Albrecht Reichle *Editor*

Evolution-adjusted Tumor Pathophysiology

The Novel Language of Tumor Biology

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Contents

Part I
Introduction

Chapter 1
Communication—Evolution—Pathophysiology: An Endogenous Conjunction—Instead of an Introduction

Albrecht Reichle

Abstract The communicative expression of participators in tumor systems, for example, different cell compartments, pathways, oncogenes, tumor suppressor genes, etc., results in the constitution of tumor-immanent normative notions, i.e. tumor-associated immune response, tumor metabolism, etc., and their respective ways of rationalization. The orientation towards more than one read-out 'system' for conceiving rationalizations of tumor-immanent normative notions allows the exemplary investigation of central questions of communication influenced by systems-immanent constraints. Communication processes concertedly express themselves in evolution histories, outcome reports, medical imaging, identification, and quantification of tumor-associated structures and functions, and—last but not least—in the situatively evaluated communicative expression of tumor systems participators. The central task of the current book is to critically scrutinize the automatic transfer of communicative expression—associated with a detectable tumor systems participator—from one tumor system to another. The formal-pragmatic communication theory and the evolution theory shall help find answers to the following critical questions: When can an identical communicative expression of systems participators within different histological or molecular-genetic tumor types (evolutionary-preserved communicative expression) be assumed, and which communicative circumstances are able to alter the communicative expression of identical systems participators in a therapy-relevant manner? Answers to these two questions are important because they may contribute to bridging medical theory and therapeutic practice. Such bridging efforts are embedded in an ethical framework, because therapeutical consequences may be delineated. Evolution-adjusted tumor pathophysiology presents the situatively evaluated constitution of rationalization processes for tumor-promoting normative notions, and, thus, a novel therapeutically accessible level for overcoming cytogenetically and molecular-genetically based tumor heterogeneity.

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Introduction

The clinical efficacy of combined modularized (biomodulatory) therapies for metastatic tumors together with observations of distinct cell compartments and their capacity to initiate tumor growth in heterologous cell types provide excellent opportunities to point to central communication problems among tumor systems participators.

For pragmatic purposes, communication primarily turns all tumor systems participators into abstract and scientifically assignable 'artifacts' that transmit information and concertedly constitute communicative expression of tumor systems via physically accessible tumor-immanent rationalization processes. Evolutionarily organized rationalization processes for shaping tumor-immanent normative notions provide functions, such as angiogenesis, inflammation, immune response, etc., but also decision maxims (hubs, nodes) and tumor-associated molecular or morphological structures.

In particular, the 'metabolism' of tumor evolution cannot be operationalized without the inclusion of stringent ideas on how biological communication processes are realized in developing tumors. Also theories on evolution-historical processes have to consider the fact that communication is the medium by which evolutionary processes are promoted, even if communication is assumed to be unidirectional in Darwinian 'selection processes' and selection aims at arbitrarily chosen normative notions.

The extensive and so far not systematically organized material on the novel tumor pathophysiology compelled to an exploratory approach, guided by a formal-pragmatic communication theory. The main goal of this theory is to provide instruments for uncovering the rules underlying the organization of the communicative expression of systems participators and the therapeutic modulation or redirection in steadily evolving biologic systems, such as tumors.

The communicative expression of tumor systems participators results in the constitution of tumor-immanent normative notions, i.e. tumor-associated immune response, tumor metabolism etc., and their respective ways of rationalization.

The orientation towards more than one read-out 'system' for conceiving rationalizations of tumor-immanent normativity allows the exemplary investigation of the central questions of communication influenced by systems-immanent constraints, such as evolutionarily restricted or diversified communication-derived rules, the multifaceted types of communication within tumor diseases, and evolutionarily based intersystemic exchange processes. In combination, these factors concertedly express themselves in evolution histories, out-come reports, medical (molecular) imaging, the identification and quantification of tumor-associated structures and functions, and—last but not least—in the situatively evaluated communicative expression of tumor systems participators provided by the systems context for generating tumor-immanent normative notions.

The contradictions between the phenomenology of an individual tumor disease and methodologically offered scientific explanations are uncovered by a more sharpened and diversified view on the single lines of traditions that prefer particular forms

of scientific perception for comprehending communicative expression of systems participators. The different perspectives are indicated by the selection of respective read-out systems.

Usually, we assume that functions of molecular-genetic and genetic aberrations in tumors may be simply added up or interconnected in a systems-biological manner without acknowledging their evolutionarily confined situative communicative expression in distinct systems stages. Upon closer examination, this 'modus operandi' is frequently less successful in explaining the natural history of a tumor disease. In daily clinical practice, theoretical explanations often prove to be contrary to the observed natural history of a tumor disease.

In tumors, systematic reconstructions of the communicative expression of situative systems participators may allow a better understanding of the seemingly irresolvable nexus between theory and practice.

Because the discussed results of the combined modularized therapies for metastatic tumors are not common knowledge, data on clinical trials including combined modularized therapies are required. Showing that the tradition of reductionist data interpretation is insufficient for explaining the concerted activity of drug components with poor or no single agent activity is rather simple. However, this conjuncture is not important, because the theoretical and practical methodological instruments discussed for reconstructive activities may be generalized and also used in a different biological context.

The point is: no refutation of reductionist-derived considerations and their application in an evolutionary context. Ideas for explaining communicative processes in biology and their principles of organization, exemplified by the two different pillars of evolution history and evolution theory, originate from rather diverse scientific disciplines. These ideas should be reconstructed and operationalized to constitute an evolution theory that explains the 'metabolism' of evolution.

Traditional evolution historical considerations may neither sufficiently explain the activity profiles of combined modularized therapies nor the clinical hints that such therapy approaches have the capacity to induce a therapeutically relevant biological memory for long-term tumor control; therefore, the focus inevitably turns to evolution theories. Simultaneously, the data show to what extent the scientific history of data interpretation is shaped by traditional patterns of recycling reductionist thinking. Such patterns remain in the collective memory to be transformed and adapted in due course to changing interests and specific therapeutic purposes. To some extent, such adaptations are 'distortions', because originators are unable to foresee how the after-world is going to deal with their conceptions. Vice versa, a novel conception for describing the 'metabolism' of evolution and its successful application in a formal-pragmatic communication theory may confirm the continuance and necessity of the original reductionist considerations.

It is in our interest to comprehend the therapeutically all important situative validity and denotation of systems participators within the evolutionarily developing novel contexts beyond the multifaceted communicative behavior of tumor systems participators. Here, the hermeneutic approach is used for highlighting ways how to systematically reconstruct rationalizations of tumor-immanent normative notions

for diagnostic and therapeutic purposes and for appointing the situative function of tumor systems participators within a concrete rationalization process that is assigned to propagate a distinct tumor-associated normative notion.

When we turn away from the myriads of historical descriptions of evolution and try to address ourselves to the theoretical considerations on evolution, i.e. to the 'metabolism' of evolution and the reconstruction and modulation of tumor-immanent normative notions, we reductionistically undermine the multifaceted evolution histories with the aim to comprehend communicative rules to which evolutionary processes adhere.

The process that initiates the constitution of an evolution theory requires the involvement of multifold scientific disciplines, whereas the theory itself should be self-explanatory. My own starting point to converge multifold scientific disciplines to evolution theory is internal medicine. Based on the diagnostic and therapeutic equipment of a physician, I tried to study the whole array of questions evolving between the conflicting priorities of medical theory and therapeutic practice and between evolution history and evolution theory. Most investigators may feel that these questions are best answered by consulting classic reductionistically working systems biology. The fact that, in the present case, a physician dares to investigate such questions is derived from systematic therapeutic experiences in combined communicative interactions with tumor-promoting systems participators. Examples are given in the current book: Tumor systems are accessible for communicative interactions by modulating and redirecting tumor-immanent normative notions.

The justification for the committed systematic transgression to other neighboring and supplementing scientific fields, including philosophy, lies in the nature of the investigated tumor systems objects and their inevitable integration and organization within tumor-immanent rationalization processes to constitute tumor-associated normative notions.

Therapeutically aligned communication with tumor systems does not only mean launching or interrupting information processes, but equally focuses on the communicative expression of tumor systems participators and the specified and individualized shaping of tumor-associated normative notions. Therefore, redirecting and modulating the communicative expression of systems participators may be a highly efficacious and clearly specifiable therapeutic goal. Furthermore, combined modularized therapies help to detect multifaceted and situatively adapted rationalization processes available for ubiquitously occurring tumor-immanent normative notions.

Artists and writers as well as their audience always have the privilege and proclivity to feel completely free from constraints imposed by single disciplines and highly sophisticated niches. The unconditional use of rather diverse scientific fields is required for uncovering the situative validity and denotation of tumor systems objects in an evolutionary context, i.e. the situative communicative expression of tumor systems objects, beyond the simple proof of 'artifacts', their identification, quantification, and suggestion of communicative expression 'per se'. Such uncovering contributes to the permanent evolution-immanent transformation of provable tumor systems objects to corresponding systems subjects that are characterized by

their systems-imposed, communication-derived situative validity and denotation as a display of their evolutionary-based communicative expression.

At that stage, the exclusively communication-related tumor systems subject with its situatively inherent communicative expression becomes the center of scientific interest.

The attempt of a multidisciplinary integrative approach—that exceeds boundaries drawn by multifold scientific niches that develop either unwarrantedly or rather justifiably—offers the opportunity to investigate the transformation processes between medical theory and therapeutic practice as well as between objects and systems subjects. Interdisciplinarity provides and organizes the methodological instruments for routinely reconstructing evolutionarily initiated transformations and for therapeutically using this novel information that is now summarized in evolution-adjusted tumor pathophysiology.

Multidisciplinarity is the adequate approach to converging the transformation problem. However, besides daily medical practice, multidisciplinarity includes communication theory, philosophy, molecular biology, systems biology, bioinformatics, genetics, etc. Thus, this approach may involve the risk of inadequately covering all neighboring scientific fields affecting evolution-adjusted tumor pathophysiology. In such cases, I would like to ask my readers to kindly overlook any oversights or inaccuracies.

This book is organized from 'bed side' to 'bench', corresponding to the 'historical' time line: (1) Combined modularized therapies for metastatic tumors are pointing to central problems of communication among 'systems participators' in tumors and may efficaciously address the therapeutic problems arising from genetically based tumor heterogeneity. (2) A communication theory provides the basis for explaining social engineering either endogenously within the natural evolutionary tumor process or by implementing non-normative boundary conditions with combined modularized therapies. (3) Observations from rather different disciplines are a prerequisite for reconstructing and operationalizing starting points for an evolution theory, and (4) for developing an evolution theory that is borne by evolution-adjusted tumor pathophysiology and that (5) aims at uncovering the 'metabolism' of evolution. As a direct consequence, tumor staging focuses on rationalization processes, the non-genetic counterpart of the genome. (6) Data on systematic reconstructions of tumor-immanent normative notions, which can be depicted in rationalization processes, are the basis of communication-derived tumor pathophysiology. Systematic descriptions of rationalization processes pave the way from a genome-centric to a rationalization-centric, namely evolution-adjusted tumor-pathophysiology. (7) The introduction of evolution-adjusted tumor pathophysiology represents a prerequisite for diversifying therapeutic instruments aimed at improving palliative care and personalizing tumor therapy. (8) Combined modularized therapy approaches, a description of tumor biology based on evolution-adjusted pathophysiology, and novel tools of biomarkers will allow the adaptive bioengineering of tumor response. (9) Evolution-adjusted pathophysiology provides a novel reification of the scientific picture about the 'objective' world by objectifying the subjectivity of systems objects in biological systems.

Following these general explanations on methodological issues, some remarks should be given on the claim for evolution-adjusted tumor pathophysiology, which may be established for the routine evaluation of the communicative expression of systems participators.

Bridging medical theory and therapeutic practice is a timeless challenge, and the success of bridging depends on the situative circumstances, traditions, and emancipatory interests. Mostly, scientists do not sufficiently acknowledge that medical knowledge is based on methodological issues of comprehension, particularly in the field of basic medical sciences, i.e. pathology, pathophysiology, molecular-genetics, and biochemistry. These disciplines are always based on medical theories that tend to be steadily recycled in daily practice.

Based on the tendency to recycle patterns of medical thinking, results gained from basic science may quickly reach the status of facticity, both intentionally and unintentionally. This status means that results derived from arbitrary biologic systems, particularly from successfully established model systems, are generalized without hesitation and transferred into newly arising biological systems contexts, as in the case of tumor diseases.

The substantiation of tumor systems objects is promptly followed by reckoning distinct 'historical' communicative expressions or generalized communicative expressions 'per se'. The catchphrase is 'from bench to bed side'. Yet, the question arises: What is the situative communicative expression of a few familiar molecular-genetic aberrations detected in tumor cells on the background of an arbitrary number of additional aberrations in tumor and stroma cells and genetically based tumor cell heterogeneity?

Pathology, molecular-pathology and cytogenetics allow the easy detection of specific tumor heterogeneities. In contrast, the current pathophysiology is overwhelmingly a theoretically-based science, and most pathophysiological case-related phenomena are only poorly integrable in daily clinical practice.

The central task of the current book is to critically scrutinize the automatic transfer of the communicative expression of tumor systems participators from one tumor system to another—even within tumor systems accomplishing identical normative notions. Formal-pragmatic communication theory and evolution theory shall help find answers to the following critical questions: When can an identical communicative expression of systems participators within different histological or molecular-genetic tumor types (evolutionary preserved communicative expression) be assumed, and which communicative circumstances are able to alter the communicative expression of identical systems participators in a therapy-relevant manner?

Attempts to detect the 'technical' prerequisites for the communicative expression of tumor systems participators reach ethical relevance: Bridging medical theory and therapeutic practice, the development and use of methodologies for reconstructing situative communicative expression of tumor systems objects within multifaceted, evolution-based systems contexts, and consequentially the purposeful modulation and redirection of tumor-associated normative notions related to tumor progression—an up-coming novel field of palliative care—are medical procedures embedded in an

ethical background. Insofar, it seems to be justified to systematize the communicative expression of systems objects, particularly rationalization processes, on the basis of situatively evolving communicative contexts that have been investigated in an equal manner. Consequently, the need arises for a systematic presentation of evolution-adjusted tumor pathophysiology as a novel language of tumor biology that may be integrated into daily routine diagnostics.

Evolution-adjusted tumor pathophysiology presents the situatively evaluated constitution of rationalization processes for tumor-promoting normative notions and, thus, a novel therapeutically accessible level for overcoming cytogenetically and molecular-genetically based tumor heterogeneity.

After these general considerations on communication, evolution, and tumor pathophysiology, I would like to thank all persons, who contributed to the publication of this book in rather different ways. G. Haegeman supported our efforts to proceed with combined transcriptional modulation in combination with metronomic low-dose chemotherapy to redirect and modulate tumor-immanent normativity for attenuating tumor growth.

My special thanks go to all the scientists and the steadily growing community of clinicians who participated in the large number of clinical phase II trials on biomodulatory therapies and to those scientists who contributed to a chapter to give the issue novel input. Ms Schoell, I want to thank for her excellent linguistic support.

The pragmatic considerations of J Habermas on communication theory inspired the systematic interpretation of the data acquired during combined modularized therapies in metastatic tumors and built the basis for formulating an evolution theory.

This book is dedicated to all students who have decided to become physicians. Famous clinical scientists and their invaluable experience do not lose their standing or charisma if we ask that the gap between medical theory and therapeutic practice should be methodologically supplemented and systematized by evolution-adjusted tumor pathophysiology to generate replicable knowledge on evolutionarily confined communicative expression with the aim to further foster the personalization of tumor therapy: Standard operating procedures are generated to further the knowledge of physicians in rather different medical fields. However, such knowledge should represent a continuous basis and challenge to methodologically bridge the conflicting poles, i.e. medical theory and therapeutic practice.

Both young physicians and basic scientists should be stimulated by the book to promote their own research—with the necessary openness to risk—for the further methodological integration of medical theory and therapeutic practice as a perpetual task in the field of evolution-adjusted tumor pathophysiology and translational medicine.

Part II
Combined Modularized Therapies for Metastatic Tumors: Pointing to Central Problems of Communication Among 'Systems Participators' in Tumors

Chapter 2
Applied Systems Biology for the Control of Metastatic Cancer: Therapeutic Top-Down Strategy for Targeting the Tumors' Normativity

A. Reichle and G. C. Hildebrandt

Abstract We hypothesized, that tumor systems-directed therapies might have the capability to therapeutically modulate and redirect the tumor systems' stability, homeostasis, robustness, and normative notions. This therapeutic 'top down' strategy may provide novel targets for the control of metastatic tumor disease. We comparatively analyzed redirection and modulation of tumor-associated normative notions, particularly inflammatory, osteoblastic activities, ECOG status, and metastatic potential in parallel with response, time to response and duration of response induced by continuously administered biomodulatory treatment modules (module M: metronomic low-dose chemotherapy; module A: pioglitazone plus etoricoxib; module A+M; module A+M/+: plus second transcriptional modulator [interferon-alpha or dexamethasone +/− imatinib or dexamethasone plus lenalidomide]) in the metastatic stages of seven different histological tumor types (ten phase II trials, two of them randomized; 333 patients; 80 % systemically pre-treated). A series of (randomized) phase II studies demonstrated differentially modularized accessibility of tumor-associated normative notions, i.e., inflammation, ECOG status, osteoblastic metastases, and metastatic tumor spread for mediating objective tumor response. Biomodulatory treatment schedules may induce long-term disease stabilization followed by prolonged objective response (3–100 %), even continuous complete remission, despite poor or no monoactivity of the respective drugs. Progression-free survival data are comparable with those of reductionist-designed standard first-line therapies. The differential response patterns indicate the therapies' systems biological activity. Clinical efficacy of 'top-down' therapy strategies (biomodulatory therapy elements administered as fixed modules) for metastatic cancer provide excellent opportunities to point to central problems of communication among 'systems participators' in tumors. Combined modularized therapies (1) help to detect multifaceted, situatively adapted rationalization processes available for ubiquitously occurring

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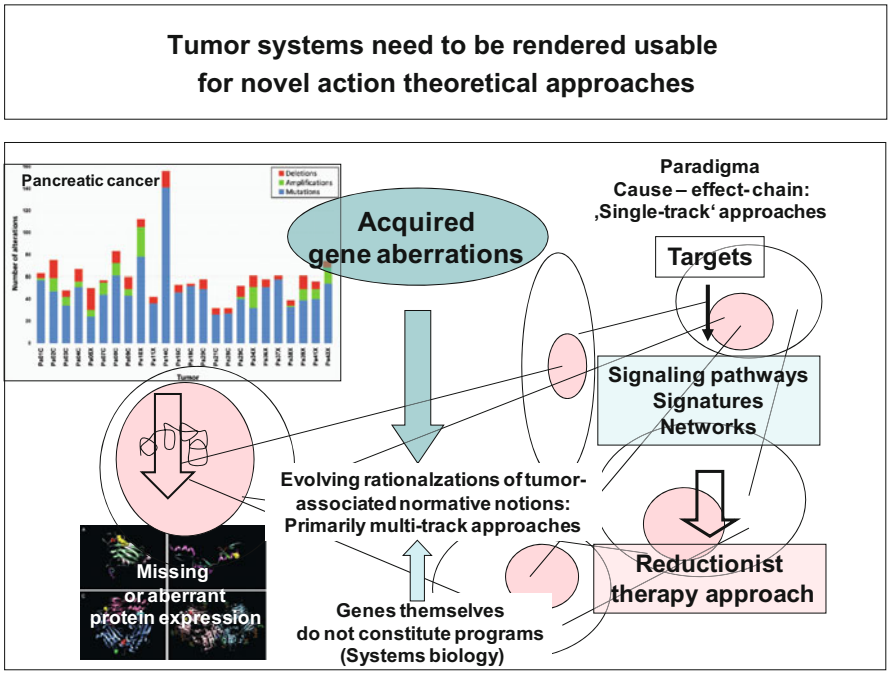


Fig. 2.1 The commonly used 'bottom-up' strategy regards as sufficient the availability of targets in cellular tumor compartments without identifying validity and denotation of targeted, presumably tumor-promoting systems participators. The communicatively cross-linked 'background', which may be functionally specified due to varying numbers of chromosomal or molecular-genetic aberrations, remains therapeutically unrecognized

tumor-immanent normative notions, (2) may uncover novel regulatory systems in tumor biology (e.g., hubs), (3) pathologies within communication processes (e.g., inconsistencies, disturbances in intersystemic exchange processes) (4) are a basis for studying communicative rules mediating the 'metabolism' of tumor evolution, and (5) may pave the way for inducing biological memory in metastatic tumors.

Keywords Metastatic tumors · Applied systems biology · Combined modularized tumor therapy · Evolution theory · Evolution-adjusted tumor pathophysiology

Introduction

Tumor-related activities that seem to be operationally induced by the diversity of tumor-immanent normative notions and their multifaceted evolutionary confined rationalization processes, such as normative functions (i.e. inflammation, neoangiogenesis, Warburg effect, immune response, extracellular matrix remodelling, cell

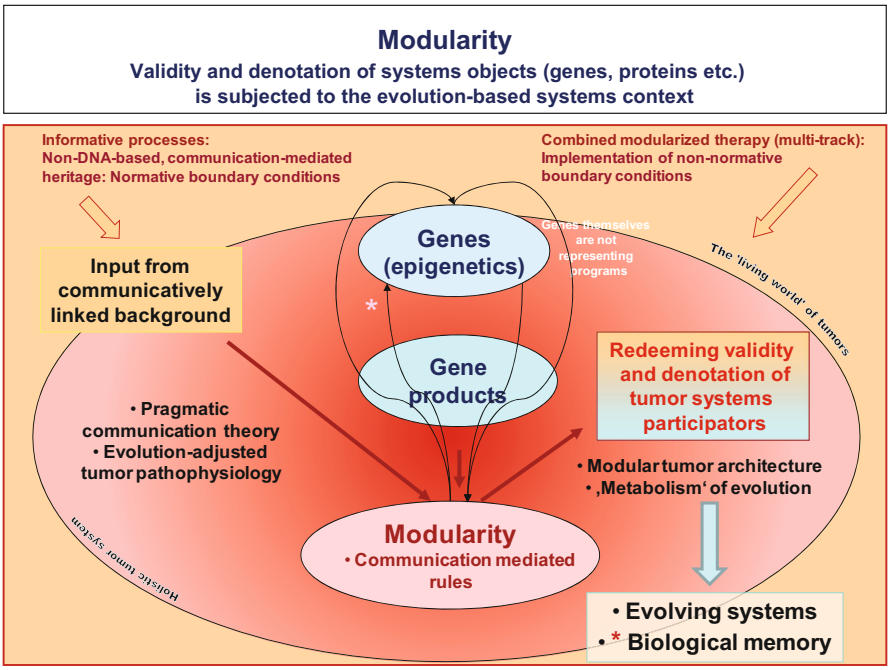
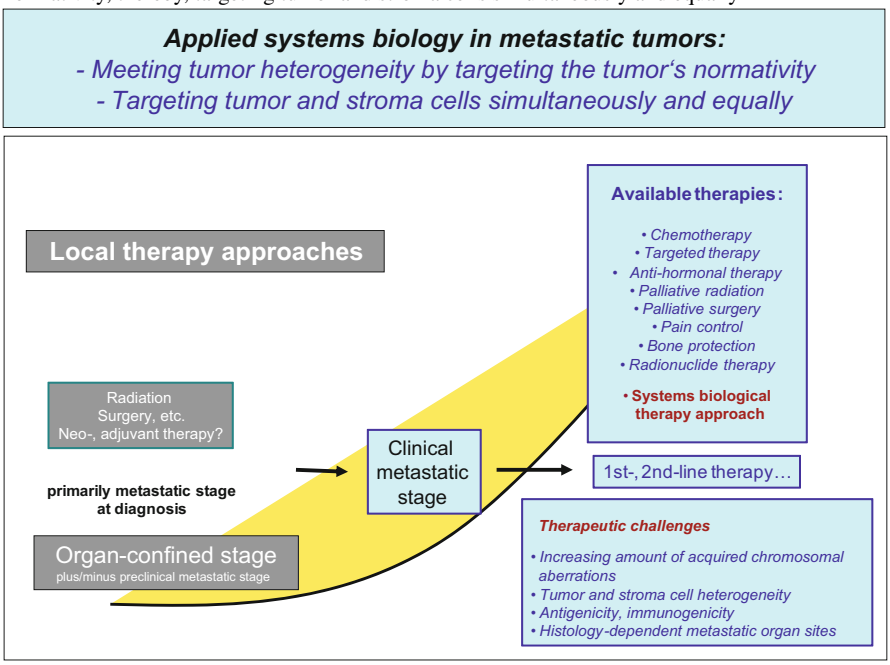


Fig. 2.2 Tumors allow experimental therapeutic access from inside in a comprehensive and reconstructive way (systems view) via modular (biomodulatory) therapy approaches and may be described as evolutionary developing systems. Modular therapies evolve the informative background, which redeems validity and denotation of tumor-associated objects. Therapeutically accessible pathologies may derive from the decoupling of functional cellular and systems 'world' and can be targeted by modular therapy approaches

proliferation rate, apoptosis, coagulation effects), normative structures and decision maxims (hubs), present itself from a systems perspective as an enhancement of complexity. So far, tumor systems have been assumed to defy experimental therapeutic access from inside, in a comprehensive and reconstructive way that means, in a communication-derived systems view, and to only comply with reductionist knowledge with regard to biochemical pathways (Fig. 2.1).

