR signaling. It binds to VEGFR-1 and -2 inhibiting growth and triggering apoptosis in leukemic cells. A phase II study to evaluate the efficacy of AZD2171 in AML evaluated a total of 23 patients. The blast count was reduced in all four patients taking the higher dose and three out of eight patients taking the lower dose. It was seen that lower dose of cediranib (30 mg daily) was better tolerated. While percentage blast in the BM decreased in some patients, due to overall poor efficacy in AML, cediranib has not been tested further as a single agent [66, 84].
2. ***Bevacizumab***: Given the role of endothelial cells in leukemic proliferations, it was hypothesized that treatment with VEGF inhibitor could be beneficial in patients with AML. It has been shown that in MDS patients, treatment with bevacizumab reduces plasma levels of VEGF [85]. In an earlier study, nine AML patients relapsed/refractory AML were treated with bevacizumab. None of the patients fulfilled the criteria of PR even though the expression of VEGF was significantly decreased during treatment. It was concluded that bevacizumab, as a single agent, did not have any significant clinical efficacy in these patients. However, in elderly patients with AML the role of bevacizumab in combination with standard chemotherapy was evaluated in a recent study. The treatment with

bevacizumab significantly increased EFS and tended to be associated with a beneficial OS for patients displaying 'low vessel count' profile. EFS and OS were not increased in patients with 'angiogenic sprouting' or 'vessel hyperplasia' suggesting that AML patients with 'low vessel count' might be good candidates for bevacizumab. Further studies are needed to determine if vascular density studies are indicated at the time of diagnosis in AML and if those with 'low-vessel count' should be routinely treated with additional VEGF inhibitors [83, 86]. HOVON81 study is a multicenter phase II trial that is evaluating prognostic importance of vascular patterns in BM and the role of addition of bevacizumab to standard chemotherapy regimen in elderly AML patients.

3. ***Aflibercept***: is a decoy fusion protein of domain 2 of VEGFR-1 and domain 3 of VEGFR-2 with the Fc fragment of IgG1 that binds to VEGF-A, VEGF-B, and placental growth factor (PlGF) with high affinity. It binds to VEGF with higher affinity than bevacizumab. The efficacy of aflibercept as a single agent and in combination with doxorubicin has been evaluated in AML cell lines and in mouse models. Aflibercept inhibited cell growth in vitro and in mouse model. Aflibercept also enhanced the antitumor effects of doxorubicin likely by increasing its concentration by at least twofold in BM and decreasing its clearance. [45]. Based on these results, trial evaluating aflibercept in MDS was conducted and a trial evaluating the efficacy of aflibercept is planned.

Wilm's tumor (WT)-1 and PR1. WT1 is zinc finger transcription factor that is overexpressed in AML cells. WT1 antigen specific cytotoxic T-lymphocytes (CTL) have been generated and used. It was shown that WT1-CTLs selectively targeted leukemic cells while sparing normal CD34+ HSCs. Although numerous HLA class-I and -II WT1 peptides have been identified, three HLA class I nonamer peptides: the HLA-A0201-restricted peptide, the HLA-A2402-restricted peptides, and its modified version are most commonly used in clinical trials. A reduction in blast number was reported in the leukemia patients receiving this vaccine. The WT1 HLA-A0201 peptide was also demonstrated to be safe and efficacious when administered to patients with AML with minimal toxicity and promising efficacy. A phase II clinical trial in seventeen patients with AML resulted in stable disease in twelve patients and complete remission in one. Molecular analysis was more promising and showed a reduction in WT1 mRNA levels (a surrogate tumor marker in AML) in about a third of patients [42].

PR1 is a HLA-A2-restricted nonameric peptide antigen derived from the proteinase-3 (P3) and neutrophil elastase (NE). Normally, NE and P3 are relatively restricted in early stages of hematopoiesis. Both NE and P3 are expressed in AML cells. Hence, targeting PR1-expressing cells would selectively target leukemic cells. PR1-specific CTLs have shown efficacy in patients with AML in phase II trials (see Table 1). Eight patients with myeloid malignancies were vaccinated with PR1 and WT1. Antigen-specific CTLs and correlative decrease in WT1 mRNA were observed in all the patients after a single vaccination [43].