We hypothesized, that tumor systems-directed therapies might have the capability to use aggregated action effects of tumor-immanent normative notions, as adjustable sizes to therapeutically modulate and redirect the tumor systems' stability, homeostasis, robustness, and normative notions, and that this therapeutic 'top down' strategy may provide novel targets for the control of metastatic tumor disease in contrast to currently provided 'bottom-up' strategies including the classic 'targeted' therapy approaches: Combined modularized therapy approaches have been designed to study the operative accessibility of tumor-immanent normative notions for tumor control (therapeutic implementation of 'non-normative' boundary conditions into the tumor systems world) by ubiquitously available, non-oncogene addicted, but differentially distributed targets among tumor and stroma cells [1–6] (Fig. 2.2, Tables 2.1, 2.2).

Table 2.1 During progression from the organ-confined stage to the clinical metastatic stage tumors acquire asynchronously multifold chromosomal and molecular-genetic aberrations. Applied systems biology in metastatic tumors may meet this therapeutic challenge by targeting the tumor's normativity, thereby, targeting tumor and stroma cells simultaneously and equally



Materials and Methods

We comparatively analyzed redirection and modulation of tumor-associated normative notions, particularly inflammatory, osteoblastic activities, ECOG status, and metastatic potential in parallel with response, time to response and duration of response induced by continuously administered biomodulatory treatment modules (Table 2.4, 2.5) (module M: metronomic low-dose chemotherapy; module A: pioglitazone plus etoricoxib; module A+M; module A+M/+: plus second transcriptional modulator [interferon-alpha or dexamethasone +/− imatinib or dexamethasone plus lenalidomide]) in the metastatic stages of different types of tumors (ten phase II trials, two of them randomized; 354 patients; 80 % systemically pre-treated; metastatic melanoma (two trials, one randomized), (angio-) sarcoma, renal clear cell carcinoma (two trials), glioblastoma, castration-resistant prostate cancer (two trials on CRPC), gastric cancer (randomized phase II trial), multi-systems Langerhans' cell histiocytosis, and multiple myeloma in third-line) (Tables 2.3, 2.6) [7–22].

Further, we analyzed the follow-up of patients discontinuing study medication due to medical indications, and who achieved objective response to module A+M/+combined with a second transcriptional modulator (dexamethasone), besides metronomically administered imatinib (400 mg once daily) in CRPC (phase

Table 2.2 The 'Top-down' approach allows redirecting and modulating the communicative 'background', which mediates validity and denotation of tumorpromoting systems participators and organizes the constitution of rationalizations for maintaining tumor-immanent rationalizations. The 'background' is modularly arranged and therapeutically accessible with primarily multi-track, modularized therapy elements

Studies' objective: *Meeting tumor heterogeneity in metastatic tumors, high therapeutic efficacy and a low rate of toxicity by applied systems biology*

'Top-down' approach	*Communication-related targets*
Redirecting the communicative expression of tumor-promoting systems participators *communication lines, pathways etc.*	**Tumors' normativity beyond the 'hallmarks' of cancer:** - *Tumor-immanent normative structures* - *Normative functions* - *Decision maxims (hubs)*
Therapeutic modulation of the communicative 'background' *Multi-track, combined modularized tumor therapy*	**Modular access to the tumors' normativity:** - *Osteoblastic processes (prostate cancer)* - *Tumor angiogenesis* - *Tumor-promoting inflammation* - *Tumor-associated immune escape* .....
Novel tool of therapeutic targets, drugs *Ubiquitously accessible targets in tumor and stroma cells* ***Combined transcriptional modulation:*** *Induction of epigentetic changes*	**Non-oncogene addicted targets** - ***COX-2, PPARbeta*** *(etoricoxib)* - *PDGF-R (imatinib); targets of lenalidomide* - ***Regulatory T-cells etc.*** *(metronomic low-dose chemotherapy)* - ***PPAR alpha/gamma receptors*** *(pioglitazone)* - ***Glucocorticoid receptor*** *(dexamethasone) or* ***interferon-alpha receptor*** *(interferon-alpha)*

I/II trial for CRPC, first-line therapy) or lenalidomide (10 or 15 mg once daily) in multiple myeloma (third-line therapy for MM, phase I, on-going phase II trial) (Chap. 19).

Results

A series of (randomized) phase II studies demonstrated differentially modularized accessibility of tumor-associated normative notions, i.e., inflammation, ECOG status, osteoblastic metastases, and metastatic tumor spread for mediating objective tumor response. Biomodulatory treatment schedules may induce long-term disease stabilization followed by prolonged objective response (3–100 %), even continuous complete remission, despite poor or no monoactivity of the respective drugs (Table 2.7). Progression-free survival data are comparable with those of reductionist-designed standard first-line therapies. The differential response patterns indicate the therapies' systems biological activity (Figs. 2.3, 2.4 and 2.5).

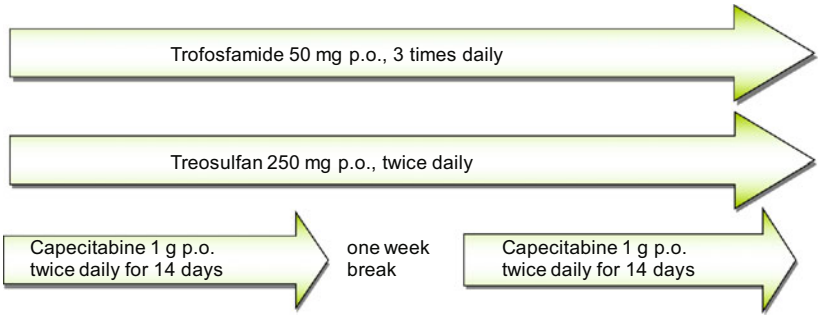
Table 2.3 For studying the capacity of combined modularized tumor therapies to redirect the tumors' normativity, we selected tumors as normative model systems, i.e., tumors with predominant pro-angiogenic component, with strong pro-inflammatory component, and tumors with pro-inflammatory characteristics in the metastatic stage

Tumors as normative model systems:
Combined modularized therapy: Antiangiogenetic/ anti-inflammatory/immun-modulatory trials

- **Tumors with high vascular density**
  - Angiosarcoma
  - Renal clear cell carcinoma (RCCC)
- **Tumors with extensive inflammation**
  - Refractory multivisceral Langerhans' cell histiocytosis (mLCH)
  - Multiple myeloma (MM)
- **Tumors with inflammation in advanced stage**
  - Sarcoma
  - Melanoma
  - Cholangiocellular carcinoma (CCC)
  - Castration-resistant prostate cancer (CRPC)
  - Gastric cancer
  - Glioblastoma

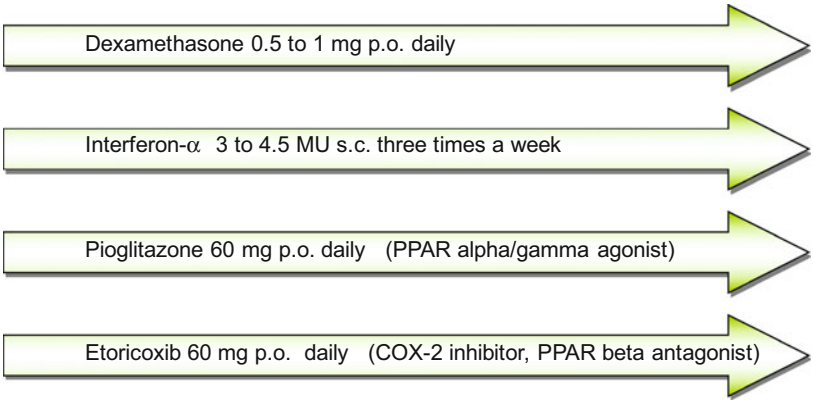
Table 2.4 Metronomic low-dose chemotherapies and mechanisms of action

Angiostatic, immunmodulatory and anti-inflammatory therapies: Metronomic low-dose chemotherapies



Angiostatic:	*Up-regulation of thrombospondin 1, reduction of circulating endothelial cells, decreased recruitment of endothelial progenitor cells, and blocking rebounds by the tumor vasculature*
Anti-inflammatory	*in gastric cancer*
Immun-regulatory:	*Reduction of tumor-induced immune-tolerance, enhanced immunity against tumor antigens, and strongly curtails immunsuppressive regulatory T-cells*

Table 2.5 Targeting the tumor systems biology: Combined transcriptional modulation of ubiquitously available targets



- **Pioglitazone/etoricoxib** (Metastatic melanoma, cholangiocellular carcinoma, sarcoma, multivisceral Langerhans' cell histiocytosis, gastric cancer, glioblastoma)
- **Combined transcriptional modulation**
  - Pioglitazone/dexamethasone/etoricoxib (Castrate-resistant prostate cancer)
  - Pioglitazone/dexamethasone (Multiple myeloma)
  - Pioglitatzone/interferon-alpha/etoricoxib (Renal clear cell carcinoma)

Table 2.6 The table gives an overview about the performed combined modularized therapy approaches. In three trial designs we used combined transcriptional modulation (castration-resistant prostate cancer, multiple myeloma and renal clear cell carcinoma)

Experimental plan: Combined modularized tumor therapy (n= 354 patients, 19 centers)
(Reichle A, Cancer Microenvironment, 2008; Reichle A, J Clin Oncol 29: 2011 (suppl; abstr 4599), Reichle A, Blood suppl. ASH 2012)
o pioglitazone, **d** selective COX-2 inhibitor, dexamethasone, *interferon-a, PPAR= peroxisome proliferator-activated receptor agonist

Metastatic cancer: Tumor type	Metronomic low-dose chemotherapy	No. of patients	*Receptor agonist/antagonist* PPARa/γ agonist[O]	PPARδ antagonist[Δ]	Gluco-corticoid	IFN-α *	Publications
Kaposi sarcoma	Trofosfamide	1	+	+	-	-	*Arch Dermatol, 2004*
(Hem)angiosarcomas	Trofosfamide	12	+	+	-	-	*Cancer, 2003/04*
Sarcomas I	Trofosfamide	21	+	+	-	-	*Cancer, 2004*
Melanoma I	Trofosfamide	19	+	+	-	-	*Cancer, 2004*
Melanoma II Arm M		35	-	-	-	-	*Melanoma Research, 2007*
Arm A/M		32	+	+	-	-	*Lancet Oncol 2007 (comment) Cancer Microenvironment 2008, 2009*
Langerhans' cell histiocytosis	Trofosfamide	3	+	+	-	-	*Br. J.Haematol, 2005*
Glioblastoma	*Capecitabine*	14	+	+			*Oncology, 2007*
Renal clear cell carcinoma (A/M)	*Capecitabine*	18	+	+	-	-	*Biomarker Insights, 2006*
Renal clear cell carinoma (A/M+)	*Capecitabine*	33	+	+	Combined transcriptional modulation	+	*World J Urol, 2012 Biomarker Insights, 2006*
Castration-refractory prostate cancer	Treosulfan *Capecitabine*	61	+	+	+ (imatinib)		*ASCO abstract, 2007; 2011*
Multiple myeloma	Treosulfan	36	+	+	+	-	*Lancet Oncology, 2006*
Cholangiocellular carcinoma		6	+	-	+ (lenalidomide)	-	Blood 2012; **120**: 5029
		21	+	+	-	-	*From molecular to modular tumor therapy*
			+	+	-	-	
Gastric c. Arm M	*Capecitabine*	42	-	-	-	-	*ASCO abstract 2009*
Arm A/M	*Capecitabine*		+	+	-	-	

Table 2.7 Overview of outcome following combined targeting of the modular tumor architecture in pre-treated patients (80 %)

Combined targeting of the modular tumor architecture in pre-treated patients (80%):Response behavior (n=321)

(Reichle A, Cancer Microenvironment, 2008; Reichle A, J Clin Oncol 29: 2011 (suppl; abstr 4599), Reichle A, Blood suppl. ASH, 2012)

Tumor types (n=9)/Therapy arm	Response			
	No. of patients	partial remission/ PSA response %	complete remission %	continuous CR %
Sarcomas I	21	19%	16%	**5%**
Angiosarcomas/Hemangiopericytomas/Kaposi s.	8/4/1	12%/25%/100%	62%/0%/0%	**12%/0%/0%**
Melanoma Arm M (R)	35	3%	0%	**0%**
Arm A/M (R)	32	9%	3%	**3%**
Langerhans' cell histiocytosis (multivisceral)	3	-	100%	**100%**
Renal clear cell carcinoma I (RCCC)	18	0%	0%	**0%**
Renal clear cell carinoma II (plus IFN-a)	33	35%	13%	**9%**
Castration-refractory prostate cancer (CRPC)	36	41%	0%	**>4 years minimal residual disease**
CRPC (plus imatinib)	61	37.7%	0%	**>5 years minimal residual disease**
Cholangiocellular carcinoma	21	24%	5%	**5%**
Multiple myeloma (plus lenalidomide, third-line, phase I)	6	67%	17%	**17%**
Gastric cancer Arm M (R)	20	20%	0%	**0%**
Arm A/M (R)	22	14%	0%	**0%**

Toxicities of combined modularized therapies were manageable by early dose reductions according protocol in case of grade 2 toxicities, and therefore, facilitate long-time administration of study therapy. Only 3 % of patients permanently discontinued therapy due to site effects ([7, 23], Chap. 5). Long-term tumor control can be achieved in elderly (up to 86 years) and medically non-fit patients (Chap. 5) on the basis of moderate treatment-related toxicity, particularly less grade 3 and 4 toxicities.

Tumor-specific and stage-specific therapeutic accessibility of inflammation-related processes to induce response in all tumor types indicate a constitutive spin-off of novel rationalized systems functions during the metastatic process [24]. Furthermore, this accessibility shows differential integration of inflammation processes into the context-dependent 'living world' of tumor compartments that is marked by tumor-specific and subtype-specific rationalization processes: Inflammation-related activities are communicatively promoted and differentially adapted during tumor evolution. Empirically, differences may be detected in the modalities of developing evolutionary systems and in the acquired functional impact of inflammation-related systems [24].

The observed response patterns, either very rapid or delayed tumor responses, indicate that communicative inconsistencies may be therapeutically met (Achilles' heels). Disturbances in intersystemic exchange processes are suggested, if biomarkers (e.g., C-reactive protein) or signatures depicting the redirection of normative

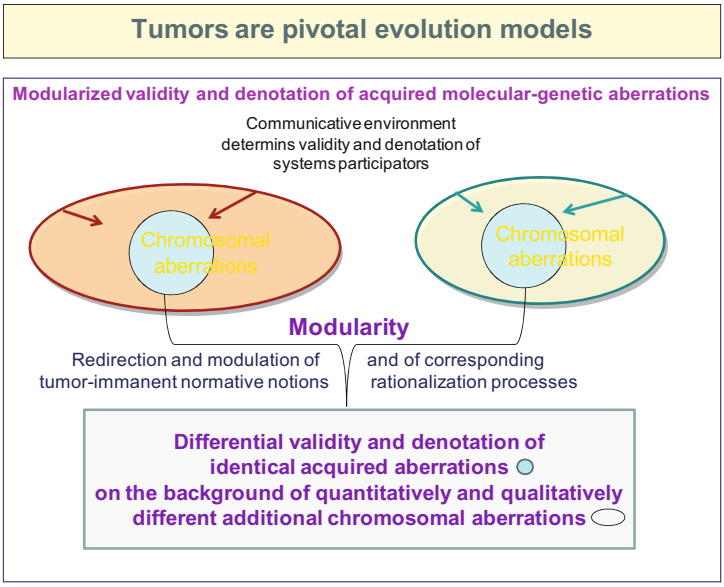


Fig. 2.3 Tumors represent pivotal evolution models, as the communicative 'background' of tumor-promoting systems objects is highly variable due to the varying numbers of chromosomal or molecular-genetic aberrations, which individualize a tumor disease to a high degree. But likewise, rationalizations of tumor-immanent normative notions are individualized and differentially accessible by modularized therapy approaches

notions (here tumor-associated inflammation) show a low sensitivity to predict clinical benefit [24].

Temporally limited metronomically administered, combined modularized therapies may facilitate biological memory for stably sustaining long-term tumor growth control (12.5–15 months) without disease specific therapy in patients, who discontinued study medication due to non-tumor-related surgical interventions (Chap. 19).

Differential approaches implementing combined transcriptional modulation plus metronomic chemotherapy demonstrated the capacity to redirect and modulate tumor-immanent normative functions in a multifaceted way (inflammation control, anti-osteoplastic effect, immunregulation, biological memory, induction of continuous complete remission; long-term maintenance at minimal residual disease), in renal clear cell carcinoma, multiple myeloma and castration-resistant prostate cancer (Tables 2.7, 2.8, Chap. 19). Targeting the tumors' normativity by combined transcriptional modulation allows to diversifying therapeutic instruments for purposive rationale therapies with the aim to specify palliative care (Chap. 15).

Metastatic lesions, pretreated with palliative radiotherapy, had frequently shown local progression in the radiation field (86 % of $n = 56$ previously locally irradiated patients), despite disease stabilization or response towards combined modularized therapies at other metastatic tumor sites.

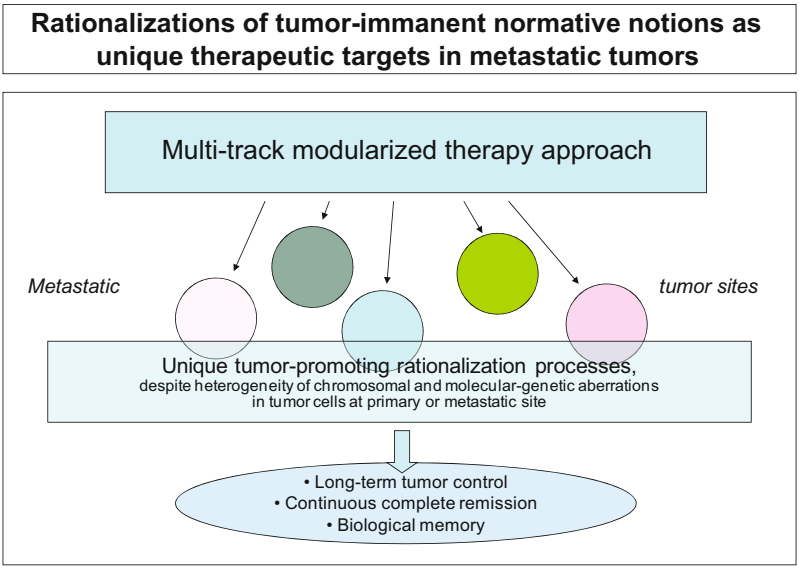


Fig. 2.4 Induction of long-term tumor response or continuous complete remission in metastatic tumors indicates that tumor-promoting rationalizations at the metastatic sites are uniquely constituted within an individual tumor disease and are efficacious targets for combined modularized tumor therapies to overcome cytogenetic heterogeneity in tumor cells

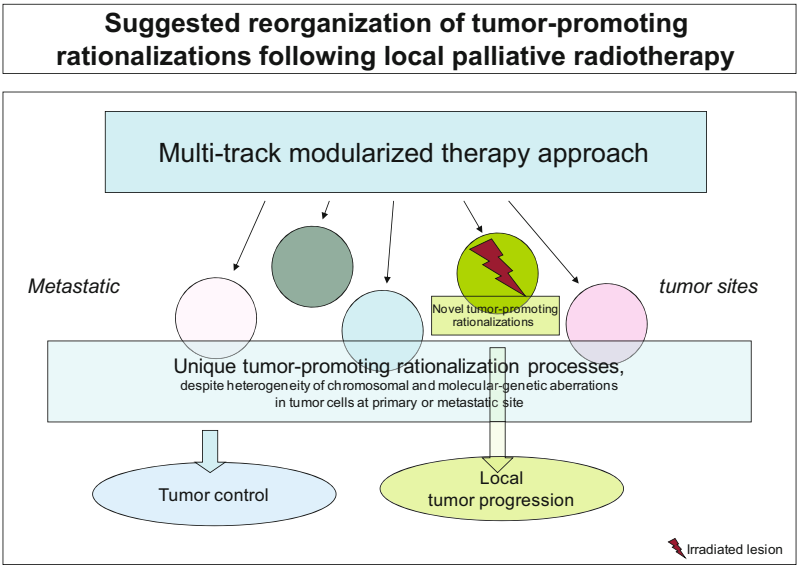


Fig. 2.5 Clinical data indicate that radiotherapy commonly leads to a reorganization of rationalizations constituting tumor-promoting normative notions and consecutive to resistance towards combined modularized tumor therapies

Table 2.8 Combined transcriptional modulation led to an impressive redirection of the tumors' normativity in quite different tumor histologies. Induction of biological memory (more than 10 fold longer times to PSA doubling after discontinuation of study medication compared to base-line PSA doubling time) and induction of continuous complete remissions are pivotal findings

Combined transcriptional modulation:
Redirection of tumor-associated normative functions
(ASH,Blood 2012;**120**:5029; *World J Urol, 2012; ASCO abstract, 2007; 2011)*

Castration-resistant prostate cancer

(pioglitazone and dexamethasone):
• *Anti-osteoblastic effect*
• *Immunregulatory effect*
• *Long-term response (> 4 years)*
• *Biological memory > 1 year (therapy discontinuation)*

Renal clear cell carcinoma

(pioglitazone and interferon-alpha):
• *Anti-inflammatory effect (interferon-alpha!)*
• *Continuous complete remission (histologically confirmed)*
• *Reduction of metastatic potential*

Multiple myeloma

(pioglitazone and dexamethasone):
• *Continuous complete remission in third-line (>1.0 year)*
• *Anti-inflammatory effect*
• *Rapid increase of serum hemoblobin*

Discussion

Clinical efficacy of 'top-down' therapy strategies (biomodulatory therapy elements administered as fixed modules) for metastatic cancer provide excellent opportunities to point to central problems of communication among 'systems participators' in tumors, i.e. the different cell compartments, pathways, oncogenes, tumor suppressor genes, etc. Combined modularized therapies (1) help to detect multifaceted, situatively adapted rationalization processes available for ubiquitously occurring tumor-immanent normative notions [2, 7, 8, 15] and (2) corresponding novel tools of biomarkers [5, 7, 9, 22], (3) may uncover novel regulatory systems in tumor biology (e.g., hubs) (Chaps. 16 and 17), (4) pathologies within communication processes (e.g., inconsistencies, disturbances in intersystemic exchange processes) [7, 24], (5) are a basis for studying communicative rules mediating the 'metabolism' of tumor evolution ([24], Chap. 12), and (6) may pave the way for inducing biological memory in metastatic tumors [25, 26] (Table 2.9, Chap. 19).

Induction of long-term tumor response or continuous complete remission in metastatic tumors indicates that tumor-promoting rationalizations at metastatic sites are uniquely constituted within an individual tumor disease and are efficaciously targeted with combined modularized tumor therapies. This way, biomodulatory therapies overcome the major obstacle of 'bottom-up' strategies, namely cytogenetic heterogeneity in tumor cells at primary and metastatic tumor sites (Figs. 2.4, 2.5).

Table 2.9 Evolution-adjusted tumor pathophysiology enables to study novel biomarkers depicting the evolvability of tumors within their 'living world' as well as the therapy-induced redirection and modulation of the tumors' normativity

Novel tool of biomarkers indicating redirection and evolvability of the tumor's normativity

Biomarkers indicating systems' evolvability within the tumor's ‚living world' (‚universal' surrogates)

- ***‚Universal' surrogates*** *for tumor-associated normative notions, e.g. inflammation: C-reactive protein response (in melanoma, renal clear cell carcinoma, multiple myeloma)*
- ***Late-stage biomarkers*** *(e.g. PPARgamma expression in metastatic melanoma)*
- ***Education:*** *Biological memory (epigenetic changes)*

Technologies recording the redirection of the tumors' normativity

- ***Cellular secretome analytics*** *(substantiating rationalizations of tumor-immanent normative notions, i.e. identity and fuction of cellular compartments)*
- ***Molecular imaging*** *of the tumors' normativity*
- ***Histology-based markers:*** *Markers developing with tumor progression (late-stage markers)*
- ***Epigenetic monitoring*** *(25, 26)*

Vice versa, preceding radiation therapy frequently induces intrinsic resistance towards biomodulatory therapy in irradiated tumor lesions. This observation suggests that radiation therapies accomplish heterogeneity of the tumor's growth-promoting rationalization processes as cause for resistance towards combined modularized tumor therapies.

Understanding systems biology as adjustable size ('top-down' technology) may break through the barrier of complex tumor-stroma-interactions in a therapeutically relevant way (Table 2.7): Comparatively high efficacy at moderate toxicity. Structured systems-directed therapies in metastatic cancer may get a source for detecting the topology of tumor-associated complex aggregated action effects accessible for combined modularized therapies (Figs. 2.4, 2.5).

Biomodulatory therapies are tools for uncovering novel modular structures and rationalizations in tumor systems, for probing cellular activation states and for identifying key hubs in both normal and diseased tissues. The concept of protein modularity is central to the field of combined modularized therapies and synthetic biology [7, 27]. With biomodulatory therapies we may design new approaches to disrupt tumor-promoting signaling via remodeling of signaling pathways based on principles derived from modular tumor pathophysiology [22, 28–30].

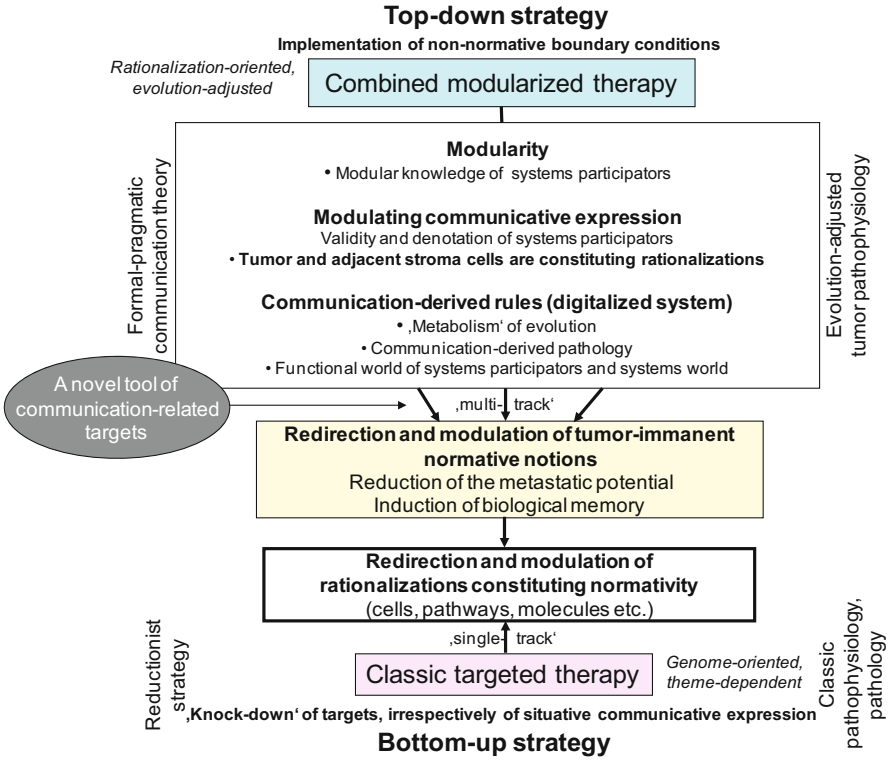


Fig. 2.6 Applied systems biology for the control of metastatic cancer: Shaping and focusing systems' communication by disrupting the holistic thicket with a 'top-down' strategy. Tumors are not any more considered as 'objects', which have to be destroyed with 'single track' or combined 'single track' targeted therapy approaches, but as subjects within a communicative context, which allow to implement multifold novel combined modularized therapies (multi-track approaches). Bottom-up and top-down strategy have in common to redirect and modulate the tumors' normativity as prerequisite for tumor growth control

Observations on the activity profile of combined modularized therapies, i.e. rapid versus delayed response, induction of biological memory, the possibility to achieve response induction via purposive-rational modulation and redirection of tumor-associated normative notions, and the successful application of drug repurposing may not be explained with traditional evolution historical considerations, but facilitate the development of an evolution theory, i.e. the formal-pragmatic communication theory [31, 32].

Specifying 'top-down' approaches necessitates novel pathophysiological considerations, the application of an evolution-adjusted tumor pathophysiology, which focuses on the systematic comprehension of tumor systems objects' communicative expression, their validity and denotation in a distinct evolutionary context (Fig. 2.6).

Evolution-adjusted tumor pathophysiology provides contently and methodologically novel approaches to succeed in personalizing tumor therapy. By targeting the tumors' normativity, genetically based tumor heterogeneity is efficaciously addressed [33]. Uniquely constituted rationalization processes within a distinct tumor disease are a common denominator for successful modular therapeutic access and long-term tumor control.

Evolution-adjusted tumor pathophysiology should be introduced as clinically orientated discipline, equivalent with traditional disciplines, thereby increasing their value and accomplishing ethical demands. A tumor type-specific, systems stage-specific, metastatic site-specific or disease trait-orientated therapy seems to be within grasp.

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Chapter 3
Targeting the Proangiogenic Network in Angiosarcomas: Biomodulatory Therapy

S. Singer and L. Größer

Abstract Over the last years, thirteen metastatic vascular tumors (8 AS, 1 KS, 1 hemangioendothelioma, 3 hemangiopericytomas) were treated with a biomodulatory therapy consisting of metronomic low-dose chemotherapy (trofosfamide), PPAR δ-blockage (rofecoxib) and the transcription modulator pioglitazone. Among these, one continuous complete remission (> 5 years), four complete remissions, two partial remissions, three cases of stable disease and three cases of progressive disease were achieved. Taking these findings together, further investigations are necessary to turn promising therapeutic starting points into viable therapeutic regimens for the treatment of AS.

Angiosarcomas (AS), representing about 2 % of soft tissue sarcomas [1], are rare malignant vascular tumors with a very poor prognosis. These tumors are most commonly located on the head and the neck and mainly occur in older men (median age about 71 years). In contrast, AS below the clavicle—except for AS on the breast—are equally distributed in both sexes (median age about 52 years) [2]. The 5-year overall survival rate for localized disease is about 30–40 %. For metastatic patients, the estimated progression-free survival rate is about 4 months, the overall survival time about 8 months [3]. The incidence of AS is increasing, probably owing to increased radiotherapy and higher clinical awareness.

In the case of localized disease, primary treatment of choice is radical surgery with wide margins. Because of the tumor location (e.g., AS of the head) or its diffuse tissue infiltration, R_0 resection is often hard to achieve; therefore, local and distant recurrences are common. For this reason, surgery should be followed by adjuvant radiotherapy [4, 5].

Considering the poor prognosis of AS, new therapeutic options should be investigated. Development of such novel therapeutic approaches has been delayed by the low incidence of the disease. To this day, no standard therapy has yet been established for patients with advanced, recurrent, and metastatic disease. A considerable number of chemotherapeutic regimens have been described with limited success but severe toxic side effects.

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Angiogenesis plays a crucial role in the growth and metastatic spread of malignant tumors. Targeting the proangiogenic network seems to be a promising approach because it is independent from the genomic instability and heterogeneity of the tumor itself. Particularly in AS, both the malignant endothelial tumor cells as well as the benign proliferating vasculature may be targeted.

Bevacizumab is a humanized monoclonal antibody against the vascular endothelial growth factor A (VEGF-A), which plays a crucial role in angiogenesis. VEGF-A is an agonist ligand of the VEGF receptor-1 (VEGFR-1) and VEGF receptor-2 (VEGFR-2), i.e., two tyrosine kinase receptors that are mainly found on the surface of vascular endothelial cells. VEGF-A and VEGFR-1 have been shown to be highly expressed in different subtypes of AS [6]. A prospective multicenter phase II trial including 30 patients with advanced AS and epithelioid hemangioendothelioma showed a moderate success of monotherapy with bevacizumab: four patients (17 %) had a partial response, and 15 patients (50 %) showed stable disease with a mean time to progression of 26 weeks [7]. Sorafenib, another VEGFR inhibitor, that also blocks tyrosine kinases such as PDGFR or RAF kinases, had a beneficial therapeutic effect on patients with AS [8]. Further anti-VEGF/R agents such as sunitinib and pazopanib require additional investigation [9].

Taking these data into account, monotherapy does not seem to be the most promising approach. Indeed, a combination of multiple drugs with angiostatic, antiproliferative, anti-inflammatory, and immunomodulatory effects may provide a much more effective palliative regimen. The idea of an angiostatic, anti-inflammatory, immunomodulatory and proapoptotic metronomic therapy can be realized by administration of low-dose trofosfamide, pioglitazone, and rofecoxib. In a pilot trial, six patients with advanced and pretreated but progressive malignant vascular tumors (including 5 AS and 1 hemangioendothelioma) were treated with this triple combination: two patients responded with complete remission, one patient with partial remission, and one patients showed at least stabilization of the disease [10].

The small sample size did not allow a statistical analysis, but the idea itself remains promising. In 2004, a patient with endemic Kaposi Sarcoma (KS), another malignant vascular tumor, which is associated with HHV-8 infection, was treated with the same medication. Like AS, KS has a poor prognosis. The female patient responded with a partial remission, which showed to be stable for 18 months [11]. In another trial, 21 patients with advanced soft tissue sarcoma (including 3 AS and 3 hemangiopericytomas) were treated with the same triple therapy: 50 % of the vascular tumors responded with complete remission (among these one patient with a continuous complete remission of more than 5 years), 50 % with progressive disease [12].

In summary (Table 3.1), over the last years, 13 vascular tumors (8 AS, 1 KS, 1 hemangioendothelioma, 3 hemangiopericytomas) were treated with a biomodulatory therapy consisting of metronomic low-dose chemotherapy (trofosfamide), PPAR δ-blockage (rofecoxib) and the transcription modulator pioglitazone. Among these, one continuous complete remission ($>$ 5 years), four complete remissions, two partial remissions, three cases of stable disease and three cases of progressive disease were achieved. Table 3.1 gives an overview of the different trials using biomodulatory therapy.

Table 3.1 Overview of trials using biomodulatory therapy in the treatment of vascular malignancies

Vascular tumor	Regimen	Results	References
5 Angiosarcomas 1 Hemangioendothelioma	pioglitazone hydrochloride (60 mg/d), rofecoxib (25 mg/d), trofosfamide (3 × 50 mg/d p.o.)	2 × complete remission, 1 × partial remission, 3 × stable disease	Vogt et al. 2003
1 non-endemic Kaposi sarcoma	pioglitazone hydrochloride (60 mg/d), rofecoxib (25 mg/d), trofosfamide (3 × 50 mg/d p.o.)	Partial remission, which was stable for 18 months	Coras et al. 2004
21 soft tissue sarcoma (among six vascular sarcomas): Angiosarcoma (n = 3), Hemangiopericytoma (n = 3)	pioglitazone hydro-chloride (60 mg/d), rofecoxib (25 mg/d), trofosfamide (3 × 50 mg/d p.o.)	within vascular sarcomas: 2 × complete remission, 1 × continuous complete remission (> 5 years), 3 × progressive disease	Reichle et al. 2004

Cyclooxygenase-2 (COX-2) has been shown to be not only present in inflammation but also in tumors and neovasculature [13]. COX-2 inhibitors such as Rofecoxib, Celecoxib, and others do not only have anti-inflammatory effects but can also induce cancer cell apoptosis by suppressing the peroxisome proliferator-activated receptor δ [14]. PPAR δ seems to be involved in cell differentiation and apoptosis. Furthermore, a blocking of AKT activation, a downregulation of bcl-2, and an upregulation of Bax has been described.

Pioglitazone and rosiglitazone are thiazolidinediones and agonist ligands of the peroxisome proliferator-activated receptor γ (PPAR γ) that are broadly used as antidiabetic drugs. PPAR γ is not only expressed in fat but also in many other human cell lines. Several trials have shown the PPAR γ-dependent and PPAR γ-independent antitumor effects of thiazolidinediones that include the induction of apoptosis, angiostatic activity, and inhibition of pro-inflammatory transcription factors [15]. Recently published data reveals a synergistic antitumor effect of the PPAR γ agonist 15d-PGJ2 on melanoma cells, as well as on the surrounding stroma [16]: antiproliferative effects on melanoma-associated fibroblasts and anti-angiogenic effects owing to inhibition of tube formation were shown. The expression of PPAR γ is turning out to be a predictive marker for a response to a stroma-affecting therapy within the treatment of melanoma [17]. The effect on AS cells could be similar but this still has to be evaluated. Interestingly, PPAR γ agonists have been shown to induce hemangiosarcomas in rodents by increased proliferation and survival of endothelial cells [18, 19]. This effect was not seen in human cells, yet.

A tumor cell is not an isolated entity but it has to be understood in the context of its microenvironment. Tumor progression is always a result of an emerging interaction between genetically altered tumor cells and their surrounding tissue. "Normalization" of the stromal environment seems to be able to slow or even reverse tumor progression [20], which is also the intention of a biomodulatory therapy. Main target

of metronomic therapy is the endothelial cell and tumor angiogenesis. Owing to the genetic stability of stromal cells (in contrast to tumor cells), metronomic therapy seems to be less susceptible to the development of drug resistance. Therefore, the main concept could turn out to be an emerging and striking regime for multiple far advanced malignancies in palliative condition. Metronomic therapy seems yet to be effective in advanced malignancies as melanoma and soft tissue sarcoma [12], Ewing Sarcoma [21], Langerhans' cell histiocytosis [22], prostate cancer [23], and more [24].

So far, no curative therapeutic regime for metastatic AS has been found. Particularly the vasculature, the tumor itself, as well as the tumor stroma requires further investigation. Recently, the tunica internal endothelial cell kinase 2 (Tie2) has been identified as a potential novel therapeutic target. Tie2 is an endothelial-specific receptor tyrosine kinase, and its most important agonist ligand is angiopoietin 1. Tie2 kinase inhibition showed reduced cell survival *in vitro* and delayed tumor growth *in vivo* [25]. This effect was shown to be even potentiated by combining Tie2 kinase inhibitors with bevacizumab.

Taking these findings together, further investigations are necessary to turn promising therapeutic starting points into viable therapeutic regimens for the treatment of AS.

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Chapter 4
Long-Term Results of Combined Modularized, Immune-Modulatory, Angiostatic, and Antiinflammatory Therapy in Systemically Pre-Treated Multi-Systems Langerhans Cell Histiocytosis

A. Reichle, M. Vogelhuber, T. Vogt, L. Kunz-Schughart, T. Bretschneider, K. Bross and R. Andreesen

Abstract Up to now, systemically pretreated multi-systems Langerhans cell histiocytosis has been an incurable disease, and therapeutic options for patients have been limited. Acknowledging the fact that the continuous production of pro-inflammatory cytokines contributes to the resistance to commonly used agents, we hypothesized that a multi-targeted approach consisting of continuously administered anti-angiogenic, anti-inflammatory, immune-modulatory, and anti-tumor components may overcome such resistance mechanisms. To clinically test this hypothesis, we initiated a phase II trial with a combination of pioglitazone 60 mg daily, trofosfamide 50 mg thrice daily, and etoricoxib 60 mg daily (rofecoxib 25 mg). In the absence of toxicity > grade 2, patients were eligible to continue the treatment regimen until tumor progression or complete tumor remission. Key inclusion criteria were progressive or relapsing MS-LCH after at least two previous lines of systemic therapy. We here report on the long-term results of three consecutive patients treated at one center. Between November 2003 and December 2011, three patients with progressive multi-systems Langerhans cell histiocytosis (MS-LCH) were included into our phase II trial. Each patient was male and had been heavily pretreated. The

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median number of previous treatment lines was 3.3 (range 3–5). The median age was 34 years (range 22–50 years). Remarkably, none of the patients experienced dose-limiting toxicity (defined as any toxicity with NCI-CTCAE grade ≥ 3 during the first treatment cycle). Therefore, all patients continued treatment. Serious adverse reactions observed during the follow-up period were infections ($n = 2$, grade 2 NCI-CTCAE toxicity). Response assessment showed two patients with continuous complete remission (CR) and two patients with histologically confirmed CR in the respective lesions. One of the patients had progressive disease after long-term CR and responded again to study medication. Our prospective phase II trial showed that the combination of low-dose chemotherapy, COX-2 inhibitor, and pioglitazone administered as a long-term treatment on a daily basis has not only a very favorable toxicity profile but also represents a feasible treatment regimen applicable in an outpatient setting. Because of the high tolerance of this therapy and the encouraging long-term response data, this study protocol should be evaluated in a multi-center trial.

Keywords Metronomic low-dose chemotherapy · Multi-systems Langerhans cell histiocytosis · Cyclo-oxygenase-2 · Peroxisome proliferator-activated receptor gamma

Introduction

Langerhans cell histiocytosis (LCH) is a chimeric disease that affects people of all ages. LCH manifests at different organ sites and initially presents as a focal or systemic disease; thus, LCH is the prototype of an inflammation-driven disease [1, 2]. Growing evidence suggests that LCH may be classified as a malignant disease from a molecular-genetic view (mutant BRAFV600E protein, p53 expression) (Fig. 4.1) [3, 4]. However, the presence of oncogenes and tumor suppressor genes alone is no proof of malignancy [5]. Cell-specific gene expression in LCH lesions shows a distinct profile in contrast to normal epidermal Langerhans cells. The expression profile of CD3(+) cells of LCH lesions is consistent with an activated regulatory T cell phenotype with the increased expression of FOXP3, CTLA4, and SPP1 [6]. Recent advances in LCH immunology suggest that the aberrant immune interaction with T cells may be caused by clonal changes in dendritic cells. These changes lead to the pathological picture that combines the features of cancer and chronic inflammation [7].

Because of its biological behavior and metastatic potential, LCH must be treated like a malignant disease, i.e., with cytotoxic drugs [8]. However, both immunosuppressive and anti-inflammatory approaches have failed to bring a decisive breakthrough in therapy.

Langerhans cells are characterized by a close interaction with the adjacent microenvironment [9]. Microscopically, the lesions present as a heterogeneous mixture

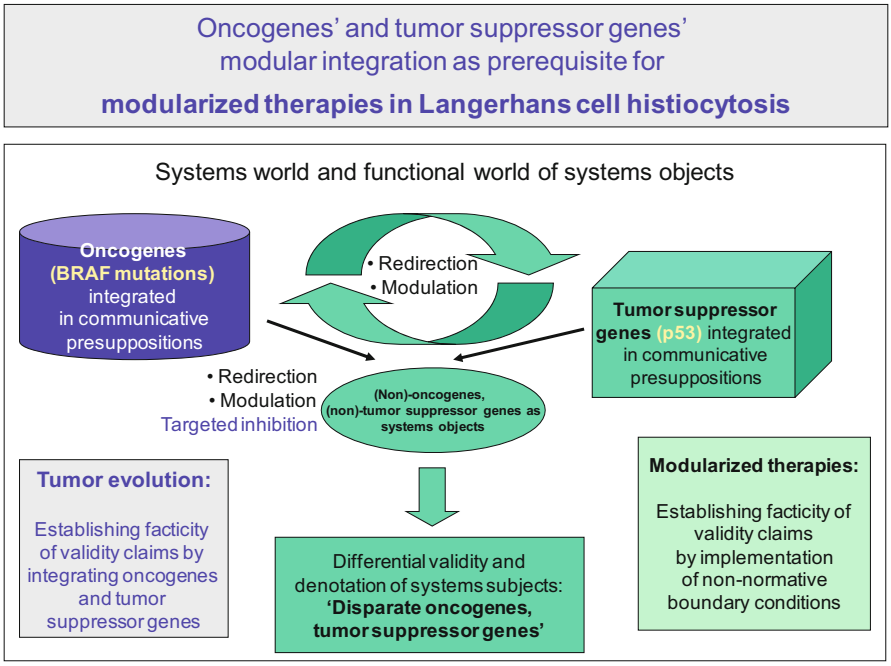

Fig. 4.1 Oncogenes and tumor suppressor genes are communicatively integrated into a systems context to constitute normative notions that facilitate growth promotion and dissemination of LCH-associated lesions. Combined modularized therapies aim at redirecting or impeding tumor-promoting normative notions for attenuating tumor growth or inducing remission

of cell populations with varying proportions of macrophages, giant cells, lymphocytes, and a large number of eosinophilic granulocytes and histiocytes. Currently, the diagnostic standard for LCH is the detection of both CD1a and CD207 in Langerhans cells of LCH lesions.

The strong lesional overexpression of prostaglandins, cyclo-oxygenase-2 (COX-2), and NFkappa-B [1, 2] characterizes the disease as inflammation-dependent. In addition to COX-2 overexpression, an important inflammation-regulating nuclear receptor, i.e. the peroxisome proliferator-activated receptor gamma (PPAR gamma), has been shown to be overexpressed in LCH [10] (Fig. 4.2). The activity mediated by the pharmacological ligand activation of PPAR gamma could be delineated by the action profile of PPAR gamma ligands (pioglitazone) on dendritic cells (DCs): PPAR gamma ligands may promote apoptosis in DCs [11]. An additional therapeutic option in LCH may be angiostatic because significantly increased serum levels of vascular endothelial growth factor (VEGF) were reported in pediatric patients with multisystem (MS)-LCH [12]. Major mechanisms of action of metronomic low-dose chemotherapy are the up-regulation of an antagonist of VEGF, i.e., thrombospondin-1, and the down regulation of regulatory T cells [13].

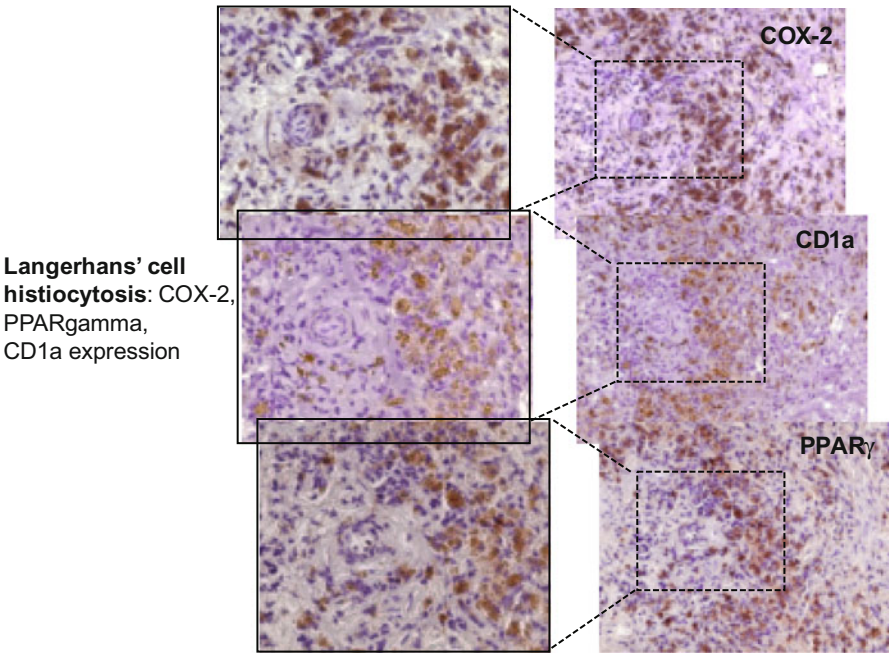

Fig. 4.2 Histiocytes in the inflammatory infiltrate of patient B overexpressed COX-2 and were also clearly positive for PPAR gamma [10]

Therapy of Chemo-Resistant Multi-Systems Langerhans Cell Histiocytosis (MS-LCH)

In our current phase II trial on chemorefractory MS-LCH, we combined anti-inflammatory, angiostatic, and immune-modulatory therapy. Treatment of patients A and B consisted of pioglitazone 45 mg and rofecoxib 25 mg once daily p.o., day 1 +, and angiostatic scheduled (metronomic) low-dose chemotherapy with trofosfamide 50 mg three times daily p.o., starting on day 15 +. Patient C was given pioglitazone etoricoxib 60 mg once daily p.o., day 1 +, with angiostatic scheduled (metronomic) low-dose chemotherapy with trofosfamide 50 mg three times daily p.o., starting on day 1 +. Each of the three patients continued to be treated throughout the trial with pioglitazone and rofecoxib or etoricoxib, respectively (Fig. 4.3) [10, 14, 15]. The patients had given written informed consent, and the trial had been approved by the local ethics committee.