Competitive CXCR4 inhibitor (AMD3100, Plerixafor). AMD3100 is a competitive antagonist of CXCR4. The treatment of leukemic mice with chemotherapy plus

AMD3100 resulted in decreased tumor burden and improved OS compared to mice treated with chemotherapy alone providing a proof-of-concept for role of CXCR4 inhibitors in treatment of AML [35].

Molecular Mechanisms of All Trans-Retinoic Acid (ATRA) and Arsenic Trioxide (ATO)

As mentioned above, the unique molecular feature of APL is fusion oncoprotein PML-RARα. Retinoic acid is the ligand for retinoic acid receptor or RAR. In the absence of retinoic acid, RARα heterodimerizes with retinoic X receptor-α (RXRα) and mediates transcriptional repression resulting in blockage of differentiation and augmentation of self-renewal pathways. In the presence of retinoic acid, RARα/RXRα complex is dissociated from repressive machinery resulting in relief from transcriptional repression. This ultimately leads to differentiation and maturation of leukemic cells into mature myeloid cells inducing remission.

ATO as well is known to induce similar effects on leukemic cells. ATO acts by causing PML and PML-RARα (but not wild-type RARα) to be tagged by small ubiquitin-like modifier (SUMO) in a process known as SUMOylation. Once SUMOylated, protein undergoes ubiquitination and ultimately proteosomal degradation. Thus, ATO ultimately hastens degradation of PML-RARα resulting in differentiation and maturation of promyelocytic cells [3, 11].

ATRA acts on RARα subunit of PML-RARα oncoprotein, while ATO acts upon PML subunit. This double-pronged approach explains the synergistic action exerted by the combination of these two agents.

AML and the Cancer Stem Cell Paradigm

The origin and propagation of cancer cells have always puzzled scientists. A question of great importance to both scientists and clinicians is whether all tumor cells are equal. The stochastic model predicts that the properties of proliferation and differentiation would be present in every cell fraction and hence there would not be hierarchy of the cells within tumor mass. In 1930s, it was shown that a single leukemic cell transplanted into mice can cause leukemia—leading to an alternative model suggesting that some or even few cells being responsible for 'entire' tumor burden. In later years, with refinement of the stem cell concept (including hematopoietic stem cell), the theory was proposed that not all cancer cells are created equal. Cancer stem cells (CSC) were proposed to be a small fraction of cells within the tumor mass that were responsible for proliferation. While the idea of CSC gathered steam in 1960s, no concrete proof existed until recently.

It was in AML that not only CSC paradigm was validated, but also the phenotype of CSC was shown. CSC in AML is called leukemic stem cell (LSC) to differentiate it from the normal hematopoietic stem cell (HSC). In AML, the initial cell transforming into LSC is derived from HSCs. LSCs are able to differentiate and proliferate, and have the capacity of self-renewal in vivo—an essential characteristic of any stem cell. Further work in AML proved that just like the hierarchy of normal hematopoiesis, there exists a hierarchy among leukemic cells. LSCs divide at different speed and may undergo self-renewal rather than commitment. LSCs are now broadly divided into three classes from the most 'long-term' and stem-like to the least long-term and stem-like: quiescent LSC, long-term LSC, and short-term LSC.

In an elegant study done by Pollard et al., it was seen that leukemia in which *FLT3*-ITD mutations were found in $CD34^{+}/CD33^{-}$progenitor cells had a higher risk of relapse and an overall inferior prognosis compared to those when *FLT3*-ITD was detectable in more mature phenotype defined by $CD34^{+}/CD33^{+}$ cells [87]. An important and interesting question that arises is at what stage of leukemogenesis does FLT3 mutations occur? In 84% of patients with *FLT3* mutated AML, *FLT3*-ITD was present at the time of relapse. Interestingly, in *FLT3*-TKD mutations, *FLT3* mutation persisted in less than half of the cases. These data suggest that while *FLT3*-ITD mutation is acquired by LSCs in most cases, *FLT3*-TKD mutation may be accrued either by LSCs or by a subclone [25, 88].