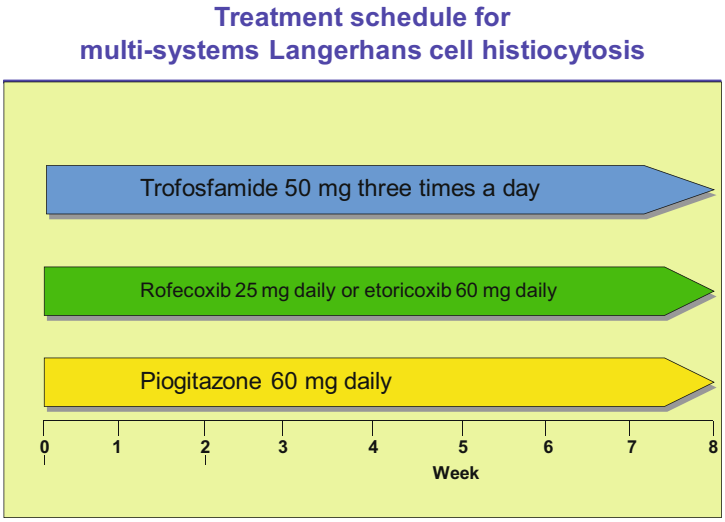

Fig. 4.3 Combined modularized, anti-inflammatory, immune-modulatory, and angiostatic therapy for controlling multiply pre-treated chemorefractory MS-LCH

Three Case Reports

A 31 year-old Caucasian man (**patient A**) had a 5-year history of progressively feeling thirst, coxitis 3 years previously, recurrent otitis media, and progressive respiratory distress. The second patient, a 22 year-old Caucasian man (**patient B**), had a 1-year history of progressive back pain and drenching night sweats. Both were smokers (Figs. 4.4–4.6). The third patient **(patient C)**, a 50 year-old Caucasian man, had a 26-year history of undiagnosed LCH. Symptoms had started with diabetes insipidus and partial hypophyseal insufficiency; additionally, he had suffered diabetes type 2 for 15 years. At diagnosis, an LCH lesion had caused colon perforation. Moreover, multi-focal osteolytic bone lesions, hypophyseal insufficiency, and cutaneous as well as joint involvement had been diagnosed. Upon admission, **patients A** and **B** presented with seborrheic dermatitis, tachypnoea, and a honeycomb lung with multiple small infiltrations, multifocal liver lesions, and hepatosplenomegaly.

Patient A, who was overweight (112 kg, 176 cm), had a painful swelling, a scaly scalp, punched-out osteolytic lesions in the skull, ossa femura, infiltrations in the anterior and posterior pituitary, a thickened pituitary stalk, multifocal dural lesions, and lesions in the medulla oblongata. **Patient B** additionally presented with secondary hypogonadism and os spine involvement, but without any cerebral affliction. Biopsies of the skin (**patient A, C**), the liver (**patient B**), and the hip joint (**patient C**) confirmed the diagnosis of LCH by positive S-100 and CD1a staining of LCH cells. For patient C, LCH was additionally confirmed by the (immuno-) histology of the resected colon lesion.

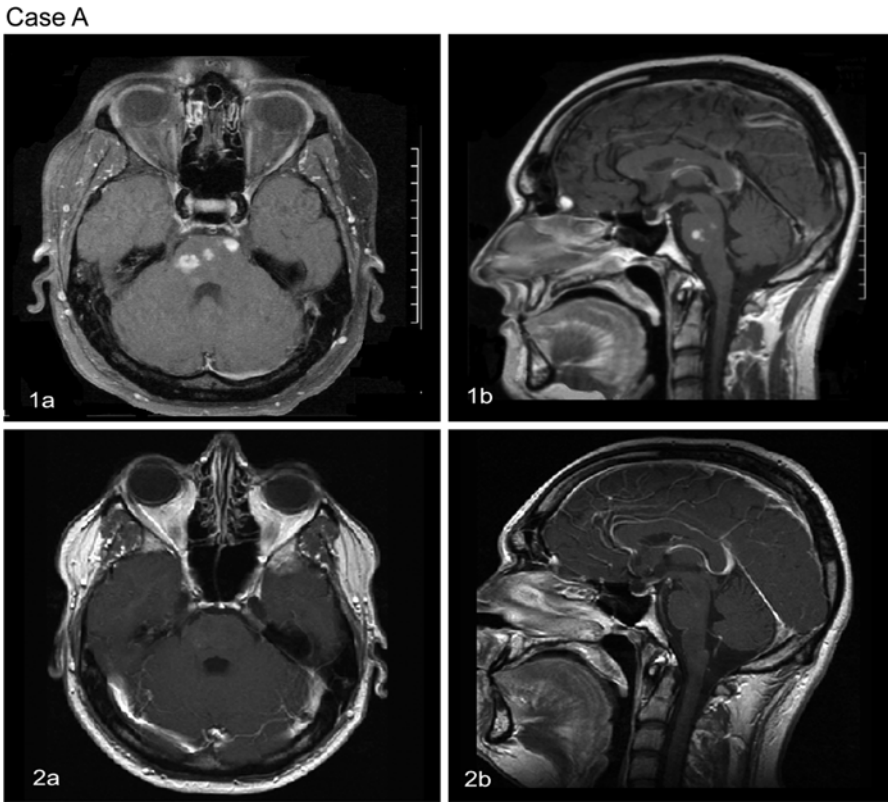

Fig. 4.4 Patient A: Topography of the brain lesions (1**a**, 1**b**) and their resolution after combined modularized therapy (2a, 2b)

Six courses (**patient C**), two courses (**patient A**), and one course (**patient B**) of vinblastine and prednisolone, administered according to the LCH-III trial of the 'Histiocyte Society' [16], had failed to stop the progression of MS-LCH, as had the second-line protocol according to arm B of LCH-III (which included additional methotrexate) in **patient A** and azathioprin in **patient C**.

During further disease progression, **patient A** had suffered from pulmonary embolism (hyperhomocysteinaemia) and purulent cholecystitis (cholecystectomy) on the basis of sclerosing cholangitis. The health status of **patient C** had not improved because of refractory MS-LCH and vinblastine-induced polyneuropathia. Because of cutaneous disease progression, **patient C** had received therapy with Azathioprin, but osseous involvement had been continuously progressive. During the maintenance therapy with mercaptopurine, **patient B** had shown improved lung function and abating skin lesions but unchanged liver and bone lesions [16, 17].

Under combined study medication, we observed continuous tumor resolution (**patient A**) and improvements (**patient C**) of the skin, liver (**patients A and B**),

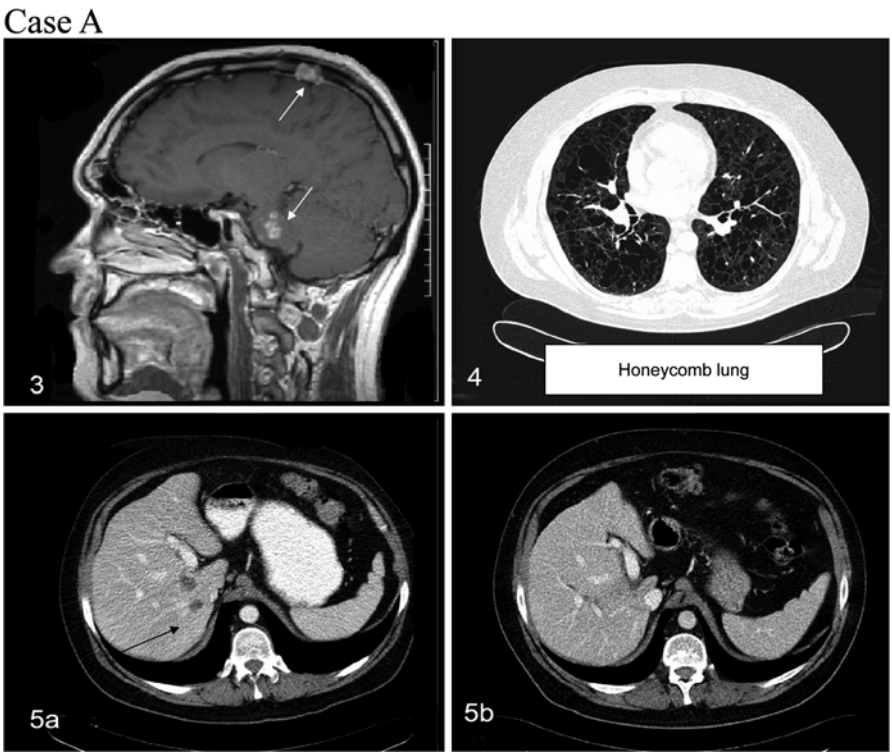

Fig. 4.5 Patient A: Topography of the brain lesions (3), the honeycomb lung (4), and the topography of the liver lesions and their resolution after combined modularized therapy for MS-LCH (5**a**, 5**b**)

lung (**patients A and B**), and brain lesions (**patient A**) but persisting osteolytic lesions (**patients A, B and C**) over a study period of 21 (**A; B**) and 8 months (**C**), respectively. Tumor resolution was confirmed by the biopsy of a residual liver lesion after 6 months on treatment (**patient A**). Functional pituitary deficiencies improved (**patient A**) during the resolution of LCH-associated brain lesions.

Patient A and B had complete remission (CR) after 25 months and 28 months on treatment, respectively. **Patient B** has been disease-free for > 5.5 years after discontinuation of the study medication. **Patient A** relapsed after 74 months and responded again to study medication. **Patient C** remained stable for 9 months (constant osteolytic lesions). The pathological workup of the purulent hip joint tissue (hip endoprothesis) did not show any LCH as histologically verified before the start of the study medication. Hypophyseal parameters did not improve for patient C, nor did his status in diabetes mellitus. This patient continued study medication after his recovery from surgery (10 months+).

The study medication was well tolerated and discontinued 3 months after achieving CR in patient A, B and is on-going in C. In **patients A** and **C**, the trofosfamide

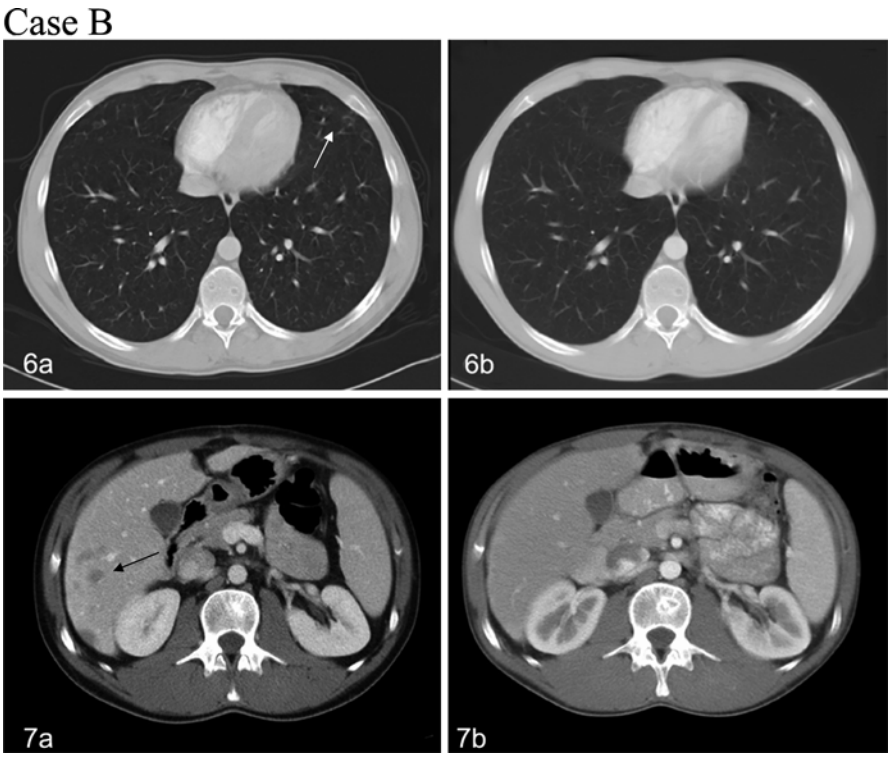

Fig. 4.6 Patient B: Topography of the small lung lesions (6**a**) and liver lesions (7a) and their resolution (6**b**, 7**b**) after combined modularized therapy for MS-LCH

dose was reduced to 50 mg twice daily p.o. after 1 year and after 3 months, respectively, and rofecoxib was reduced to 12.5 mg/d [edema, NCI-CTCAE Grade 1]. No other toxic side effects were observed according to the NCI-CTCAE criteria. two patients had to be hospitalized due to infections in the honeycomb lung and in the hip joint.

Discussion

This report is the first to show induction of continuous complete remission of chemorefractory MS-LCH after combined treatment with metronomic low-dose chemotherapy, a COX-2 inhibitor, and a PPARgamma agonist. The successful clinical courses in three patients with chemorefractory MS-LCH should induce further investigations into combined modularized, immuno-modulatory, anti-inflammatory, and angiostatic therapy in the framework of a multi-center MS-LCH trial (Fig. 4.7).

The activity of combined modularized therapies in systemically pre-treated MS-LCH proves the hypothesis that therapies directed at tumor systems may be able to use

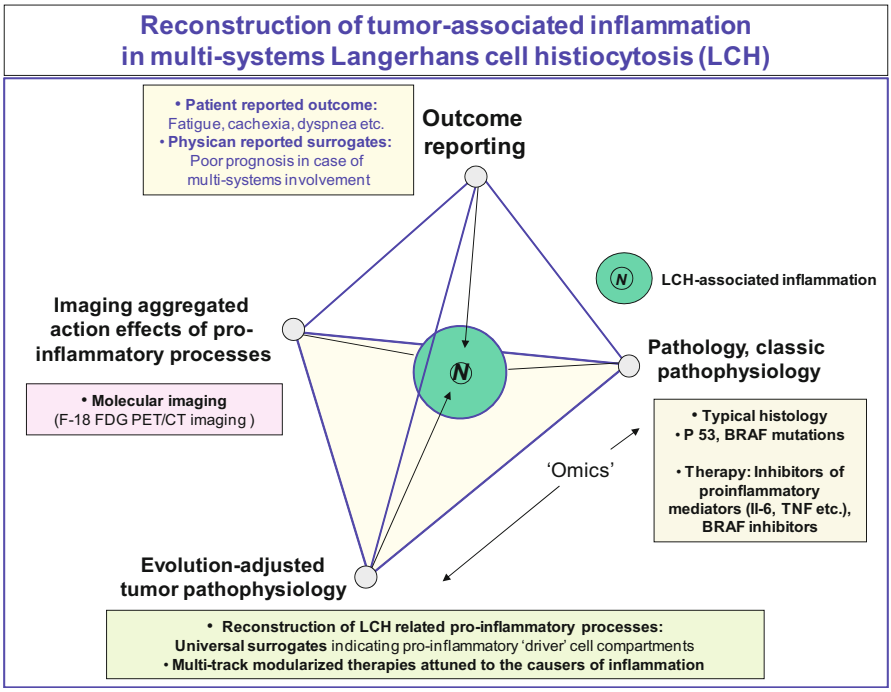

Fig. 4.7 LCH-associated inflammation may be concertedly monitored by classic outcome reporting, classic pathology and pathophysiology, evolution-adjusted tumor pathophysiology, and molecular imaging strategies

aggregated action effects as adjustable sizes to therapeutically modulate the stability, homeostasis, and robustness of tumor systems. In MS-LCH, combined modularized therapies may attenuate tumor-promoting inflammation and induce long-term disease stabilization that is followed by a prolonged objective response, despite the poor monoactivity of the respective drugs.

The still continuing discussions about the origin of the disease and the degree of malignancy were not helpful in generating more efficacious reductionistically derived therapy strategies for MS-LCH. The crucial factor seems to be targeting rationalizations of pivotal disease promoting normative notions, i.e., inflammation, immune response, and angiogenesis, as shown by the capacity of a combined modularized therapeutic strategy for treating chemo-resistant MS-LCH, even with a long-term response.

Impressive rapid responses in systemically pre-treated MS-LCH following the presented multi-track top-down strategy are now contrasted by short-term responses to a typical single-track approach with the B-RAF inhibitor vemurafenib, but at the expense of a higher rate of toxicities [18]. Up to now, continuous complete remission could be only shown for the combined modularized approach.

Understanding systems biology as a therapeutically adjustable size may break through the barrier of complex tumor-stroma-interactions in a therapeutically relevant way: Comparatively high efficacy at moderate toxicity. Structured therapies directed at tumor systems in MS-LCH may provide a source for detecting the topology of tumor-associated aggregated action effects as adjustable sizes available for targeted biomodulatory therapies (Fig. 4.7).

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Chapter 5
Redirecting and Modulating Rationalizations of Tumor-Immanent Normative Functions in Castration-Resistant Prostate Cancer

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Abstract With a median survival period of approximately 19 months, therapeutic options for patients with castration-resistant prostate cancer (CRPC) remain limited. In a multicenter phase II trial, 65 patients with histologically confirmed CRPC continuously received a biomodulatory regimen during the 6-month core period for redirecting tumor-promoting normative notions, i.e. angiogenesis, inflammation, immune response and the osteoplastic process. Treatment comprised daily doses of imatinib mesylate, pioglitazone, etoricoxib, treosulfan, and dexamethasone. The primary endpoint was prostate-specific antigen (PSA) response, defined as a confirmed reduction in serum PSA of $\geq$ 50 % in patients with a baseline value of $\geq$ 5 ng/mL. Responders could enter an extension phase until disease progression or presence of intolerable toxicity. Mean PSA was 45.3 ng/mL at baseline, and 77 % of the patients had a PSA doubling time of $<$ 3 months. Twenty three (37.7 %) out of the 61 evaluable patients were PSA responders, who showed a mean PSA decrease from

Clinical trial registration: NCT00427999 (http://clinicaltrials.gov/ct2/show/NCT00427999)

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278.9 ± 784.1 ng/mL at baseline to 8.8 ± 11.6 ng/mL at the final visit (24 weeks or LOCF). The remaining 38 non-responders included 14 patients (23.0 %) with stable disease. In one center, 6 out of 16 patients showed nearly complete resolution of bone metastases. Out of the 947 adverse events observed, 57.6 % were suspected to be drug-related, 13.8 % led to dose adjustment or permanent discontinuation of the study medication, and 40.2 % required concomitant medication. Twenty seven patients experienced serious adverse events. This novel multi-targeted approach led to an impressive PSA response rate of 37.7 % in CRPC patients despite the fact that individual components had shown limited efficacy when applied on their own. The good PSA response rate and the manageable toxicity profile suggest that this combination may offer an alternative treatment option to present therapeutic regimens.

Introduction

The natural history of metastatic prostate cancer may be divided into two phases: the castration-sensitive stage and the castration-resistant stage, the latter stage requiring a complete change of treatment strategy (Fig. 5.1).

In recent years, a variety of novel compounds have shown a survival benefit in castration-resistant prostate cancer (CRPC), for instance, the vaccine taxane cabazitaxel (Sipuleucel-T) and the androgen biosynthesis inhibitor abiraterone acetate [1, 2]. Further agents are currently under investigation [3], for example, androgen receptor inhibitors (MDV3100), androgen biosynthesis inhibitors, immune-modulating compounds (PROSTVAC-VF), as well as angiogenesis inhibitors (thalidomide, lenalidomine, aflibercept, tasquinimod), orteronel (TAK-700), and radium-223 chloride (Alpharadin). The availability of these new therapeutic approaches allows the exploration of sequential treatment regimens in an attempt to balance the risks and benefits of novel compounds for individual patients with CRPC.

After exploiting novel therapy strategies for achieving hormonal ablation, the standard therapy during the castration-refractory stage is chemotherapy (docetaxel) combined with prednisone. Our novel biomodulatory therapy approach aimed at achieving at least the same efficacy levels (PSA response rate) as chemotherapy

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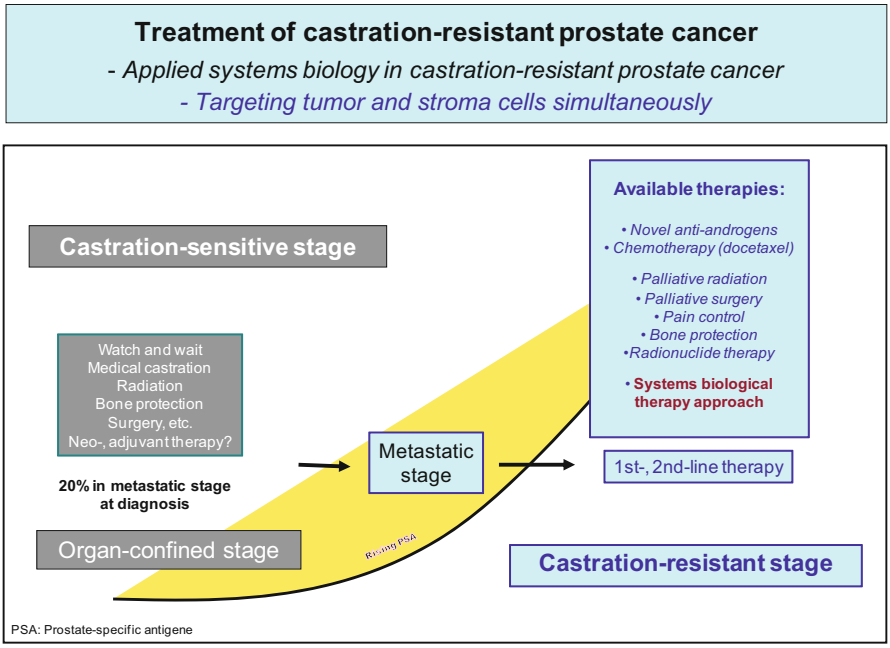
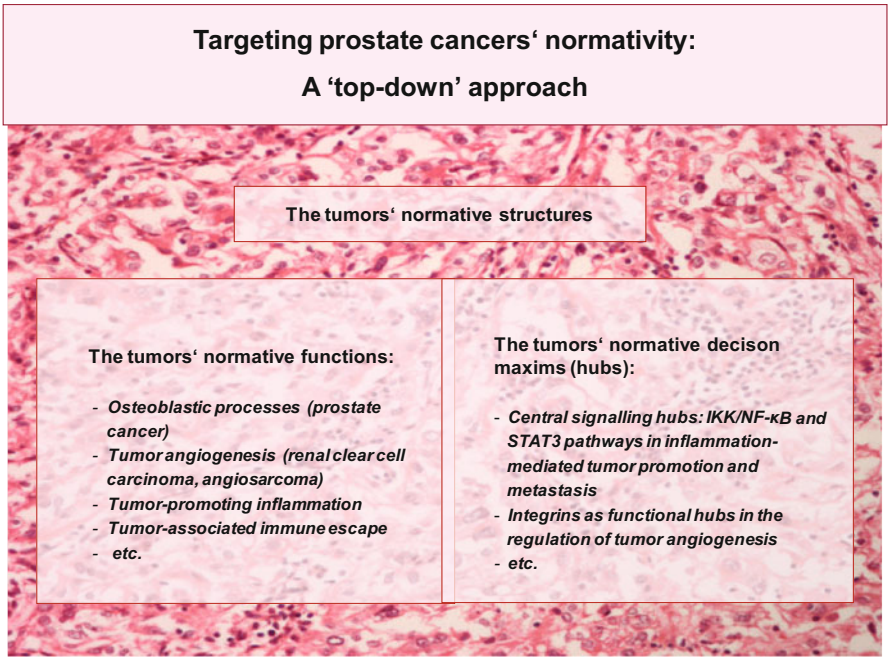


Fig. 5.1 During progression from the organ-confined stage to the clinical metastatic stage, tumors acquire multifold asynchronous chromosomal and molecular-genetic aberrations. Applied systems biology in metastatic tumors may meet this therapeutic challenge by targeting a tumor's normativity, thereby simultaneously and equally targeting tumor and stroma cells

while simultaneously avoiding particularly grade 3 and 4 toxicity levels that are commonly associated with pulsed chemotherapy.

We report the findings of a phase II, single-arm, multicenter trial, in which patients with CRPC received a combination of biomodulatory agents. In contrast to conventional treatment strategies, our multi-targeted therapy exploited the molecular and genetic heterogeneity of both tumor and stroma cells in CRPC [4–6]: A biomodulatory approach of this type aims at comprehensively targeting the pathogenic mechanisms of CRPC, including some of the classic 'hallmarks' of cancer, such as tumor-associated inflammation, angiogenesis, and immune response. Each of these conditions is highly relevant in prostate cancer: Inflammation plays a crucial role in its pathogenesis [7–9], tumor-associated angiogenesis is obligate, and prostate cancer is known to principally be an immunogenic tumor [8–12]. In addition, the current regimen targets the contribution to tumor growth in CRPC made by osteoblasts because osteoblastic metastases represent up to 80 % of organ metastases in prostate cancer [13]. The aim of the multi-targeted treatment regimen in this trial is to modulate and redirect the tumor and stroma cells via ubiquitous and non-oncogene-addicted targets including PDGFR (imatinib) [14, 15], the PPARalpha/gamma receptor (pioglitazone) [16–21], the glucocorticoid receptor (dexamethasone) [20, 21], and the cyclooxygenase-2 inhibitor (etoricoxib) [24, 25]. Such effects are coupled with

Table 5.1 The 'top-down' approach allows redirecting a tumor's normativity by modulating the communicative 'background' that mediates the validity and denotation of tumor-promoting systems participators and organizes the constitution of rationalizations for maintaining tumor-immanent normative notions. The 'background' is modularly arranged and therapeutically accessible with primarily multi-track modularized therapy elements



the pleiotropic/immunomodulatory and angiostatic activity of metronomic low-dose chemotherapy using treosulfan via regulatory T-cells and thrombospondin-1 [26, 27] (Table 5.1). In the current regimen, these drugs were administered to achieve concerted biomodulatory activity by imposing therapeutic boundary conditions in the normative growth of CRPC. Two of the drugs, dexamethasone [22, 23, p. 223] and metronomic low-dose chemotherapy with alkylating agents [28–30], have previously shown mono-activity in CRPC (Fig. 5.2). Other drugs have shown activity in in vitro or animal models but have failed to induce a response in vivo (pioglitazone [20]) or to improve response when added to taxotere (imatinib [15]).