These new findings have potential impact on the way therapeutic targets of the future will be designed (Fig. 1). Most of the chemotherapeutics agents act upon dividing cells. Since the LSCs, especially the quiescent LSCs, divide rather infrequently, chemotherapeutic agents are only minimally toxic to these cells. One can also infer that it is the presence of these residual LSCs that drive relapse of the disease. Hence, in a very strict sense, most traditional chemotherapeutic agents are very efficient at decreasing the burden of tumor, but do not result in eradication of the disease or 'cure'. Since the phenotype of LSCs is well known in AML, it is hoped that targeted therapeutics against these LSCs will be successful in eradicating the disease with minimal toxicity. It is possible that CD33 antibodies are unable to 'cure' leukemia as they do not target CD34+/CD33− progenitor cells that are higher in the hierarchy of LSCs.

Summary

AML is one of the most common leukemias of adults. AML is a subject of myriad research. A likely explanation for such popularity of AML as a model for cancer is the prevalence of the disease, ease of obtaining samples, and the ability to achieve high purity of the cancer cells free of 'contaminant' normal cells. AML has been at the forefront of many paradigms in cancer biology such as cancer stem cells, whole genome sequencing. Hence, the developments in AML represent advances in the fields of cancer, targeted therapeutics, and stem cell biology.

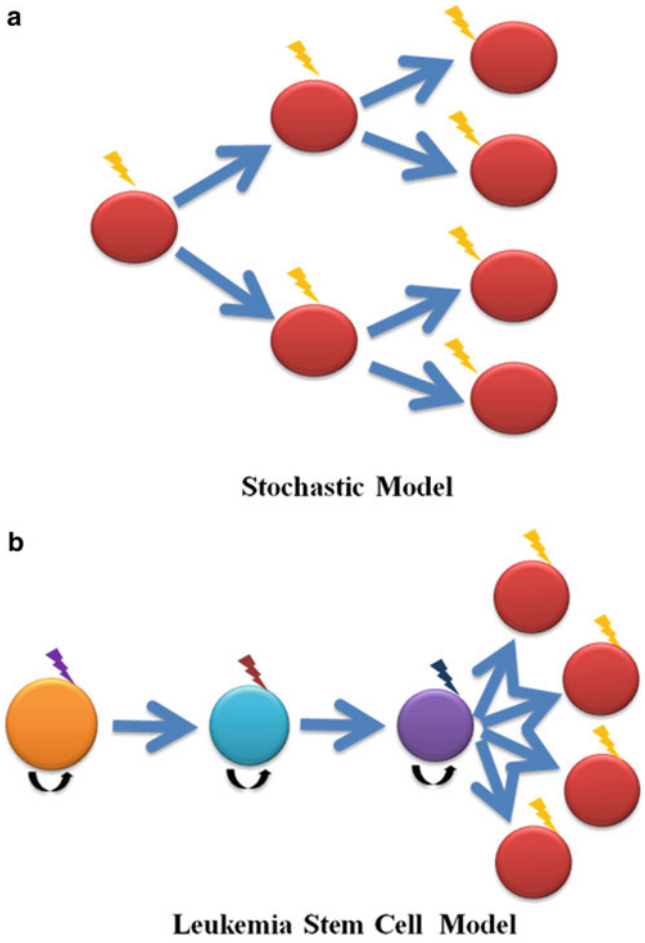

Fig. 1 Two models of leukemogenesis and its effects of treatment. (**a**) The stochastic model predicts that all the leukemic cells are equal. Hence, the effect of chemotherapy is equal on all the tumor cells. This therapy is supported by the fact that current chemotherapy is able to 'cure' at least some leukemias. (**b**) The leukemia stem cell (LSC) model predicts that just like normal HSCs, the LSCs also have a hierarchy and that the conventional chemotherapy only targets the most differentiated of the tumor cells. LSCs, by their virtue of prolonged hibernation and the capacity of self-renewal, are inherently resistant to conventional chemotherapy. This model predicts that in order to ensure cure a two-pronged approach is warranted—(1) chemotherapy to reduce tumor burden; and (2) the targeted therapy against LSCs.