Patients and Methods

Study Design and Conduct

This single-arm, open-label, 6-month phase II trial was conducted at 11 German oncology centers. Patients with CRCP received imatinib mesylate, pioglitazone, etoricoxib, treosulfan, and dexamethasone until progression of the prostate-specific

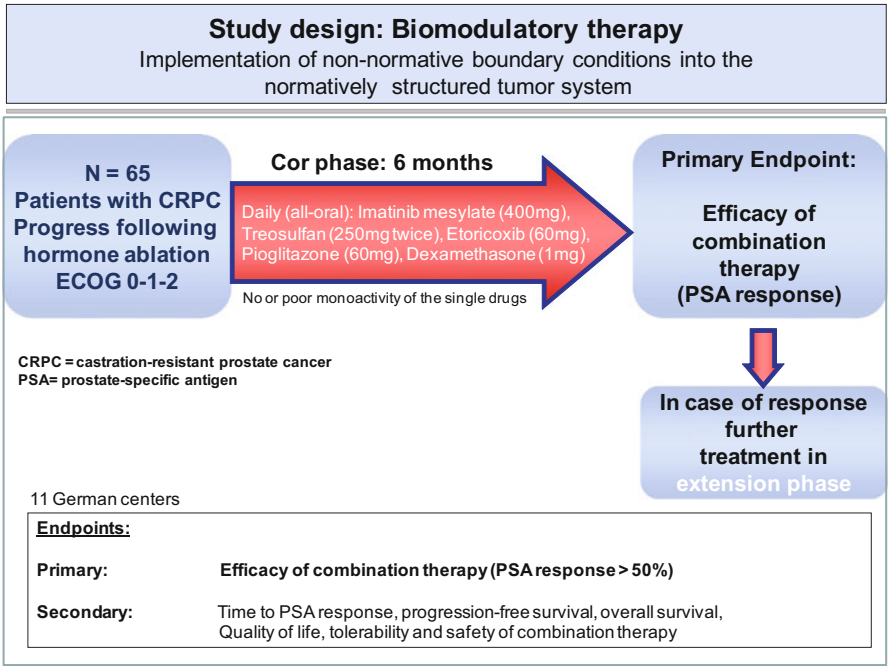


Fig. 5.2 Patients received the all-oral biomodulatory therapy during the core phase. In case of stable disease or > 50 % PSA response, patients were allowed to proceed to the extension phase until disease progression

antigen (PSA) (Fig. 5.3). At the end of the core 6-month trial, patients responsive to study medication (defined at a PSA decrease of ≥ 30 % from baseline and a 24-week treatment period without any signs of disease progression) proceeded to an extension phase until disease progression or presence of intolerable toxicity levels. The study protocol had been approved by the institutional review board of the participating centers and by the health authorities. Written informed consent from patients had been obtained before enrolment.

The trial was sponsored by Novartis Pharma GmbH and registered at ClinicalTrials.gov (NCT: NCT00427999).

Study Population

Male patients aged ≥ 18 years were included, who had histologically confirmed prostate cancer with proven progression after androgen deprivation therapy (surgical or medical castration). For inclusion, patients had to have total serum testosterone of < 1.72 nmol/L (50 ng/dL). Patients also had to be castration-resistant, which needed to be confirmed by three consecutive elevated (≥ 50 % above nadir) serum PSA tests separated by at least two weeks, and the last 2 PSA measurements had to be ≥ 5.0 ng/mL despite secondary hormonal manipulations (according to the European

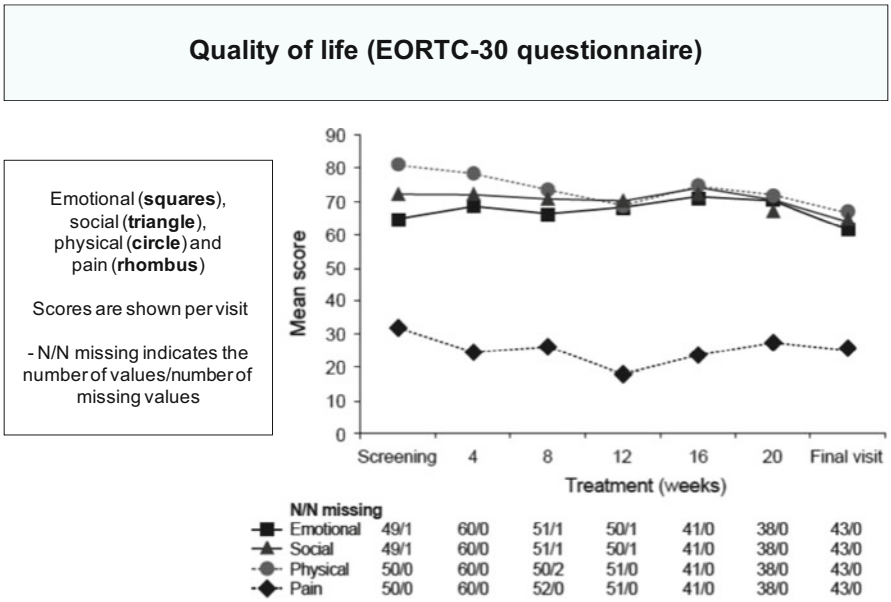


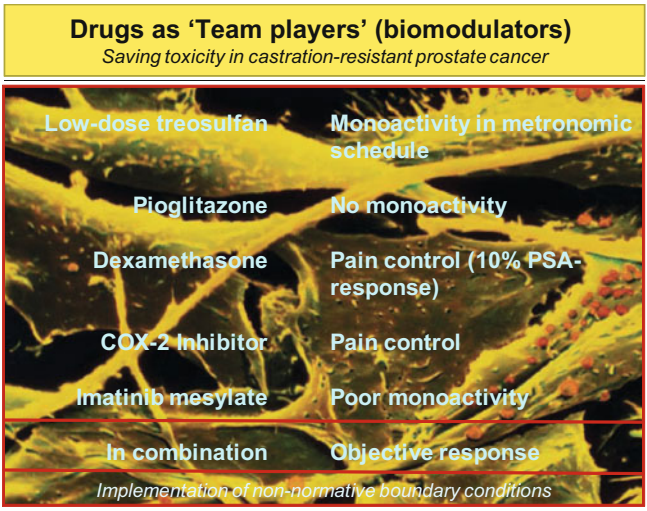
Fig. 5.3 Quality of life scores remained stable throughout the core phase for all patients regardless of response behavior

Association of Urology (EAU)) [31]. Additional inclusion criteria were a performance status of ≤ 2 according to the Eastern Cooperative Oncology Group (ECOG), an adequate hematological status (defined as absolute neutrophil count $> 1{,}500/\text{mm}^3$, platelet count $> 75{,}000/\text{mm}^3$), normal hepatic, renal, and cardiac function, and a life expectancy of at least 6 months. Key exclusion criteria included use of chemotherapy, treatment with imatinib or other tyrosine kinase inhibitors, concomitant therapy with other tumor treatments except for LHRH agonists, concomitant warfarin, phenprocoumon or other oral anticoagulant treatment, radiotherapy of > 25 % of the bone marrow, systemic radioisotope therapy, uncontrolled brain metastases, regular blood transfusions, and previous secondary malignant disease within the past 5 years. Patients with the following comorbidities were excluded: Symptomatic congestive heart failure, unstable angina or recent myocardial infarction, uncontrolled diabetes, chronic hepatic or renal disease, active uncontrolled infection, chronic inflammatory intestinal disease, autoimmune disease or a known diagnosis of HIV, or hepatitis B or C infection.

Interventions

Eligible patients received oral doses of imatinib mesylate (400 mg daily), pioglitazone (60 mg daily), etoricoxib (60 mg daily), treosulfan (250 mg twice daily), and dexamethasone (1 mg daily) until PSA progression. Patients with PSA progression were switched to a dose of 400 mg imatinib twice daily and treosulfan 250 mg daily;

Table 5.2 The 'top-down' approach allows combining drugs with poor or no mono-activity in the respective cancer type. A primarily multi-track approach may be important for efficaciously implementing non-normative boundary conditions to redirect and modulate a tumor's normativity



in case of further progression, patients were withdrawn from the trial. Dose reductions were permitted for intolerable non-hematologic or hematologic grade 2 toxicity or any grade 3 or 4 toxicity. Study medication was restarted after the toxicity of the respective drug(s) had resolved or decreased to less than grade 2 or less than grade 3, depending on the toxicity and respective drug. If toxicity recurred despite dose reduction(s), the relevant drug was withdrawn. Dose reductions, if required, were specified as follows: Reduced dose for imatinib depending on dose and toxicity grade (between 200–600 mg/day, 60 mg every second day for etoricoxib, 45 mg/day for pioglitazone, 0.5 mg/day for dexamethasone, and 250 mg/day for treosulfan). Study participation was discontinued if medication could not be maintained at a minimum of treosulfan 250 mg/day in addition to one biomodulator (etoricoxib or pioglitazone or imatinib) plus dexamethasone after a 4-week interruption because of grade 2–4 toxicity. Patients were also to discontinue the trial if they refused to continue therapy, in response to protocol violations, or administrative problems (Table 5.2).

Concomitant use of bisphosphonates was allowed.

Evaluation

During screening, all patients underwent imaging by CT, MRI, or plain radiography as necessary to confirm metastatic sites. A radioisotope bone scan was conducted for patients with bone metastases. Pre-treatment evaluations included medical history, ECOG performance status and vital signs, physical examination, electrocardiogram laboratory screening including PSA and testosterone levels, coagulation assessment, urinalysis, electrocardiography, and assessment of quality of life (EORTC-30 questionnaire). During the 6-month core trial, PSA values, ECOG performance status,

and quality of life were assessed monthly. Physical examination, vital signs, and blood tests were conducted after 1, 2, 4, 8, 12, and 16 weeks, and coagulation was measured after 4 weeks and subsequently, if clinically indicated. Urinalysis and imaging by CT, MRI, plain radiography, or bone scanning were conducted as clinically indicated. At the final visit of the core trial, ECOG performance status, vital signs, and concomitant medication and therapies were recorded, and physical examination, laboratory screening including PSA, coagulation and urinalysis, quality of life assessment, and imaging (if clinically indicated) were conducted. Adverse events were monitored throughout the trial and graded according to the Common Terminology Criteria for adverse events established by the National Cancer Institute (version 3.0).

Study Endpoints

The primary endpoint was PSA response, defined as a reduction in serum PSA of $\geq$ 50 % compared to baseline value; confirmation was obtained by a second PSA value 3–4 weeks later. Patients who did not fulfill these criteria were defined as PSA non-responders and were categorized as having PSA progression or stable disease. PSA progression was defined as a PSA increase of at least 50 % over baseline or an increase of at least 25 % over baseline with an absolute PSA increase of 5 ng/L, which had to be confirmed 3–4 weeks later. PSA non-responders were considered to have stable disease if they did not meet the criteria for progressive disease. Tumor response was evaluated by the Response Evaluation Criteria in Solid Tumors (RECIST) [13], if adequate imaging data were available for follow-up.

Secondary endpoints included the time to PSA response (defined as the time from the first administration of the study drugs to the first confirmed PSA response), progression-free survival (defined as the time from the first administration of the study drugs to the first date of PSA progression, overall survival during the extension phase of the trial, quality of life including pain response (EORTC-30 questionnaire), and safety and tolerability of the combined therapy.

Statistical Analysis

The sample size calculation estimated that 46 evaluable patients would be required to distinguish between the two rates 40 % (p_1) and 25 % (p_0) with a one-sided alpha of 10 and 80 % power under the assumption of a 20 % dropout rate [2]. Sample size was estimated according to exact binomial probabilities. The first design (and hence the lowest number) that satisfied the design criteria (alpha and power constraints) was chosen.

The intent-to-treat (ITT) population was defined as patients who received at least one dose of the study medication and who provided two consecutive post-baseline PSA values. The number of PSA responders is presented with the corresponding 95 % confidence interval for ITT populations. Data on quality of life as assessed by the EORTC-30 questionnaire are presented descriptively.

Table 5.3 In case of grade 2 toxicities, patients were scheduled according to protocol to receive dose reductions

Interventions
• **Dose reductions**, *if required, were specified as follows:* – 60 mg every second day for etoricoxib – 45 mg/day for pioglitazone – 0.5 mg/day for dexamethasone – 250 mg/day for treosulfan *(about 3 mg/kg body weight)* – imatinib depending on dose and toxicity grade (between 200-600 mg/day) • **Study withdrawal was to take place** *if medication could not be maintained at a* – minimum of treosulfan 250 mg/day plus one – biomodulator (etoricoxib, or pioglitazone or imatinib) – plus dexamethasone following a four-week interruption due to grade 2-4 toxicity.

Results

Patients

Between February 2007 and October 2009, 65 patients received at least one treatment with the study medication; 61 out of these 65 patients provided two consecutive PSA values and were thus included into the ITT population (Table 5.3). Thirty-two patients discontinued the trial prematurely, mostly because of disease progression ($n = 13$), consent withdrawal ($n = 9$), or withdrawal of medication because of side effects ($n = 6$). The mean time in the core trial was 141 days, and the mean duration of at least minimal therapy was 121 days.

Eighteen patients entered the extension phase of the trial, which is still ongoing. One patient has been followed in the extension trial since June 2008 without any disease progression or occurrence of intolerable toxicity levels. Mean PSA at baseline was 45.3 ng/mL with values ranging from 5 to 3603 ng/mL. Approximately 78 % of the patients had bone metastases, 34 % had measurable lymph node involvement, and 8 % had measurable organ involvement (Table 5.1).

Combined Modularized Therapy

PSA response Twenty three patients showed PSA response (37.7 %, 95 % CI 50.1, 74.5). Among responders, mean PSA levels decreased from 278.9 ± 784.1 ng/mL at

baseline to 8.8 ± 11.6 ng/mL at the last visit. The remaining 38 patients (62.3 %, 95 % Cl 25.5, 49.9) were considered PSA non-responders, and 14 of them (14/61, 23.0 %) showed stable disease. Altogether, 37 patients (60.6 %) responded or had stable PSA levels during the trial. The evolution of PSA level in these three categories of patients is presented in Fig. 5.1. In total, 26 patients (42.6 %) had a PSA decline of ≥ 50 %, and a further 12 patients (19.6 %) had a PSA reduction of < 50 % during the 6-month core trial at one measured time point. A PSA reduction of > 50 % was also observed in five patients with a baseline PSA doubling time of < 3 months.

Twelve patients received an imatinib dose increased from 400 to 800 mg as per protocol because of PSA progression, but this increase did not improve PSA response in any of the patients. PSA response occurred independent of the presence of distant metastases and the metastatic site.

Seventy-seven percent of the patients required some type of dose modification or a temporary interruption of the study drug because of a non-hematologic or hematologic toxicity grade 2 or because of adverse events. Nevertheless, over 60 % of the study population either showed a PSA response or maintained a stable disease course.

Neither median time to PSA response nor overall or progression-free survival could be achieved.

Time to doubling of the PSA level at baseline (ITT population) For correctly estimating the PSA response rate, it should be mentioned that 77 % of the patients had a PSA doubling time of < 3 months.

Adverse events and serious adverse events Each of the 65 patients experienced one or more adverse event (Table 5.2). The majority of adverse events (97.1 %; $n = 919$) did not result in a permanent discontinuation of the study medication. Out of the 947 adverse events reported, 545 (57.6 %) were suspected to be drug-related, 131 (13.8 %) led to dose adjustment or temporary interruption, 27 (2.9 %) led to permanent discontinuation, and 381 (40.2 %) required concomitant medication or non-drug therapy.

The most frequently reported drug-related adverse events (> 20 % of the patients) were peripheral edema (56.9 %), nausea (38.5 %), edema (36.9 %), fatigue (35.4 %), dyspnea (35.4 %), anemia (33.8 %), leukopenia (29.2 %), diarrhea (23.1 %), vomiting (23.1 %), facial edema (23.1 %), muscle spasms (21.5 %), and increased weight (21.5 %). In total, 98 serious adverse events were reported in 27 patients (41.5 %); 32 of these events that occurred in 14 patients were drug-related and led to permanent discontinuation of the study drug in five patients. The most frequent drug-related serious adverse events (> 2 % of the patients) were general disorders and administration site conditions (7.7 %), blood and lymphatic system disorders (6.2 %), infections and infestations (4.6 %), nervous system disorders (4.6 %), and gastrointestinal disorders (3.1 %) (Table 5.4 and 5.5).

Four patients (6.2 %) died during the core trial, either as a result of tumor progression [1], acute respiratory insufficiency because of progression of prostate cancer [1], acute respir atory distress syndrome because of pneumonia [1], or cardiac arrest and pulmonary arrest [1].

Table 5.4 Patients characteristics compare to large randomized trials, except that the present patient population had a high rate of short PSA doubling times at base-line (< 3 months)

Patient demographics and clinical characteristics (ITT population, N=61)	
Age, mean (range)	67 (50-83)
→ PSA at baseline, mean (range) (ng/mL)	45.3 (5-3603)
ECOG performance status	
ECOG 0, N (%)	49 (80.3)
ECOG 1, N (%)	11 (18.0)
ECOG 2, N, (%)	1 (1.6)
Previous therapy, N (%)	
Prostatectomy	22 (36.1)
Radiation	35 (57.4)
Hormone therapy	61 (100)
Tumor staging (initial diagnosis), N (%)	
T1	4 (6.6)
T2	10 (16,4)
T3	21 (35.0)
T4	16 (36,7)
NA/NX	10 (16.3)
Lymph nodes	
N0	21 (34,4)
N1	16 (26,2)
N2	2 (3,3)
NA/NX	22 (36,1)
→ Metastasis (78% bone metastases)	
M0	19 (31,1)
M1	32 (52,5)
M2	1 (1,6)
NA/NX	9 (14,8)

77% of the patients had a PSA doubling time < 3 months

Quality of life (EORTC-30 questionnaire) Quality of life assessment showed that social, emotional, and physical function scores remained stable throughout the core phase of the trial (Fig. 5.3).

Redirecting and Modulating Tumor-Immanent Normative Functions

Clinically, the trial shows the modulation and redirection of important normative functions maintained by castration-resistant prostate cancer.

Immunmodulation Rapid tumor response and recovery from tumor-associated lupus erythematodes could be shown after metronomic low-dose chemotherapy in addition to combined transcriptional modulation with pioglitazone and dexamethasone. This therapy was followed by an objective response in liver metastases (Fig. 5.4).

Table 5.5 The rate of grade 3 and 4 was low, as dose reductions already took place in case of grade 2 toxicities. Cumulative grade 3 and 4 toxicities compare with abiraterone trials [2]

Adverse events and serious adverse events

	Patients N (%)	
All adverse events	**65 (100)**	
With suspected relation to study drug	64 (98.5)	
Leading to dose adjustment or temporary interruption	50 (76.9)	
Leading to permanent discontinuation	15 (23.1)	
Requiring concomitant medication/non-drug therapy	62 (95.4)	
All serious adverse events	**27 (41.5)**	
Deaths	4 (6.2)	
With suspected relation to study drug	14 (21.5)	
Leading to permanent discontinuation	5 (7.7)	
Frequent adverse events (>20%)[a]	**All grades**	**Grade => 3**
Peripheral edema	38 (58.5)	1 (1.5)
Nausea	30 (46.2)	3 (4.6)
Fatigue	29 (44,6)	8 (12.3)
Diarrhea	29 (44.6)	0 (0.0)
Dyspnea	26 (40.0)	5 (6.2)
Edema	25 (38.5)	0 (0.0)
Anemia	24 (36.9)	4 (6.2)
Leukopenia	20 (30.8)	5 (7.7)
Vomiting	19 (29.2)	2 (3.1)
Muscle cramps	16 (24.6)	1 (1.5)
Facial edema	15 (23.1)	2 (3.1)
Increased weight	14 (21.5)	0 (0.0)
Increased blood lactate dehydrogenase	14 (21.5)	1 (1.5)

Marked reduction of technetium up-take after biomodulatory therapy A marked reduction or disappearance of bone metastases in control bone scans could be observed at one center during 6 out of 16 examinations (according to protocol, routine diagnostic investigations did not include follow-up bone scans). Figure 5.5 shows the example of a patient who experienced a steep decrease in the PSA level (from 2137 to 0.73 ng/mL at month 12) accompanied by an impressive decrease in bone metastases. Two patients with extensive lymphatic metastases showed calcifications in the lymph node tissue and partial remission according to the RECIST criteria.

Induction of a biological memory In 3 patients, the study medication was discontinued after a PSA response of > 50 % due to hip or knee replacement (degenerative diseases). The PSA doubling time was up to 10-fold higher (12.5–15 months) compared to baseline (Fig. 5.6). All patients responded to retreatment. One patient who had been progressive during retreatment responded again to additional treatment with a gonadotropin-releasing hormone (GnRH) agonist.

These results indicate that the biomodulatory therapy approach may induce a biological memory for tumor growth control, presumably based on epigenetic changes mediated by the preceding combined transcriptional modulation with pioglitazone plus dexamethasone (Fig. 5.7).

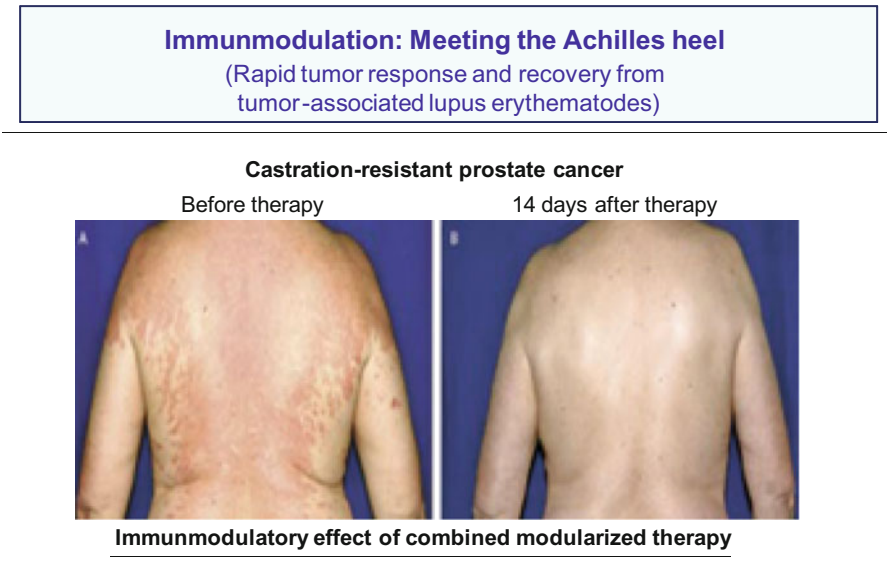
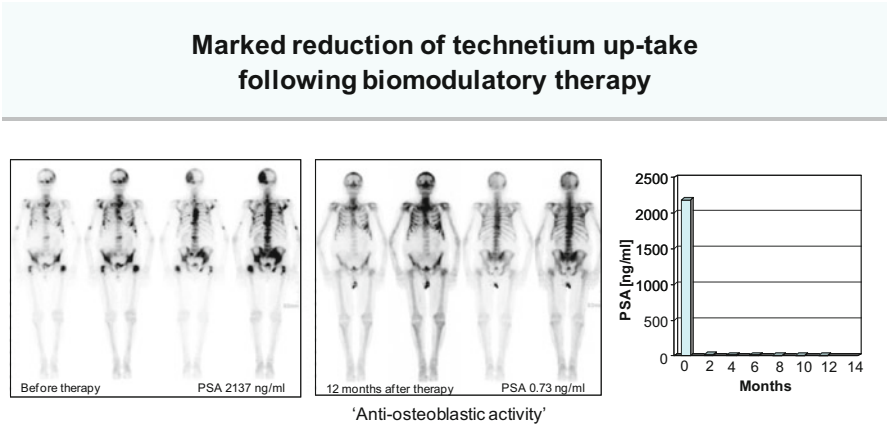


Fig. 5.4 The immunmodulatory activity of the schedule is underlined by the rapid control of paraneoplastic lupus erythematodes before the onset of objective tumor response



Example: 80 years old patient:

- Dramatic decrease of technetium up-take in bone scans
- Steep decrease of PSA levels in serum to 0.7 ng/ml during therapy

Fig. 5.5 Steep PSA decrease was accompanied by the resolution of skeletal lesions visible from the missing technetium up-take in the bone scan. Strong antiosteoplastic activity is assumed on the background that pioglitazone may inhibit the maturation of mesenchymal cell to osteoblasts

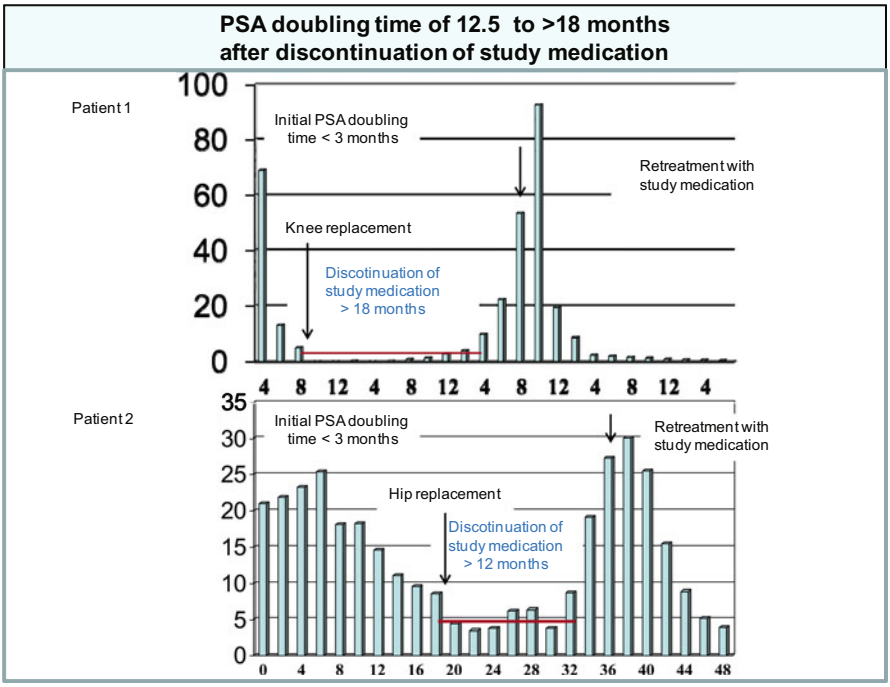


Fig. 5.6 The rapid base-line PSA doubling time of < 3 months was up to ten times prolonged after the discontinuation of the study medication due to surgeries. Retreatment again was efficacious

Are there different rationalizations for tumor-associated normative functions? Biomodulatory therapies are useful to uncover different rationalization processes for tumor-immanent normative functions, because they simply implement non-normative boundary conditions in a tumor system to force the system to start communicative activities by modularly rearranging tumor-associated systems functions.

Sixty percent of the patients responded to biomodulatory therapy with disease stabilization or a > 50 % PSA response. However, 40 % of the patients did not respond, although the frequency of osteoblastic metastases in this group was the same (80 %). Therefore, normative notions in the latter patient population are differentially rationalized and not accessible by the administered combined modularized therapy approach. Figure 5.8 presents PSA changes by patient from baseline.

Discussion: Top-Down Strategy

The results of this phase II trial suggest that the combination therapy with the oral biomodulatory active drugs imatinib, treosulfan, etoricoxib, pioglitazone, and dexamethasone induces PSA responses of ≥ 50 % in almost 40 % of patients when used as a first-line therapy for CRPC. This response rate is comparable with that achieved with standard chemotherapies, such as docetaxel (45 %) or mitoxantrone (32 %)

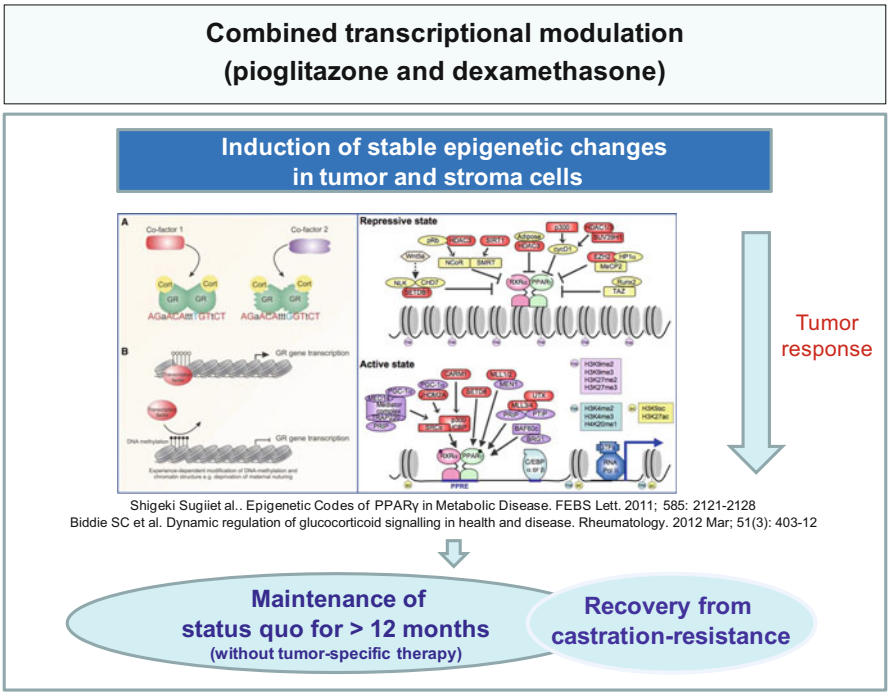


Fig. 5.7 Combined transcriptional modulation is assumed to induce stable epigenetic alterations in a tumor that might be responsible for maintaining response

[32–34] and presumably much higher than in 'low-risk' patients receiving glucocorticoids only (7 % up to 67 %) [22, 23]. Phase II trials on abiraterone achieved PSA response rates between 36 to 67 % [2], again indicating that response is dependent on disease characteristics of included patients. Moreover, this encouraging finding was accompanied by a low rate of acute toxicity of the study regimen, as indicated by patient-reported outcomes (quality of life assessments). Early dose reductions as a response to increased toxicity levels allowed the continuation of the treatment regimen over an extended period. These findings increase the possibility that this biomodulatory strategy could achieve long-term tumor control with a very low tumor burden.

Previous phase II trials have shown that dexamethasone 2 mg daily or metronomic low-dose cyclophosphamide or combinations can achieve a PSA response (again defined as $\geq$ 50 % decrease in PSA from baseline) in more than 50 % of asymptomatic patients [26]. However, the novel regimen used in the current trial may induce an objective response even in patients with rapid PSA doubling times (a majority of patients in the present study population) and extensive tumor load (Fig. 5.5). In addition, a marked reduction or nearly complete disappearance of bone metastases was observed in bone scans at one center in 6 out of 16 patients (the patients were not systematically screened during the follow-up). These patients experienced long-term tumor control at a low tumor burden. Two out of 16 patients at one center showed tumor necrosis with saponification as indicated by lymph node calcification.

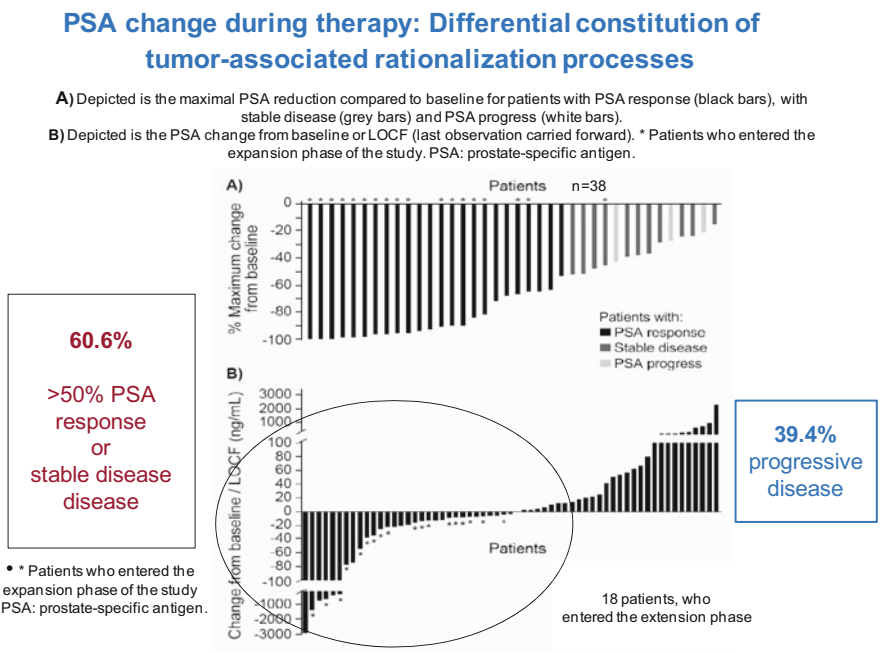


Fig. 5.8 The combined modularized therapy approach may control the osteoplastic metastases in about 60 % of the patients. Although non-responders had the same percentage of bone involvement, therapy was inefficacious in about 40 % of non-responders. This inefficacy indicates that the osteoplastic process in prostate cancer is differently rationalized

Our therapeutic schedule did not include any classic cytotoxic agent, thus drug-related toxicity levels of standard chemotherapy regimens could be avoided [32, 33]. Although all patients experienced at least one adverse event, drug-related toxicity was generally manageable after prompt dose modifications for events of grade 1 or 2 toxicity. These changes did not appear to markedly limit the efficacy of the regimen: Although 77 % of the patients required some type of dose modification or a temporary interruption of the study drug, over 60 % of the study population showed either a PSA response or maintained a stable disease course. In addition, quality of life was maintained throughout the trial.

The combined activity of individual compounds in this regimen and particularly the concerted effect of metronomic low-dose chemotherapy and other biomodulators has been proven previously [28]. Using a similar therapeutic strategy by combining etoricoxib, pioglitazone, dexamethasone, and metronomically administered capecitabine after first-line chemotherapy, a high PSA response rate was observed (41 %), which was superior to that of standard-dose capecitabine alone in historical controls (12 %) [30, 35]. In biomodulatory regimens, the activity of one single drug cannot be defined, because mono-activity is not a prerequisite for concerted activity. The combination, however, must facilitate non-normative boundary conditions to redirect tumor-promoting action norms. Monitoring biomodulatory activity requires serum analytics of the secretome derived from specific cellular compartments in the tumor and could provide novel functional signatures [36, 37]. Such

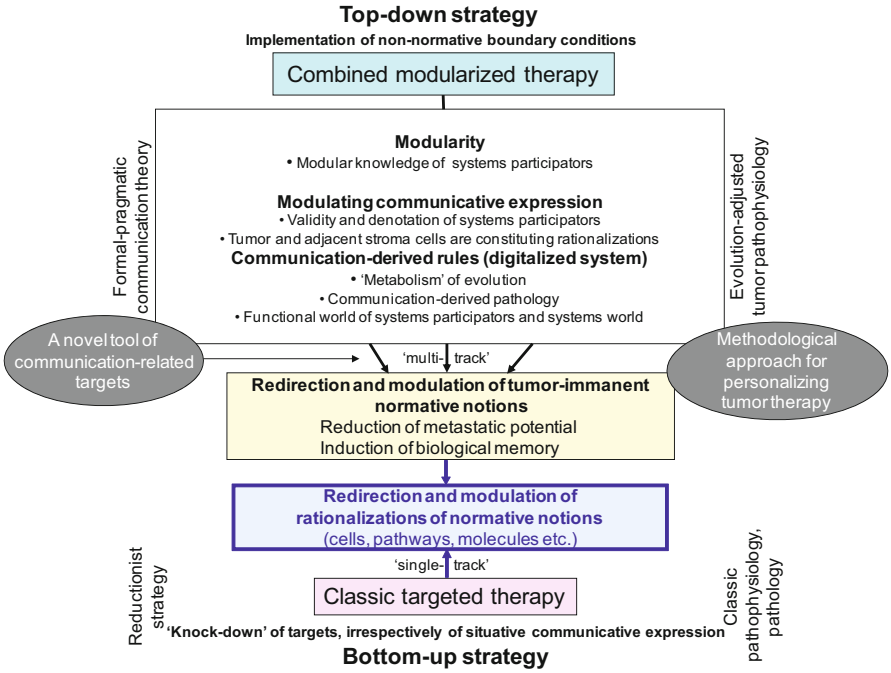


Fig. 5.9 Finally, either the top-down approach or the bottom-up approach has to redirect or modulate rationalizations of normative notions to attenuate tumor growth. The modes how the approaches achieve this aim are rather different: The traditional bottom-up approach tries to knock-down the function of assumed tumor-promoting pathways with single-track or combined single-track methods, irrespective of their communicative expression in the concert of additional tumor-relevant aberrations. The top-down approach aims at targeting a tumor's normativity with a primarily multi-track approach by redirecting the communicative background of assumed tumor-promoting pathways

an analysis could determine which components of the cocktail are redundant or essential and which have additive or synergistic effects. Moreover, this analysis may also provide clues for repurposing drugs and for establishing adaptive trial designs [38–41].

The central therapeutic problem of tumor heterogeneity, particularly in CRPC [42–44], may be addressed by targeting selected normative notions, including particularly the 'hallmarks' of cancer by a 'top-down' approach (Fig. 5.9). Such a novel therapy strategy aims at redirecting the communicative expression of tumor-promoting systems participators, pathways, communication lines, etc. by modulating their communicative 'background'. Such an approach primarily necessitates a multi-track approach to facilitate concerted biomodulatory drug activity, aiding the inclusion of drugs with poor or no mono-activity in the respective tumor type.

Particularly the combined transcriptional modulation opens up completely new therapeutic strategies, such as the implementation of a presumably epigenetically maintained biological memory. Additionally, biomodulatory therapies contribute

to uncovering differently organized rationalizations for tumor-specific normative functions.

Promising clinical data indicate that combined modularized therapies that modulate tumor-associated angiogenesis, inflammation, and immune response in CRPC need to be explored further. In addition, biomodulatory therapies targeting tumor-immanent normative notions may also be effective for the large and expanding group of elderly and frail patients because of its favorable toxicity profile [45–47]. Finally, biomodulatory therapy schedules offer the important opportunity of combining tumor-specific medications with relatively mild mono-activity to achieve synergistic effects.

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Part III
Social Engineering: Biomodulation, Either Endogenously Initiated or by Implementation of Non-normative Boundary Conditions

Chapter 6
Non-Hierarchically Organized Operations in Malignancies: Stromal Dysfunction Induces and Maintains Hematopoietic Malignancies

J. Grassinger and R. Schelker

Abstract Hematopoiesis within adult mammals takes place in a special microenvironment within the bone marrow (BM), the stem cell niche. Cellular and extracellular components of the niche support hematopoiesis by maintaining hematopoietic stem cells (HSC) in a quiescent state but also provide growth signals in response to extrinsic hematopoietic stress. According to the cancer stem cell (CSC) hypothesis, leukemogenesis is a hierarchical process induced by malignant transformation of HSC into leukemia stem cells (LSC). The dogmatic view of leukemogenesis so far suggested a primarily intrinsic cause for malignant transformation of hematopoietic cells due to genetic or epigenetic disarrangements. Recent data, however, proposes that the induction of LSC and their maintenance is associated with an aberrant stem cell niche. In this chapter we summarize basic features of the physiological and aberrant stem cell niche focusing on mesenchymal stromal cells (MSC) and osteoblasts.

Introduction: An Advanced Concept of Leukemogenesis

The hematopoietic system is the best characterized stem cell system in human and animal organisms. Therefore it is not surprising that the cancer stem cell (CSC) model was initially established in hematologic malignancies. The existence of CSC was proposed over 50 years ago as animal models demonstrated that not all cells within a tumor could form the malignant disease in vivo [1]. This hierarchical concept of tumor cell heterogeneity suggests that only a small subset of tumor cells have the ability to sustain tumor development and growth [2].

In 1994 the experimental proof for this hypothesis was made as it was shown that only a subset of acute myeloid leukemia (AML) cells could engraft in immunodeficient mice [3]. Interestingly, theses cells were enriched in the $CD34^{+}$ $CD38^{-}$ fraction resembling physiological HSC. Likewise in chronic myeloid leukemia (CML) it was demonstrated that the underlying LSC share common features with physiological HSC and that the malignant bcr/abl translocation occurs most likely within HSC [4].

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In the meantime the CSC concept was established in a variety of other malignancies [5]. However, recent data also suggests that in some tumors a hierarchical organization of the malignancy is missing or heterogeneous phenotypes within a tumor can be defined as CSC populations [6–9]. This is not surprising as aberrant expression of surface markers in malignant cells is not uncommon. Also, the origin of LSC does not have to be HSC but mature progenitors that transform malignantly and regain stem cell like features by settling into the niche [10].

The CSC hypothesis explains phenomena like therapy resistance and tumor relapse after initial sufficient therapy as CSC have distinct functional properties in contrast to leukemic blasts. This includes anti-apoptotic properties, multi drug resistance (MDR) activity and quiescence [11, 12]. Recent data suggests that these properties are not only intrinsic but induced and regulated by the microenvironment [13]. Especially in acute leukemia, myelodysplastic or myeloproliferative neoplasms and myeloma the aberrant conversion of the microenvironment augments or maybe even induces malignant transformation of hematopoietic cells. This is supported by reports of genetic alterations within the microenvironment of patients with AML or myelodysplastic syndrome (MDS) being different to the genetic alterations within the corresponding leukemia cells [14]. Walkley et al. reported in 2007 that the disruption of the intercellular cross talk between HSC and bone marrow stromal cells by inactivation of retinoblastoma protein (rb) caused a sustained myeloproliferation. In this case, reduced signaling through rb caused an amplified cell cycle of the HSC [15].

In regard to this new concept it is necessary to strongly consider dynamic interactions between CSC and environmental elements as the basis of tumor evolution rather than hierarchical operations. In this non-hierarchical concept, a therapeutic approach has to be individual, dependent on the particular evolutional changes in both the environment cells and the CSC and on the communication inside the tumor microenvironment. This holds true for solid tumors and hematological malignancies.

In general, two not necessarily distinct possibilities of tumor generation and propagation within the microenvironment are conceivable. Ajar the “two hit” model of tumorgenesis in solid tumors the first event in leukemogenesis can occur within the HSC itself as a result of genetic or epigenetic events leading to an autonomic growth and tumor development. This leads to a restricted differentiation of LSC into leukemic blasts but also to an aberrant microenvironment modulated by factors expressed by the LSC. In this case, the LSC generate a supportive environment for themselves (Fig. 6.1a). The second possibility is that the first event of leukemogenesis occurs within the stromal cells leading to an aberrant microenvironment within the BM and a secondary malignant transformation of the physiological HSC into LSC (Fig. 6.1b). In this model it is also conceivable that not only HSC but also mature hematopoietic progenitors can transform into malignant cells and gain stem cell like characteristics due to regulatory signals from the microenvironment as it is known in physiological hematopoiesis [10]. As a consequence of both models the regulation of the physiological HSC is disrupted leading to a decreased hematopoietic potential [16].

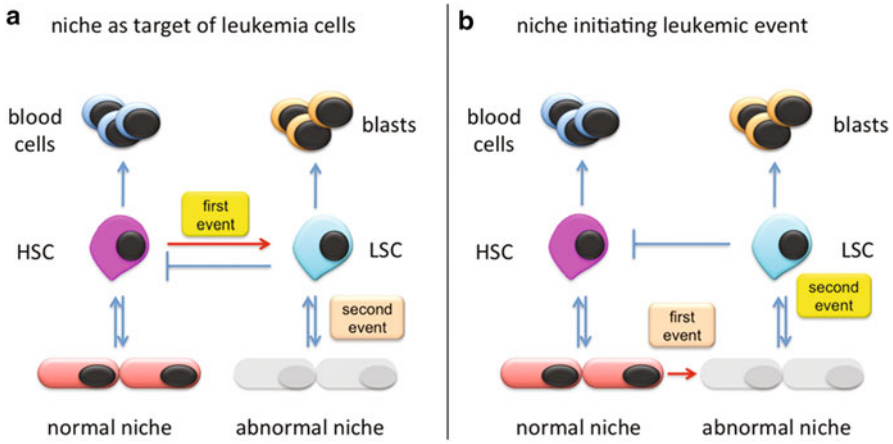

Fig. 6.1 Initiation and support of leukemogenesis. a After the initial first event resulting in the development of LSC from HSC by genetic or epigenetic defects the LSC modulate the niche to achieve growth advantage. **b** In this model the first event occurs in niche cells whereby an aberrant niche induces leukemogenesis in normal HSC resulting in the development of LSC. (Modified from [17])

In this chapter we sought to summarize the current knowledge regarding the importance of the microenvironment in induction and propagation of hematopoietic malignancies focusing on the two most studied mesenchymal cells: multipotent MSC and osteoblasts.

Hematopoietic Niches

Definitive hematopoiesis in adults is predominantly located within the BM of long bones, the sternum and calvarium. Transplantation assays revealed the endosteal region of the proximal and distal metaphysis of the femur to be the primary homing site of primitive HSC [18]. There, BM stromal cells like osteoblasts, vascular endothelial cells and MSC offer a unique microenvironment supporting stem cell quiescence or differentiation in response to extrinsic stress signals [19]. In adult mammals, extramedullary hematopoiesis is also known to occur in locations like liver and spleen associated with BM dysfunction [20]. One example is primary myelofibrosis where an autonomous proliferation of megakaryocytes and consequent stimulation of fibroblasts results in a progressive fibrotic rebuilding of the BM. This results in a decreased expression of CXC-motive receptor ligand 12 (CXCL12) within the BM as well as a decrease in CXC-motive receptor 4 (CXCR4) expression on HSC and a subsequent egress of these cells followed by a colonization of secondary hematopoietic organs [21]. Interestingly, primary myelofibrosis is a not well-understood risk factor for the development of acute myeloid leukemia strongly suggesting a role of the microenvironment in leukemogenesis [22]. Another example for extra-medullar

occurrence of hematopoietic tissue is the myeloid sarcoma. In this case myeloid differentiated blasts reside in non-hematopoietic tissues, commonly as a form of relapse after allogeneic stem cell transplantation [23]. So far it is not clear whether LSC or blasts are in need of a specific microenvironment within soft tissue spaces to form these myeloid sarcomas. Extra-medullar hematopoiesis might however be a result of HSC lodgment to vascular niche components provided by formerly hematopoietic organs or active reconstruction of non-hematopoietic tissue due to HSC derived extrinsic signals.

During ontogenesis early hematopoiesis starts at the primitive streak where hemangioblasts, able to form vascular and hematopoietic cells, differentiate from mesoderm. These hemangioblasts are ancestors of hematopoietic precursors that reside within the aorta-gonad-mesonephros (AGM) and subsequently migrate to the yolk sac and placenta. After colonizing the fetal liver a massive expansion and differentiation of HSC occurs before the HSC finally migrate into the BM as the site of live-long hematopoiesis [24]. Little is known about the factors that provide supportive signals to assist in HSC expansion within these sites, though BMP, IL-3, TPO, Notch and Rac1 pathways were shown to play a key role in embryonic and adult HSC regulation suggesting a defined microenvironment at these sites [25, 26]. Interestingly, mainly erythroid hematopoietic cell types can be found in early hematopoietic organs like fetal liver and spleen whereas in BM predominantly myeloid progenitors arise. This data suggests distinct morphological differences and different extrinsic signals influencing HSC differentiation during maturation [24, 27]. Colonization of Spleen and BM occurs late in fetal development. Vascularization and blood flow through the bone cavity are prerequisite for the homing of circulating fetal liver HSC to the BM niches. The attraction of HSC to the BM microenvironment was shown to be dependent on the expression of CXCL12 and stem cell factor (SCF) within the BM cavity [28]. The influence on HSC and possible leukemogenic effects of mesenchymal stromal cells and osteoblasts, the major source of CXCL12 and SCF in adult BM will be discussed in the next chapters.

Osteoblastic Niche in Physiological and Aberrant Hematopoiesis

Regulation of HSC by Osteoblasts

As described above, definitive hematopoiesis is located within the BM of adult mammals. Circulating fetal liver HSC lodge into BM niches during development where they are maintained life-long. Osteoblasts were first described as a key component for regulation of HSC within the BM over a decade ago. Using transgenic mice with constitutively active parathyroid hormone (PTH) and PTH-related protein (PTHrP) receptor in osteoblastic cells, Calvi et al. showed that concurrently with an increased number of trabecular osteoblasts within the metaphysis of the long bones the pool of lineage$^-$Sca-1$^+$ c-kit$^+$ (LSK) hematopoietic stem and progenitor cells (HSPC) was significantly increased [29]. Moreover, increased expression of CXCL12, SCF, IL-6

and Jagged 1 was measured within the BM endosteum suggesting the expansion of HSC population is a result of increased extrinsic signaling by these cytokines. Zhang et al. demonstrated similar results. By conditionally knocking out bone morphogenetic protein receptor IA (BMPRIA) in mice the number of N-cadherin^{+} CD45^{-} osteoblastic cells was significantly increased within the BM and also the number of HSC linked to osteoblastic cells by direct cell contact [30]. Direct blockage of osteoclast by zoledronsäure results in an increased bone mass and subsequently increased HSC pool [31]. Osteocytes, however, are not capable of HSC expansion [32]. The role of N-cadherin in HSC is still not clear, though, as contradicting data was presented on N-cadherin expression in HSC [33, 34].