Recent research has uncovered incredible heterogeneity in the biology of AML. CN-AML, once thought to be a single entity, is now further characterized by multiple genotypes and phenotypes of clinical importance. While incredible progress has been made in the field of diagnosis, molecular classification, and prognosis of AML, unfortunately, the therapeutic progress lags behind. For example, the outcome of older patients (age >60 years) with AML has not improved in the

last three decades. This is particularly troublesome since as mentioned above the prevalence of AML strikingly rises with age. Elderly people usually have significant co-morbidities, more complex cytogenetics, and higher expression of MDR1.

Targeted therapeutics have an inherent advantage of maximizing efficacy while minimizing toxicities. Many such modalities have shown promise in pre-clinical and early clinical research. However, it has been difficult to translate this progress into successful treatment strategies for patients with AML. A large impetus of translational research is mandated in this field to achieve this important goal.

References

1. Fernandez HF. New trends in the standard of care for initial therapy of acute myeloid leukemia. Hematol Am Soc Hematol Educ Prog. 2010;1:56–61.
2. Löwenberg B, et al. Cytarabine dose for acute myeloid leukemia. N Engl J Med. 2011;364(11):1027–36.
3. Niu C, et al. Studies on treatment of acute promyelocytic leukemia with arsenic trioxide: remission induction, follow-up, and molecular monitoring in 11 newly diagnosed and 47 relapsed acute promyelocytic leukemia patients. Blood. 1999;94(10):3315–24.
4. Park JH, et al. Early death rate in acute promyelocytic leukemia remains high despite all-trans retinoic acid. Blood. 2011;118(5):1248–54.
5. Park JH, Tallman MS. Managing acute promyelocytic leukemia without conventional chemotherapy: is it possible? Expert Rev Hematol. 2011;4(4):427–36.
6. Licht JD. Acute promyelocytic leukemia—weapons of mass differentiation. N Engl J Med. 2009;360(9):928–30.
7. Burnett AK, Knapper S. Targeting treatment in AML. Hematol Am Soc Hematol Educ Program. 2007;1:429–34.
8. Patel JP, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. N Engl J Med. 2012;366(12):1079–89.
9. Mayer RJ, et al. Intensive postremission chemotherapy in adults with acute myeloid leukemia. Cancer and Leukemia Group B. N Engl J Med. 1994;331(14):896–903.
10. Koreth J, et al. Allogeneic stem cell transplantation for acute myeloid leukemia in first complete remission. JAMA: J Am Med Assoc. 2009;301(22):2349–61.
11. Petrie K, Zelent A. Marked for death. Nat Cell Biol. 2008;10(5):507–9.
12. Sanz MA, et al. Management of acute promyelocytic leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. Blood. 2009;113(9):1875–91.
13. Nasr R, et al. Eradication of acute promyelocytic leukemia-initiating cells through PML-RARA degradation. Nat Med. 2008;14(12):1333–42.
14. Park JH, Tallman MS. Treatment of acute promyelocytic leukemia without cytotoxic chemotherapy. Oncology (Williston Park). 2011;25(8):733–41.
15. Mathews V, et al. Single-agent arsenic trioxide in the treatment of newly diagnosed acute promyelocytic leukemia: durable remissions with minimal toxicity. Blood. 2006; 107(7):2627–32.
16. Foran JM. New prognostic markers in acute myeloid leukemia: perspective from the clinic. ASH Educ Prog Book. 2010;2010(1):47–55.
17. Döhner H, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood. 2010;115(3):453–74.
18. Erba HP. Prognostic factors in elderly patients with AML and the implications for treatment. Hematol Am Soc Hematol Educ Program. 2007;1:420–8.

19. Schlenk RF, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. N Engl J Med. 2008;358(18):1909–18.
20. Shen Y, et al. Gene mutation patterns and their prognostic impact in a cohort of 1185 patients with acute myeloid leukemia. Blood. 2011;118(20):5593–603.
21. Takahashi S. Downstream molecular pathways of FLT3 in the pathogenesis of acute myeloid leukemia: biology and therapeutic implications. J Hematol Oncol. 2011;4(1):13.
22. Sallmyr A, et al. Internal tandem duplication of FLT3 (FLT3/ITD) induces increased ROS production, DNA damage, and misrepair: implications for poor prognosis in AML. Blood. 2008;111(6):3173–82.
23. Pemmaraju N, et al. FLT3 inhibitors in the treatment of acute myeloid leukemia. Cancer. 2011;117(15):3293–304.
24. Soucy TA, et al. An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. Nature. 2009;458(7239):732–6.
25. Small D. FLT3 mutations: biology and treatment. ASH Educ Prog Book. 2006;1:178–84.
26. Fathi AT, Chabner BA. FLT3 inhibition as therapy in acute myeloid leukemia: a record of trials and tribulations. Oncologist. 2011;16(8):1162–74.
27. Ley TJ, et al. DNMT3A mutations in acute myeloid leukemia. N Engl J Med. 2010;363(25):2424–33.
28. Marie J, Lundberg AS, Ajami A. Effect of the presence of P-glycoprotein (MDR1) on the ability of AML patients to achieve complete remission: results of a meta-analysis of the literature in Journal of Clinical Oncology. ASCO Annual Meeting Proceedings (Post-Meeting Edition), 2010.
29. Marcucci G, et al. IDH1 and IDH2 gene mutations identify novel molecular subsets within de novo cytogenetically normal acute myeloid leukemia: a cancer and leukemia group b study. J Clin Oncol. 2010;28(14):2348–55.
30. Lu C, et al. IDH mutation impairs histone demethylation and results in a block to cell differentiation. Nature. 2012;483:474–8. Advance online publication.
31. Metzeler KH, et al. TET2 mutations improve the new European leukemianet risk classification of acute myeloid leukemia: a cancer and leukemia group b study. J Clin Oncol. 2011;29(10):1373–81.
32. Dombret H, Preudhomme C, Boissel N. Core binding factor acute myeloid leukemia (CBF-AML): is high-dose Ara-C (HDAC) consolidation as effective as you think? Curr Opin Hematol. 2009;16(2):92–7. doi:10.1097/MOH.0b013e3283257b18.
33. Klein R, Marcucci G. Familial acute myeloid leukemia (AML) with mutated CEBPA. In Pagon R, Bird T, Dolan CR editors. GeneReviews [Internet].Seattle: University of Washington; 2010.
34. Spoo AC, et al. CXCR4 is a prognostic marker in acute myelogenous leukemia. Blood. 2007;109(2):786–91.
35. Nervi B, et al. Chemosensitization of acute myeloid leukemia (AML) following mobilization by the CXCR4 antagonist AMD3100. Blood. 2009;113(24):6206–14.
36. Meltzer PS. Cancer genomics: small RNAs with big impacts. Nature. 2005;435(7043):745–6.
37. Marcucci G, et al. MicroRNA expression in cytogenetically normal acute myeloid leukemia. N Engl J Med. 2008;358(18):1919–28.
38. Mardis ER, et al. Recurring mutations found by sequencing an acute myeloid leukemia genome. N Engl J Med. 2009;361(11):1058–66.
39. Ding L, et al. Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. Nature. 2012;481(7382):506–10.
40. Walter MJ, et al. Clonal architecture of secondary acute myeloid leukemia. N Engl J Med. 2012;366(12):1090–8.
41. Clinical Trials. 2012. http://clinicaltrials.gov/. Accessed 12 Feb 2012.
42. Alatrash G, Molldrem JJ. Vaccines as consolidation therapy for myeloid leukemia. Exp Rev Hematol. 2011;4(1):37–50.