Osteoblasts express a variety of proteins that were shown to be directly or indirectly involved in HSC regulation. Maintenance and quiescence of HSC is dependent on the binding of Angiopoietin-1 to its receptor Tie2 expressed on HSC [35]. In this context Adams et al. showed that HSC lacking expression of Calcium sensing receptor (CsR) do not home to the endosteal BM region efficiently and fail to reconstitute the BM [36]. The switch from fetal to adult hematopoiesis by lodgment of circulating fetal liver HSC might in fact be due to a divergent expression of CsR on these cells. Thrombopoietin (TPO) signaling through its receptor c-mpl is also required for HSC maintenance within the osteoblastic niche [37, 38]. In vitro experiments showed that co-culture of HSC with osteoblasts but not stromal cells maintains their hematopoietic potential mediated by Notch signaling pathways [39]. The expression level of CD166 on osteoblasts correlated with a high supportive activity suggesting different subpopulations of osteoblasts have different supportive potential. Most likely, not mature but immature osteoblasts maintain HSC predominantly [40, 41].

Osteopontin (OPN) expressed by osteoblasts was found to play a key role in HSC mobilization, quiescence and homing. OPN is a phosphorylated glycoprotein that is heavily modified after secretion into the BM microenvironment [42]. Cleavage of OPN by thrombin exposes a binding site for the $\alpha_9\beta_1$ integrin expressed by HSC playing a pivotal role in HSC homing, mobilization and maintenance [43]. Besides the endosteal (osteoblastic) niche a vascular niche was described recently. Functional and visual assays with SLAM receptor defined LSK cells in mice suggest that the majority of HSC are not located near osteoblasts but in close proximity to vascular endothelial cells. This observation led to the hypothesis that two distinctive niches exist, the vascular niche and the osteoblastic niche [44].

Using a murine transplantation model we could recently demonstrate that HSC harvested from the endosteal BM region exhibit significant higher functional properties than their phenotypically identical counterparts harvested from the central BM region [18]. Osteoblasts therefore maintain quiescent HSC that can be activated by extrinsic stress signals and migrate to vascular endothelial niches that enable proliferation and differentiation. This is reversible, though, as activated HSC can become quiescent again by lodgment into the endosteal niche [45, 46]. Different types of stromal cells exhibit different supportive potency depending on the location within the trabecular bone [47]. The strict breakup between endosteal and endothelial locations within the metaphysis of the femur is not necessarily required as all HSC within this BM region are in close proximity of osteoblasts and endothelial cells suggesting a combined niche with distinct regulatory properties [48].

This data clearly supports the hypothesis that extrinsic signals from osteoblast are a prerequisite for physiological hematopoietic development and maintenance. On the other hand it is evident that HSC do not only receive extrinsic signals from the environment but also exhibit a regular crosstalk with their niche counterparts. For example HSC induce differentiation of MSC into osteoblastic cells and therefore contribute to niche formation [49]. Erythropoietin (EPO) as an extrinsic growth factor secreted in response to hematopoietic stress stimulates HSC to secret bone morphogenetic proteins (BMP) that subsequently activates osteoprogenitors [50].

Propagation and Induction of Hematological Malignancies by Osteoblasts

The microenvironment within the endosteal BM region is required to maintain primitive inactive HSC that support life-long hematopoiesis and can be activated but also returned into a quiescent state by extrinsic niche signals [45]. There is clinical evidence that HSC are not only protected by the microenvironment in healthy individuals but also in a hostile environment during and after chemotherapy or allogeneic stem cell transplantation. Kolb et al. report of a patient transplanted with HSC from a sibling for treatment of CML. Two years after a successful transplantation, bcr/abl negative hematopoietic cells appeared in the peripheral blood during the course of a severe pneumonia [51]. This supports the assumption that physiological HSC can survive myeloablative chemotherapy and graft vs. host (GvH) effect of transplanted cytotoxic T-cells and can be reactivated by extrinsic signals. After successful treatment of the pneumonia the host hematopoiesis disappeared again.

This phenomenon holds also true for the occurrence of late relapses of the initial leukemia after chemotherapy or allogeneic transplantation and is most likely caused by an activation of quiescent LSC within the BM niche. The persistence of LSC after cytoreductive chemotherapy within the BM in numbers below limit of detection suggests that these cells are protected by a supportive microenvironment. As described above, the osteoblastic niche acts as retreat for quiescent HSC and therefore it is conceivable that osteoblasts also have the ability to protect LSC. In vivo models confirm this hypothesis. After transplantation into immunodeficient NOD/SCIDIL2R$\gamma^{-/-}$ (NSG) mice, human CD34^{+} CD38^{-} LSC from ***AML patients*** home into the endosteal BM region. Further, administration of chemotherapy results in persistence of leukemic cells in contact to osteoblasts within the endosteal BM niche suggesting an anti-apoptotic signaling by osteoblasts [52, 53]. Interestingly, injection of G-CSF sensitizes these endosteal leukemic cells for chemotherapy by disrupting their connection to the endosteal stroma [54]. G-CSF is known for mobilizing normal HSC into the peripheral blood (PB) by disrupting the CXCL12/CXCR4 axis and reducing of CXCL12 expression in osteoblasts [55]. This results also in a decreased CXCL12 signaling towards LSC and possible loss of quiescence [56] but also reduced adhesion properties as CXCR4 activates adhesion molecules like $\alpha_4\beta_1$

(VLA-4) [57]. This is supported by trials where the administration of CXCR4 inhibitors significantly reduces the homing and proliferation of AML cells and overrides the anti-apoptotic effect of the CXCR4 activation [58, 59]. CXCR4 inhibition also improves cytoreductive chemotherapy for chronic lymphoid leukemia [60].

Using a anti-CD44 antibody the Dick laboratory achieved a significant reduction in reconstitution ability of AML cells [61]. Beside Hyaluronic acid (HA), OPN is a major ligand for CD44. OPN was not only shown to be a negative regulator of HSC but is also involved in HSC homing to the BM and anchors HSC to osteoblasts [42, 43]. OPN is known to be a ligand for the VLA-4 expressed by HSC and LSC [62]. As seen in chronic lymphoid leukemia (CLL), VLA-4 signaling prevents apoptosis of leukemic cells via binding to fibronectin (FN), [63] suggesting a similar activity of VLA-4 after binding to OPN. Treatment of AML cells with internal tandem duplication of the fms-related tyrosine kinase 3 (FLT3) receptor, a high-risk leukemia with poor prognosis with a CXCR4 antagonist, showed a negative effect on the proliferation of these cells in co-culture models with bone marrow stromal cells [64]. On the other hand, FLT3 antagonists decrease VLA-4 modulated adhesion [65] underlining the importance of cross-talk between endosteal regulatory proteins with LSC.

In chronic myeloid leukemia (CML) the up-regulation of CXCR4 by tyrosine kinase inhibitor (TKI) activity in CML cells supports migration to hypoxic BM areas allowing them to obtain a quiescent and therefore chemotherapy resistant state [66].

The occurrence of secondary hematopoietic malignancies after chemotherapy or radiation has two possible causes. On the one hand malignant transformation of HSC or progenitor cells is a result of direct genetic damage [67]. On the other hand, however, it is conceivable that BM stromal cells are also affected by the leukemogenesis. Early in vitro co-culture experiments by Naparstek et al. demonstrated that the induction of an abnormal growth factor expression in irradiated bone marrow stromal cells could induce leukemic transformation of growth factor dependent hematopoietic progenitor cell lines bg/bg clone 1 and FDCP-1. The cultivation of these cell lines for 4–6 weeks resulted in a growth factor independent proliferation by acquired genetic aberrations [68]. The underlying cell type for this effect is unclear, however. In a recent study Wei et al. showed that human $CD34^+$ cord blood cells, retrovirally transfected with mixed lineage leukemia (MLL)-AF9 gene, generate either ALL or AML clones. Interestingly, the transplantation of these genetically modified LSC in different mouse stains resulted in different leukemia subtypes, either lymphoid, myeloid or mixed. This strongly suggests that the microenvironment within these mice has divergent supportive potential for LSC proliferation and differentiation [69].

To achieve advantage over normal HSC within the stem cell niche LSC do actively modulate niche components. In vitro data demonstrated that the interaction of AML blasts with osteoblasts resulted in a decreased proliferation of the latter also resulting in an impaired physiological hematopoiesis [70]. In a murine model of AML it was proposed that trabecular bone loss by decreased osteoblast numbers and transient increase in osteoclasts was mediated by CCL-3, a chemokine significantly increased in AML patients. This is one explanation for failure of normal hematopoiesis in

these patients [71]. This data would contradict the hypothesis that LSC are in need of the hematopoietic niche to be protected against cytotoxic medication. Our own data demonstrates however, that the absolute number of primitive $CD34^+$ $CD38^-$ cells is not decreased in AML patients but the cells are inactive and do not differentiate into mature cell types (not published). Additionally, animal models showed that only osteoprogenitor cells but not mature osteoblasts maintain HSC within the endosteal niche and therefore loss of mature osteoblasts not necessarily results in a loss of quiescent LSC [72, 73]. This observation is supported by a study of Hu et al. that demonstrated that in a model of Notch1 induced leukemia quiescent HSC are not lost but normal blood development was exhausted by an accelerated proliferation. Transplantation of these preserved HSC into non-leukemic mice resulted in regular engraftment [74]. In another animal model it was shown that leukemic cells create supportive niches expressing high amounts of SCF. This leads to a migration of physiological HSC into the leukemic niches and a disrupted HSC differentiation. Neutralization of SCF within these niches leads to a recovery of physiological hematopoiesis [16].

Moreover, the endosteal BM region is highly vascularized [75, 76]. Neovascularization is a common feature of highly vascularized tumors like colon or rectum carcinoma. High expression of vascular endothelial growth factor (VEGF) by tumor cells results in an increased angiogenesis supporting tumor growth. This effect can be blocked by anti-angiogenetic drugs like bevacizumab [77]. Expression of VEGF was found in AML blasts suggesting a paracrine activation of leukemia cells via its receptor KDR and FLT1 but also a direct angiogenetic activity towards the microenvironment [78]. The stimulation of vascular endothelial cells with VEGF on the other hand results in an increased secretion of the hematopoietic growth factors G-CSF, GM-CSF, IL-3 and IL-6 inducting proliferation and prohibiting apoptosis in leukemia blasts [79]. In vitro data confirmed that cross talk between AML blasts and osteoblasts also results in an increased VEGF secretion of the latter further supporting microvascularisation within the BM niche [70]. As there is strong evidence that the endosteal niche is a hypoxic environment supporting HSC quiescence [80] AML blasts may achieve a growth advantage by increased delivery of oxygen to the endosteal region. In contrast, interaction with the endothelial cells via CXCR4-CXCL12 signaling might allow LSC to maintain quiescent within this activated microenvironment.

The underlying mechanism of **multiple myeloma (MM)** is a clonal proliferation of mature plasma cells within the BM. In physiological hematopoiesis B cell development is promoted by signaling from osteoblasts within the endosteal BM microenvironment depending on growth factors like VEGF, FLT3L, CXCL12 and IL-7 [81]. Additionally, IL-6 was found to be a co-factor for plasma cell development [82]. Conversely, MM cells were found to inhibit osteoblastic differentiation of MSC and induce apoptosis in osteoblasts, therefore actively remodeling the BM niche [83, 84]. The observed increased osteoclast activity leading to the pathognomic bone lesions of MM is a result of a dysregulation of RANKL expressed by BM stromal cells and osteoblasts inducing an increased osteoclast activity [85]. Propagation of myeloma cell growth by osteoblasts follows the same principal as myeloid malignancies. Due

to a high expression of VLA-4 on myeloma cells these can utilize adhesion properties to extracellular matrix proteins supporting their expansion. This mechanism can successfully be interrupted by anti-VLA-4 antibodies [86].

Direct evidence for the induction of hematological malignancies by mature osteoblasts is scarce. The group of David Scadden published the first study that connects osteoblasts with myeloproliferative neoplasms in 2010. They showed that deletion of Dicer1 in mouse osteoprogenitors resulted in a MDS and consequently a secondary AML with multiple genetic abnormalities [87]. Dicer1 is an important factor for micro RNA (miRNA) biogenesis and RNA processing. MiRNA regulates hematopoietic fate as it was shown in the case of megakaryocyte-erythrocyte-progenitors (MEP), where miR-150 drives MEP-differentiation toward megakaryocytes [88]. A consequence of Dicer1-deletion was the inhibition of the expression of Sbds, the gene mutated in Schwachman-Bodian-Diamond syndrome and simultaneously a leukemia pre-disposition condition [89]. The study by Raaijmakers et al. was one of the first to show that primary stromal dysfunction can lead to secondary hematopoietic malignancy by modifying the differentiation and proliferation capability of hematopoietic cells and their apoptotic feature.

As this effect is caused by aberrant osteoprogenitor cells and not mature osteoblasts, we want to give an overview about regulation of hematopoiesis by MSC and discuss the implication of aberrant MSC's on leukemogenesis in the following chapter.

MSC in Physiological and Aberrant Hematopoiesis

The identification of MSC's within the adult organism was a central discovery in the field of stem cell biology. MSC can be isolated from a variety of tissues like fat tissue, perinatal tissues (umbilical cord, cord blood, chorionic villi of the placenta) and BM [90] and were initially described to form colony-forming-unit fibroblasts (CFU-fibroblasts) [91]. In the meantime it is evident that MSC can differentiate into several stromal cell types like osteoblasts, adipocytes, chondrocytes and fibroblasts that form a major part of the hematopoietic stem cell niche [92].

The term mesenchymal stem cell is no longer used because of their rare self-renewing property [73]. So far no specific phenotypic marker was described but the International Society for Cellular Therapy has proposed minimal phenotypic and functional criteria for the definition of MSC: plastic adherence, differentiation capacity and phenotype: $CD73^+$, $CD90^+$, $CD105^+$, $CD11b^-$, $CD14^-$, $CD34^-$, $CD45^-$, $CD19^-$, $CD79a^-$ [93].

Recently it was demonstrated that MSC play a key role in adult hematopoiesis. Therefore we discuss how MSC regulate HSC within the stem cell niche and summarize the evidence of implication in leukemogenesis.

Regulation of HSC by Multipotent MSC

MSC are a central cellular factor within the BM niche, influencing HSC in two interconnected ways: first by maintaining HSC in a quiescent state in the endosteal niche, second by activating HSC of the perivascular niche, supporting division, differentiation and mobilization out of the BM into sinusoids through extrinsic signals [94].

There is evidence that interaction of MSC and HSC subsists since embryonic life, MSC being detected in the major hematopoietic sites during mouse development: AGM region and liver at midgestational stages and later in neonatal and adult BM [95]. Heterotopic transplantation of murine BM MSC under the renal capsule resulted in the formation of ossicles containing BM with niche elements like osteoblasts and adipocytes [96]. As described above, osteoblastic cells support hematopoietic cell growth by stimulating PTH/PTHrP and producing high levels of the Notch ligand jagged 1 [29]. Adipocytes, however, are negative regulators of HSC, probably by secreting neurophilin-1, lipocalin 2, adiponectin and TNFα, each of which can impair hematopoietic proliferation [97].

Two major studies described a direct regulatory role for MSC within the hematopoietic stem cell niche. Mendez-Ferrer et al. demonstrated that Nestin$^+$ MSC that are in close proximity to HSC and adrenergic nerve fibers, highly express soluble factors like CXCL12, c-kit ligand, angipoietin-1, IL-7, vascular cell adhesion molecule-1 (VCAM-1) and OPN that maintain HSC within the niche [98]. These factors are also known to augment osteoblastic differentiation of MSC. In vivo Nestin$^+$ cell depletion led to increased mobilization and decreased homing of murine HSC from and into the BM, respectively. Sympathetic nerve fibers regulate both their proliferation and their CXCL12 expression [99]. Adrenergic signaling correlates indirectly with the circadian release of HSC into the blood stream by MSC inhibition, being highest during the resting periods [100]. In contrast, CD169$^+$ macrophages were shown to communicate in an antagonistic manner with Nestin$^+$ MSC as compared to the sympathetic nerve system (SNS), facilitating retention of HSC in the BM by enhancing the expression of HSC-maintaining factors, most lilely by insulin-like growth factor (IGF) -1, IL-1, IL-10 and tumor necrosis factor (TNF) [101].

The second major study proving evidence that MSC directly regulate hematopoiesis focused on CXCL12-abundant reticular (CAR) cells which share several properties with Nestin$^+$ MSC [102]. Termed in 2006 by Sugiyama et al., CAR cells were found in contact with almost all HSC, regardless whether the cells were located perivascular or endosteal [14]. Depletion of CAR cells inhibited HSC proliferation by reducing the production of SCF and CXCL12 but accelerated myeloid differentiation [17].

The question still remains whether CAR cells are the same cells as Nestin$^+$ MSC. Both cell types increase the egress of monocytes from the BM into the blood stream as response to circulating microbial molecules (for example binding of low-dose lipopolysaccharide to Toll-like receptor 4 on MSC) by up-regulating

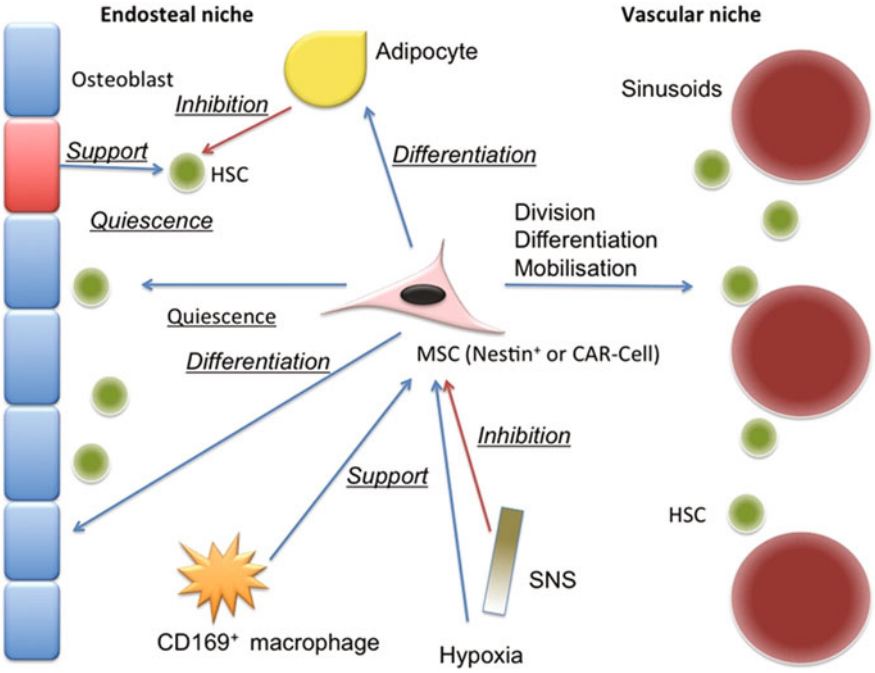

Fig. 6.2 Role of MSC and osteoblasts within the hematopoietic stem cell niche. MSC (Nestin$^+$ or CAR cells) are capable of maintaining HSC in a quiescent state and supporting their division, differentiation and mobilization. This is dependent on niche conditions, for example O_2-concentration or interaction with macrophages and the sympathetic nerve system. Besides, MSC can differentiate into osteoblasts having stimulatory ability or adipocytes, inhibiting the hematopoietic process

the expression of CC-chemokine ligand 2 (CCL2) that binds to CC-chemokine receptor 2 (CCR2) [103]. This interplay can affect HSC function as stimulation of Toll-like receptor 4 (TLR 4) in human BM-MSC inhibits the expression of jagged 1 [8]. Because MSC are able to support hematopoiesis, clinical studies investigated whether co-transplantation of MSC and HSC can accelerate HSC engraftment and/or prevent graft failure. Koc et al. demonstrated rapid hematopoietic recovery after co-transplantation of autologous MSC and HSC in advanced breast cancer patients after obtaining high-dose chemotherapy [104]. Other studies, in contrast, indicate that MSC co-transplantation is safe but the effect on hematopoietic reconstitution is weak. One possible explanation for these controversial data is the use of different HSC cell sources like cord blood or haploid allogeneic HSC [105]. The capacity of preventing graft failure on the other hand is being described to be encouraging [106]. The effect on hematopoiesis can be in part explained by HSC-promoting factors, for example SCF, CXCL12, granulocyte-macrophage colony-stimulating factor (GM-CSF), released by MSC [107, 108] and is enhanced by the hypoxic environment within the BM niche [109] (Fig. 6.2).

Induction and Propagation of Hematopoietic Malignancies by MSC

As described above, one of the landmark-studies in this field was made by Raaijmakers et al. when they showed that deletion of Dicer1 in mouse osteoprogenitors but not in mature osteoblasts, resulted in a MDS and a secondary AML with multiple genetic abnormalities [87]. Blau et al. demonstrated that MSC have other genetic abnormalities than leukemic blasts in **patients with MDS or AML**, without any evidence of chromosomal abnormalities in healthy subjects, suggesting that unstable MSC may facilitate the generation and/or expansion of malignant cells [14]. Interestingly, a study by Ardianto et al. identified a unique cell line (HPB-AML-I) in the peripheral blood of a patient with AML which may be derived from MSC's independent from the hematopoietic progenitors suggesting the possibility of leukemic transformation of MSC [110]. In vitro data of co-cultivation of MSC from the BM of patients with MDS and AML showed a severe dysfunction and even loss of the suppressive immune-regulatory effect on the proliferation of T-cells suggesting aberrant functional properties of MSC [111]. It remains unclear, however, whether the abnormal immune-regulatory functions are conditioned by inhibitory effects of the malignant hematopoietic cells or if this is an intrinsic deregulation within the MSC induced by genetic or epigenetic abnormalities. However, this reduced inhibitory effect on T-cells within the malignant BM could enhance the graft vs. leukemia (GvL) effect of cytotoxic allergenic T-cells in allogeneic stem cell transplantation.

There is also evidence for a negative effect on leukemic cell proliferation and therefore support of quiescence LSC. Dickkopf-related protein-1 (DKK-1) expressed by MSC acts as a negative regulator of the Wnt signaling pathway, a network of proteins responsible for cell proliferation and differentiation. This process is regulated by NANOG, a transcriptional factor expressed commonly in stem cells. Initially this was demonstrated on K562 cells, a myeloid leukemia cell line [112]. Another pathway identified to inhibit K562 cell expansion by MSC is the phosphinositide 3-Kinase (PI3K)-Akt-Bad signaling pathway via phosphorylation of the Akt (proteinkinase B, PKB) and Bad (Bcl-2-associated death promoter) proteins. The expression of Akt and Bad was increased in K562 cells after co-culture with MSC from patients with leukemia. The addition of the specific inhibitor LY294002, which competes with PI3K for ATP binding sites, resulted in a dramatic decrease in levels of both phosphorylated proteins [113]. This data suggests that MSC are able to maintain leukemic cells in a quiescent state.

Interestingly, depending on Focal adhesion kinase (FAK) expression – a key partner in integrin signaling – MSC can support but also suppress cell growth. FAK^+ MSC from patients with AML were described to inhibit DKK-1 and increase Wnt signaling whereas FAK^- MSC inhibit Wnt signaling by up-regulation of DKK-1 [114].

CXCL12, a key regulatory factor is overexpressed in MSC derived from patients with MDS, being an important determinant of leukemic quiescence [115]. This fact was described also by Vianello et al. in CML patients, showing that the interaction of

MSC and CML cells protects from TKI-induced cell death in a CXCL12/CXCR4-dependent manner. AMD3100, a CXCR4 inhibitor, restored CML cell sensitivity to TKI's [116]. In MSC derived from the BM of AML patients, however, CXCL12 was shown to be down-regulated and MSC consequently facilitated proliferation of leukemic cells [117].

Transforming growth factor-β1 (TGFβ1) is another regulator being able of maintaining AML-cells quiescent and promoting their survival. TGFβ1 activates the PI3K/Akt pathway, stimulates the integrin-mediated anti-apoptotic effects of MSC in the BM niche and is able to trigger G_0/G_1 cell-cycle arrest by up-regulation of C/EBPbeta, a member of the leucine zipper family of transcriptional regulators with an important role in cell proliferation, differentiation and senescence. It was also demonstrated that TGFβ1 promotes survival of myelo-monocytic leukemia cell lines (U937, THP-1, MOLM 13). A TGFβ receptor kinase inhibitor (LY2109761) and a neutralizing anti-TGFβ1 antibody inhibited this effect [118, 119].