43. Rezvani K, 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.
44. Klisovic RB, et al. Phase I study of GTI-2040, an antisense to ribonucleotide reductase, in combination with high-dose cytarabine in patients with acute myeloid leukemia. Clin Cancer Res. 2008;14(12):3889–95.
45. Lal D, et al. Aflibercept exerts antivascular effects and enhances levels of anthracycline chemotherapy in vivo in human acute myeloid leukemia models. Mol Cancer Ther. 2010;9:2737–51.
46. Juckett M, et al. Phase II study of AZD2171 for the treatment of patients with acute myelogenous leukemia, in ASCO Annual Meeting 2011; American Society of Clinical Oncology.
47. Cortes JE, et al. Safety, pharmacokinetics, and efficacy of BP-100.1.01 (L-Grb-2 antisense oligonucleotide) in patients with refractory or relapsed acute myeloid leukemia (AML), Philadelphia chromosome positive chronic myelogenous leukemia (CML), acute lymphoblastic leukemia (ALL), and myelodysplastic syndrome (MDS). ASH Annu Meeting Abstr. 2011;118(21):3639.
48. Moore JO, et al. Phase 2 study of Oblimersen sodium (G3139; Bcl-2 antisense; genasense(R)) plus gemtuzumab ozogamcin (Mylotarg(R)) in elderly patients with relapsed acute myeloid leukemia (AML). ASH Annu Meeting Abstr. 2004;104(11):865.
49. Kolitz JE, et al. P-glycoprotein inhibition using valspodar (PSC-833) does not improve outcomes for patients younger than age 60 years with newly diagnosed acute myeloid leukemia: cancer and leukemia group b study 19808. Blood. 2010;116(9):1413–21.
50. Raponi M, et al. A 2-gene classifier for predicting response to the farnesyltransferase inhibitor tipifarnib in acute myeloid leukemia. Blood. 2008;111(5):2589–96.
51. Cripe LD, et al. Zosuquidar, a novel modulator of P-glycoprotein, does not improve the outcome of older patients with newly diagnosed acute myeloid leukemia: a randomized, placebo-controlled trial of the Eastern Cooperative Oncology Group 3999. Blood. 2010;116(20):4077–85.
52. Garcia-Manero G, et al. Phase II study of vorinostat in combination with idarubicin (Ida) and cytarabine (ara-C) as front line therapy in acute myelogenous leukemia (AML) or higher risk myelodysplastic syndrome (MDS). ASH Annu Meeting Abstr. 2009;114(22):1055.
53. Castaigne S, et al. Fractionated doses of gemtuzumab ozogamicin (GO) combined to standard chemotherapy (CT) improve event-free and overall survival in newly-diagnosed de novo aml patients aged 50–70 years old. A prospective randomized phase 3 trial from the Acute Leukemia French Association (ALFA). ASH Ann Meeting Abstr. 2011;118(21):6.
54. Delaunay J, et al. Addition of gemtuzumab ozogamycin to chemotherapy improves event-free survival but not overall survival of AML patients with intermediate cytogenetics not eligible for allogeneic transplantation. Results of the GOELAMS AML 2006 IR Study. ASH Annu Meet Abstr. 2011;118(21):79.
55. Feldman EJ, et al. Phase III randomized multicenter study of a humanized anti-CD33 monoclonal antibody, lintuzumab, in combination with chemotherapy, versus chemotherapy alone in patients with refractory or first-relapsed acute myeloid leukemia. J Clin Oncol. 2005;23(18):4110–6.
56. Lancet JE, et al. Lintuzumab and low-dose cytarabine compared to placebo and low-dose cytarabine in patients with untreated acute myeloid leukemia (AML) 60 years and older: results of a randomized, double-blinded phase 2b study. ASH Ann Meeting Abstr. 2011;118(21):3613.
57. Rosenblat T, et al. Sequential cytarabine and alpha-particle immunotherapy with bismuth-213 (213Bi)-labeled-HuM195 (lintuzumab) for acute myeloid leukemia (AML). ASH Annu Meeting Abstr. 2008;112(11):2983.
58. Jurcic JG, et al. Phase I trial of the targeted alpha-particle nano-generator actinium-225 (225Ac)-lintuzumab (anti-CD33; HuM195) in acute myeloid leukemia (AML). ASH Annu Meeting Abstr. 2011;118(21):768.
59. Stone R, Fischer T, Paquette R. A phase 1b study of midostaurin (PKC412) in combination with daunorubicin and cytarabine induction and high-dose cytarabine consolidation in patients

under age 61 with newly diagnosed de novo acute myeloid leukemia: overall survival of patients whose blasts have FLT3 mutations is similar to those with wild-type FLT3., in 51st ASH Annual Meeting and Exposition New Orleans; 2009.
60. Stone RM, et al. A randomized phase III study of induction (daunorubicin/cytarabine) and consolidation (high-dose cytarabine) chemotherapy combined with midostaurin or placebo in treatment-naive patients with FLT3 mutated AML, in ASCO Annual Meeting, Chicago, p. TPS199; 2011.
61. Fischer T, et al. Phase IIB trial of oral midostaurin (PKC412), the FMS-like tyrosine kinase 3 receptor (FLT3) and multi-targeted kinase inhibitor, in patients with acute myeloid leukemia and high-risk myelodysplastic syndrome with either wild-type or mutated FLT3. J Clin Oncol. 2010;28(28):4339–45.
62. Knapper S, et al. A phase 2 trial of the FLT3 inhibitor lestaurtinib (CEP701) as first-line treatment for older patients with acute myeloid leukemia not considered fit for intensive chemotherapy. Blood. 2006;108(10):3262–70.
63. Levis M, et al. Results from a randomized trial of salvage chemotherapy followed by lestaurtinib for patients with FLT3 mutant AML in first relapse. Blood. 2011;117(12):3294–301.
64. Yee KWH, et al. Synergistic effect of SU11248 with cytarabine or daunorubicin on FLT3 ITD—positive leukemic cells. Blood. 2004;104(13):4202–9.
65. Fiedler W, et al. A phase 1 study of SU11248 in the treatment of patients with refractory or resistant acute myeloid leukemia (AML) or not amenable to conventional therapy for the disease. Blood. 2005;105(3):986–93.
66. Fiedler W, et al. A phase I/II study combining sunitinib with standard ara-C/daunorubicin chemotherapy in patients 60 years or older with FLT3 mutated AML. ASH Ann Meeting Abstr. 2010;116(21):3285.
67. Cortes J, et al. A phase I dose escalation study of KW-2449, an oral multi-kinase inhibitor against FLT3, Abl, FGFR1 and Aurora in patients with relapsed/refractory AML, ALL and MDS or resistant/intolerant CML. ASH Ann Meeting Abstr. 2008;112(11):2967.
68. Lee SH, et al. Complete resolution of leukemia cutis with sorafenib in an acute myeloid leukemia patient with FLT3-ITD mutation. Am J Hematol. 2009;84(10):701–2.
69. Safaian NN, et al. Sorafenib (Nexavar®) induces molecular remission and regression of extramedullary disease in a patient with FLT3-ITD+ acute myeloid leukemia. Leuk Res. 2009;33(2):348–50.
70. Ravandi F, et al. Phase I/II study of combination therapy with sorafenib, idarubicin, and cytarabine in younger patients with acute myeloid leukemia. J Clin Oncol. 2010;28(11):1856–62.
71. Serve H, et al. Sorafenib in combination with standard induction and consolidation therapy in elderly AML patients: results from a randomized, placebo-controlled phase II trial. ASH Annu Meeting Abstr. 2010;116(21):333.
72. Zhang W, et al. Acquired point mutations of TKD are responsible for sorafenib resistance in FLT3-ITD mutant AML. ASH Ann Meeting Abstr. 2011;118(21):3505.
73. Ferrara F. Tipifarnib and etoposide for older AML patients: from bench to bedside. Blood. 2009;113(20):4824–5.
74. Karp JE, et al. Phase II trial of tipifarnib as maintenance therapy in first complete remission in adults with acute myelogenous leukemia and poor-risk features. Clin Cancer Res. 2008;14(10):3077–82.
75. Schlenk R, et al. Interim results of a phase I/II clinical trial of belinostat in combination with idarubicin in patients with AML not suitable for standard intensive therapy. ASH Ann Meeting Abstr. 2008;112(11):1953.
76. Holkova B, et al. Phase I trial of belinostat and bortezomib in patients with relapsed or refractory acute leukemia, myelodysplastic syndrome, or chronic myelogenous leukemia in blast crisis. ASH Ann Meeting Abstr. 2011;118(21):2598.
77. Meng F, et al. Synergistic effect of panobinostat and bortezomib on chemoresistant acute myelogenous leukemia cells via Akt/NF-{kappa}B Pathway. ASH Annu Meeting Abstr. 2011;118(21):3621.
78. Tan PT, et al. Determination of the maximum tolerated dose of panobinostat in combination with a 5-day schedule of azacitidine in high-risk myelodysplastic syndrome and acute myeloid

leukemia: planned interim analysis of a phase Ib/II study. ASH Annu Meeting Abstr. 2011;118(21):1529.
79. Carter BZ, et al. Antagonizing IAPs by SMAC mimetic TL32711 induces apoptosis in AML cells including AML stem/progenitor cells alone and in combination with chemotherapy. ASH Annu Meeting Abstr. 2011; 118(21):66
80. Meng F, et al. Synergistic effect of panobinostat and bortezomib on chemoresistant acute myelogenous leukemia cells via Akt/NF-κB pathway, in 53rd American Society of Hematology Annual Meeting and Exposition, San Diego; 2011.
81. Swords RT, et al. MLN4924, a novel first in class small molecule inhibitor of the Nedd8 activating enzyme (NAE), has potent activity in preclinical models of acute myeloid leukemia. ASH Ann Meeting Abstr. 2009; 114(22):1021.
82. Nawrocki ST, et al. Disrupting NEDD8-mediated protein turnover with MLN4924 significantly augments the efficacy of cytarabine. ASH Annu Meeting Abstr. 2010; 116(21): 3255.
83. Weidenaar AC, et al. Patterns of bone marrow micro vessel morphology in AML and high risk MDS predict treatment outcome following intensive chemotherapy and bevacizumab. ASH Annu Meeting Abstr. 2011;118(21):1555.
84. Fiedler W, et al. An open, phase I study of cediranib in patients with acute myeloid leukemia (AML). ASH Annu Meeting Abstr. 2007;110(11):895.
85. Legros L, et al. Treatment of MDS with excess blasts by bevacizumab is well tolerated and is associated with a decrease of VEGF plasma level. ASH Ann Meeting Abstr. 2009;114(22):2770.
86. Zahiragic L, et al. Bevacizumab reduces VEGF expression in patients with relapsed and/or refractory acute myeloid leukaemia (AML) without clinical antileukemic activity. ASH Annu Meeting Abstr. 2006;108(11):4543.
87. Pollard JA, et al. FLT3 internal tandem duplication in CD34+/CD33-precursors predicts poor outcome in acute myeloid leukemia. Blood. 2006;108(8):2764–9.
88. Shih L-Y, et al. Heterogeneous patterns of FLT3 Asp835 mutations in relapsed de novo acute myeloid leukemia. Clin Cancer Res. 2004;10(4):1326–32.

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W.S. El-Deiry (ed.), *Impact of Genetic Targets on Cancer Therapy*, Advances in Experimental Medicine and Biology 779, DOI 10.1007/978-1-4614-6176-0,