As in physiological hematopoiesis low oxygen concentration induces leukemic engraftment and resistance to chemotherapy. Hypoxia-inducible factor α (HIFα) is shown to be a critical component for the engraftment of leukemic cells by directly up-regulation of CXCL12 expression. Knockdown of HIFα in MSC reduced human leukemic cell engraftment and chemoresistance [120, 121].

In contrast to AML, genetic aberrations in MSC of **patients with ALL** were shown to be leukemia-associated (TEL-AML1, E2A-PBX1, MLL rearrangement), indicating a clonal relationship between MSC and leukemia cells [122]. This data is not consistent, however, as Menendez et al. detected only MLL-AF4 fusion gene in MSC from patients with ALL. Other fusion genes (like TEL-AML1, BCR-ABL, AML1-ETO, MLL-AF9, MLL-AF10, MLL-ENL) were absent. They concluded that MLL-AF4 might arise in a population of pre-hematopoietic precursors, being able to differentiate in mesenchymal and hematopoietic cells concurrently [123]. These findings suggest that mesenchymal and hematopoietic cells are ontogenetically related and might be able to trans-differentiate. Another ALL cell line, GM-490, expresses the low affinity nerve growth factor receptor $p75^{LNGFR}$ that is also found in MSC, thus leading to the hypothesis that GM-490 has MSC properties. Fibroblast growth factor 2 (FGF2) and TGFβ1 expressed by GM-490 are reported to be responsible for juxtapositioning hematopoietic cells with stromal cells, cytokines and other extra cellular matrix proteins and thereby promote quiescence, cell proliferation or differentiation [124].

Recent reports demonstrate that resistance of ALL-cells to chemotherapy seems to be depending on the interaction with MSC. By enhancing bcl-2 (B-cell lymphoma 2) gene expression through interaction with MSC, the ALL cell line K562/A02 achieves chemotherapy-resistance against Adriamycin [125]. Also, co-culture of MSC with ALL cells reduces the asparaginase sensitivity of the latter. This protective effect can be attributed to the higher expression of asparagine synthetase in MSC than in leukemic lymphoblasts leading to an increased affinity to MSC derived asparagine [126]. A similar effect was demonstrated by co-culture of CLL cells with MSC. Due to a MSC mediated inhibition of dGuo triphosphate (dGTP) accumulation and adenosine/guanosin triphosphate (ATP/GTP) depletion the sensibility of CLL cells to forodesine was significantly decreased [127].

Two leukemogenic factors, CCL2 and IL-8 were found to be abundantly expressed in the leukemic BM niche, enhancing the capacity of BM-MSC to support adhesion of ALL cells by stimulating VCAM-1 expression on MSC [128]. The ability to support the survival of B-ALL cells was reverted in one study by inhibiting the Notch signaling pathways, suggesting that some signaling-proteins of the Notch family are involved in stromal-cell mediated rescue of B-ALL cells from apoptosis. Blocking and stimulating experiments demonstrated that Notch ligands Jagged-1/-2 and Delta-like ligand-1 (DLL-1) synergistically interact with Notch-3 and −4 on both cell types and promote B-ALL cell survival [129]. In CLL, besides Notch-4, also Notch-1 and Notch-2 signaling can facilitate leukemic cells survival and resistance to chemotherapy [130].

Moreover, MSC of **CLL-patients** produce high levels of hepatocyte growth factor (HGF) that interacts with its receptor (c-MET) expressed on leukemic cells and turns on STAT3 (Signal Transducer and Activator of Transcription protein) phosphorylation, contributing to survival of CLL cells within BM [131]. Ding et al. described another pathway related with disease progression: CLL cells secrete Platelet-derived growth factor (PDGF) into the microenvironment under low oxygen conditions. Binding of PDGF to PDGF receptor (PDGFR) on MSC activates the PI3K-Akt pathway. PDGFR-PI3K-Akt activation results in increased VEGF production subsequently leading to neovascularization [132]. Other researchers pointed out Zeta-associated protein 70 (ZAP70), playing an important role in chemotaxis and/or the adhesion process between B-lymphocytes and MSC. Only $ZAP70^{+}$ cells could respond to microenvironment stimuli like changes regarding the CXCL12/CXCR4 axis [133].

MSC in **patients with MM** were shown to develop distinctive genomic profiles compared to MSC from healthy donors. This altered gene expression may have an impact on the pathogenesis of the disease. Augmented levels of FBLN5, a protein similar to epidermal growth factor, promote the growth of myeloid cells [134]. However, clonal markers as identified in myeloid plasma cells were not described in MSC from patients with MM [135].

The immune-suppressive-capacity of MSC is aberrant in MM, with evidence of IL-6 overproduction, stimulating plasma cell proliferation. This is illustrated in murine models where MM can be induced by IL-6 producing inflammatory feeder cells that are created after peritoneal irritation [135]. Additionally it was shown, both on the mRNA and protein level, that GDF15 supports the growth of MOLP-6, a stromal-cell dependent myeloma cell line, and that this was increased in MSC from myeloma patients as compared to MSC from healthy donors [136] (Fig. 6.3).

Summary

We demonstrate that MSC and their progeny osteoblasts have central regulatory properties on HSC within the stem cell niche and share similar features. Osteoblasts maintain HSC in a quiescent state and are directly correlated with the stem cell pool

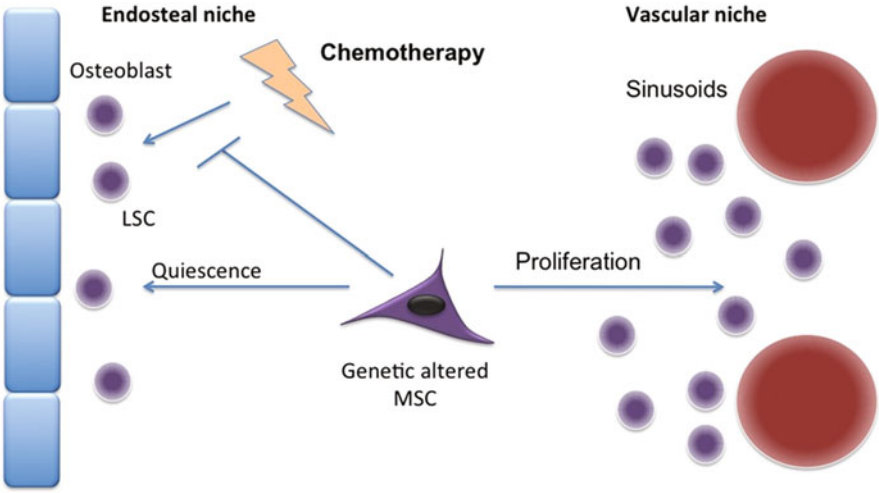

Fig. 6.3 The leukemic niche. MSC and osteoblasts can enhance chemoresistance of leukemic cells by preserving them quiescent. MSC can also stimulate their proliferation, maintaining the malignant process

size. There is good evidence, however, that rather immature than mature osteoblasts are involved in this process.

Several soluble factors provide extrinsic signals to HSC within the niche, among them CXCL12, that is also highly expressed by MSC. These multipotent cells can differentiate into a variety of key components of the stem cell niche, osteoblasts and adipocytes but also have a direct impact on hematopoiesis. Interestingly, MSC seem either to keep HSC in dormant state or activate them supporting differentiation and mobilization. This has to be seen most likely in context with other cellular or extracellular niche factors like vascular endothelial cells or neuronal cells.

The hematopoietic stem cell niche is a flexible system that supports physiological and aberrant hematopoiesis in a similar manner. Both cell types are able to provide signals preserving HSC but also LSC in a quiescent state augmenting chemotherapy resistance. This was shown for a large number of hematological and in the case of MSC also oncological malignancies that could not be discussed in this chapter. Cross talk between osteoblasts, MSC and the malignant cells conversely rebuilds the niche to support malignant cell expansion. The most intriguing fact is that genetically aberrant niche components can not only maintain leukemogenesis but also induce malignant transformation of hematopoietic cells. Thus, an integrated therapy should include not only cytoreductive regimens hitting dividing malignant cells but beyond that integrate modulatory elements diminishing the supportive factors by the niche.

The use of systemic chemotherapy without considering tumor-evolution or rational reconstruction of the malignant process ousts the delicate pathogenetic findings of the last decades.

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Chapter 7
Biomodulatory Therapy Approaches in Renal Clear Cell Carcinoma: A Perspective

Albrecht Reichle

Abstract The remarkable efficacy of multi-targeted biomodulatory therapies may be exemplarily shown in renal clear cell carcinoma (RCCC), particularly the biomodulatory activity of VEGFR-tyrosine kinase inhibitors and transcriptional modulators (pioglitazone and interferon-alpha). Biomodulation remodels tumor-immanent normative notions by therapeutically implementing non-normative boundary conditions in such a way that tumor control becomes possible. Normative notions in a tumor are morphological structures that are not only built differently in an evolutionary context, but are also evolutionarily constrained action norms, such as the hallmarks of cancer, and multifaceted patterns of inherent decision maxims, i.e., hubs and nodes. The biomodulatory activity of VEGFR-tyrosine kinase inhibitors is underlined (1) by different mechanisms of action dependent on the type of tumor (hepatocellular carcinoma, RCCC, de novo and relapsed acute myelocytic leukemia), (2) by profiles of side effects related to the type of tumor, and (3) by efficacy dependent on the metastatic organ site. The specific activity of transcriptional modulators in RCCC has been shown in recent phase II trials by the combined administration of pioglitazone and interferon-alpha. Established biomodulatory first-line therapies in RCCC need to be supplemented by further biomodulatory therapeutic principles for circumventing the following two dilemmas: The difficult combination of VEGFR-tyrosine kinase inhibitors with other classic targeted therapies because of cumulative toxicities, and the inefficacy of VEGFR-tyrosine kinase inhibitors in patients with bone (about 30 %) and brain (4–48 %) metastases from RCCC. As shown in clinical trials, multi-targeted biomodulatory therapy approaches have in principle the ability to induce continuous complete remission in metastatic renal clear cell carcinoma at a low rate of side effects.

Standard Therapy in Metastatic Renal Cell Carcinoma

Long-term control of metastatic RCCC remains a therapeutic challenge, although therapeutic progress has been made over the past years: The aim is resection of the primary lesion because of its favorable effect on overall survival [1]. Adequate

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imaging procedures of metastatic sites and the stratification according to risk scores (e.g., the Heng Score) are a prerequisite for resecting the metastatic site or for systemic therapy, the latter representing the most frequently chosen option for primary therapy in the metastatic stage [1–4]. No randomized trials on resecting metastatic tumor sites are available, but a small percentage of patients may be cured by a purely surgical approach [1].

After years of focus on chemo-immune and immune therapy, chemotherapy has completely disappeared from scientific interest; however, immunotherapy with interleukin-2 still plays an important role in some patients with a favorable risk score. Interferon-alpha with bevacizumab remains in first-line therapy. M-Tor inhibitors as well as VEGFR tyrosine kinase inhibitors are established in risk patients as a first- and second-line therapy.

The broad spectrum of available VEGFR tyrosine kinase inhibitors, which has recently been supplemented by axitinib [5], inhibits different patterns of tyrosine kinase receptors [6]. VEGFR tyrosine kinase inhibitors are not cross-resistant, have various profiles of side effects, and may be administered sequentially with different preferences [4].

VEGFR Tyrosine Kinase Inhibitors as Biomodulators

The action of VEGFR tyrosine kinase inhibitors is described by more or less specifically inhibiting VEGF receptors, PDGF-R, etc.: How colorful action profiles seem to be in 'reality' may be best delineated from therapies in other histological types of tumor: Patients with Flt-3 positive acute myelocytic leukemia benefit to a low degree from the addition of sorafenib to standard induction chemotherapy. Patients with Flt-3 positive leukemia in relapse after allogeneic transplantation, however, may even achieve complete remission after the administration of sorafenib alone. Therefore, biomodulatory activity in vivo seems to be the real cause that defines the extent of therapeutic success [7, 8]. VEGFR tyrosine kinase inhibitors may even induce complete remission in some patients with RCCC [4].

How selectively biomodulatory and organ-specific the activity profile of VEGFR tyrosine kinase inhibitors may be was shown in the case of sunitinib: This agent is not active in brain metastases, and patients with bone metastases have significantly worse overall survival rates [9, 10].

Tumor systems biology, i.e., the system of tumor-associated normative notions, may be uncovered on the basis of comparative trials on VEGFR tyrosine kinase inhibitors for various indications [11]: Combining all data on one single VEGFR tyrosine kinase inhibitor, such as sorafenib, shows that either the mechanism of action or the profile of side effects are strictly dependent on the particular tumor histology (hepatocellular carcinoma, RCCC, acute myelocytic leukemia) and the stage of therapy (de novo acute myelocytic leukemia or allogeneic relapse) [7, 8, 12–15]. Different activity profiles and side effects may be considered a further indication that the substance group meets rather different normative systems structures in the respective

Table 7.1 Different activity profiles of VEGF-R inhibitors

Differential activity profiles of VEGF-R inhibitors		
Malignant disease	**Activity/toxicity profile**	**Literature**
Acute myelocytic leukemia, flt3+, in allogeneic relapse	Monoactivity of sorafenib: Remission induction	Metzelder S et al. Blood 2009;113(26): 6567–71.
Acute myelocytic leukemia (AML)	Sorafenib no additional activity, also not in flt3+ AML	Serve H et al. J Clin Oncol 2013 (34)
Renal clear cell carcinoma (RCCC)	Sunitinib activity dependent on the metastatic organ site (less efficacious in case of bone and/or brain metastases)	Molina AM et al. Long-term response to sunitinib for metastatic renal cell carcinoma. J Clin Oncol 29: 2011 (suppl; abstr 4615)
Hepatocellular carcinoma	In comparison to RCCC differential activity and toxicity profile of sorafenib	Hoffmann K. et al. BMC Cancer. 2008;8:349. Escudier B.et al. J Clin Oncol. 2009;27(20): 3312–8.

tumor types. Thus, VEGFR tyrosine kinase inhibitors target multifaceted evolution-biological normative structures, action norms, and decision maxims within different histological tumor types. On this tumor-specific and evolution-typical normative background, VEGFR tyrosine kinase inhibitors may exert extremely different action profiles. Only normative systems structures describing the communicative impact of single receptor-triggered communication lines in a diagnostically and therapeutically relevant manner may be causally used to explain the broad activity and side effect profile of VEGFR tyrosine kinase inhibitors, such as sorafenib, in different tumor types.

Study results on VEGFR tyrosine kinase inhibitors in different histological tumor types show that the very same therapeutically targeted receptor pattern may have rather different validity and denotation dependent on the basic evolutionarily constrained communicative structure in the respective tumor type: Therefore, the validity of communication lines, in which VEGFR tyrosine kinases are involved, depends on the communicative prerequisites in their surroundings, particularly on the cell and tissue type, as shown by the immunomodulatory activity of sorafenib in acute myelocytic leukemia (Table 7.1, 7.2).

Table 7.2 Context-dependent validity and denotation of the target sites of tyrosine kinase inhibitors determine response to tyrosine kinase inhibitors in different tumor types, stages, and metastatic sites

VEGFR inhibitors: Modular activity profile
• Activity disease-specific (histology) • Stage-dependent activity profile in acute myelocytic leukemia • Side effects: disease-specific • Activity profile is correlated to side effects (hypertension) • Activity dose-dependent (axitinib?) • Activity dependent on the metastatic site (sunitinib)
• Activity related to tyrosine kinase target-profile • Activity related to distribution of target sites in tumor and stroma cells • Communicative validity and denotation of target-profiles in tumor and stroma cells

Impact of Biomodulatory Therapies

Biomodulatory therapy aims at refraining from therapeutic indications generated according to classic theme-dependent therapy principles, namely the inhibition of distinct, reductionistically ascertained communication lines, for instance, by 'specific' VEGFR tyrosine kinase inhibitors, to finally establish evolution-adjusted therapies. Such therapies accomplish the situative validity and denotation of respective communication lines, as, for example, triggered by VEGFR tyrosine kinases, as a basis for the evolution-adjusted administration of VEGFR tyrosine kinase inhibitors [16]. Simultaneously, biomodulatory therapies disassociate from the perception that the inhibition of 'driver' genes is only therapeutically relevant for attenuating tumor growth. Furthermore, non-oncogene-addicted targets, which constitute normative structures, action norms, and decision maxims in tumors, may be used as therapeutic targets for attenuating tumor growth and even for inducing continuous complete remission as in RCCC [4, 17, 18]. Specific patterns of non-oncogene-addicted targets arise from the background of frequently complex genetic and molecular-genetic aberrations [11, 19]. The toxicity of biomodulatory therapy approaches may be kept at a low range on the basis of the biomodulatory activity profile [19].

C-Reactive Protein as a 'Universal' Marker Indicating the Redirection of Tumor-Promoting Pro-Inflammatory Processes

During the past years, many scientists have investigated a wide range of tumor markers in metastatic renal cell carcinoma. The Heng Score—that is also applicable in case of VEGF- or VEGFR-inhibition—is able to retrospectively stratify patients according to its three risk groups, who had been previously itemized with other scoring systems. Thus, the newly introduced therapy principles VEGFR tyrosine kinase inhibitors, bevacizumab, and mTOR inhibitors, which are currently used as standard therapies in RCCC, adhere to the same prognostic scores and may therefore only gradually improve the total outcome of this patient cohort [20]. However, these therapy principles do not decisively improve individual prognoses within distinct stages, or RCCC-specific normative notions and their corresponding rationalization processes. Only then would the spectrum of personalized therapies be extended on a broader basis.

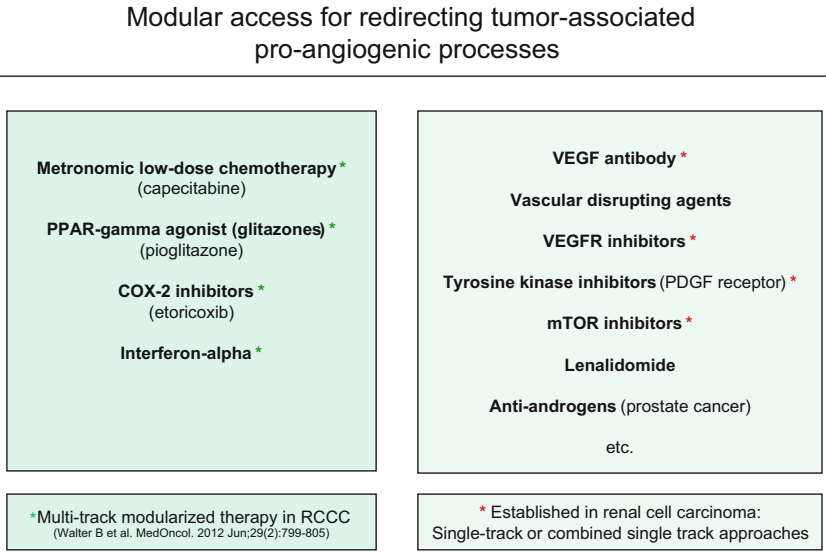
For the therapy of RCCC, markers—indicative for specific tumor biology or for the therapeutic availability of targets—are slowly being established in clinical practice. The currently most valuable biomarkers are C-reactive protein (CRP) in serum and a 'bio-score' (Survivin, B7-HI and Ki-67), but validating trials are necessary [3]. The question whether VEGF levels in serum play a significant role for therapy is still being investigated.

CRP levels are of interest in many respects: RCCC is the only tumor known so far, in which CRP is produced by the tumor cell itself and not only by the liver. Furthermore, CRP symbolizes a characteristic variable in metastatic tumors that characterizes tumor-associated inflammation as a 'hallmark' of cancer. Therefore, CRP is automatically an optimal parameter for describing a tumor feature of normative systems, serving as a 'universal' tumor marker that may indicate the redirection of tumor-promoting pro-inflammatory processes [21]; chapter 20, 22. Therefore, normative structures in a tumor are not fictive parameters but may be depicted by biomarkers or patterns of biomarkers: Functional structures are suitable to be evaluated more extensively and more specifically by cellular secretome analytics. Thereby, cellular functions may be attributed to distinct identities of cellular compartments [22]. Molecular imaging also mirrors normative systems structures [23] in a tumor.

Biomodulatory Active Combination Therapies

Two sequentially conducted multi-centric phase II trials have been initiated to prove the hypothesis whether interactive biomodulatory drugs with low or no mono-activity that ubiquitously target available structures in RCCC may modulate tumor-specific normative structures and functions for attenuating tumor growth. Both trials, which included biomodulatory active drugs (metronomic low-dose chemotherapy, coxib, PPAR alpha/gamma agonist plus/minus interferon-alpha), showed that a patient cohort with initially elevated CRP levels and thus generally unfavorable prognoses

Table 7.3 Rather divergent drugs provide modular access for redirecting tumor-associated pro-angiogenic processes correspondingly to the evolutionarily constrained multifaceted rationalizations of tumor-associated angiogenesis



benefits from therapy (improved progression-free survival) in case of a CRP response of $> 30\,\%$ from baseline. Optimized biomodulation including interferon-alpha may frequently decrease CRP levels and induce pathological and continuous complete remission in patients with pulmonary and/or bone disease [18, 19].

As shown, the single substances combined in biomodulatory intention must not exert mono-activity to induce concerted tumor control. Stimulating agents may also be included in biomodulatory therapy schedules. Comparative trials have indicated (prostate cancer, melanoma, angiosarcoma, gastric cancer) that both metronomic low-dose chemotherapy and transcriptional regulators have rather different activity profiles dependent on the tumor histology, similar to the different activity profile of VEGFR tyrosine kinase inhibitors in different tumor types [11, 24] (Table 7.3).

How Do Biomodulatory Therapies Operate?

The developmental possibilities of biomodulatory therapy schedules comprise the different therapeutic implementation of non-normative boundary conditions for tumor control, for instance, by combining metronomic therapies including transcriptional modulators, classic 'targeted' therapies, such as VEGFR tyrosine kinase inhibitors, metronomic low-dose chemotherapy, etc. These measures redirect tumor-specific, stage-specific and/or metastatic site-specific normative systems structures,

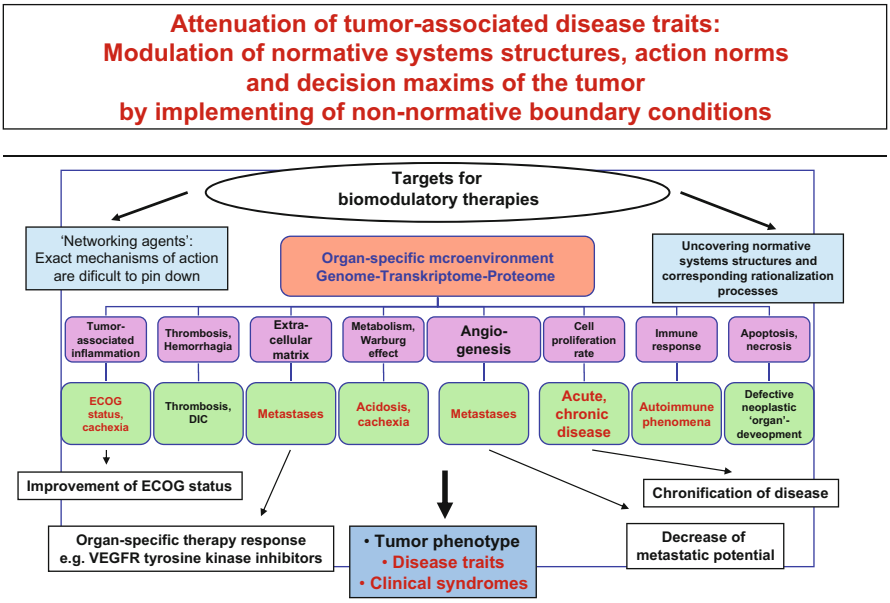


Fig. 7.1 Implementation of non-normative boundary conditions for redirecting rationalization processes that concretely constitute structures and functions, which are necessary to maintain tumor-associated normative notions, i.e., structures, action norms, and decision maxims

action norms, and decision maxims (Fig. 7.1). The tumor system is forced to respond to therapeutically established non-normative boundary conditions according to communication-derived rules by modifying communicative expression of pathways with tumor-pathological relevance in such a way that therapeutically criticizable validity claims of tumor systems objects (i.e., cells, pathways etc.) are redeemed for attenuating tumor growth and for inducing therapy-relevant evolutionary processes. The induction of evolutionary processes for tumor control does not seem to involve any changes in the genome but possibly epigenetic alterations [11, 25–28].

Conclusion

In RCCC only, the efficacy of active biomodulatory drug combinations could be shown on a rather broad basis. Supplementing established first-line therapies with further biomodulatory principles is obviously an interesting option for circumventing the dilemma of the often observed toxicities of combined targeted therapies in RCCC and the weak activity of VEGFR tyrosine kinase inhibitors in the frequent occurrence of bone and brain metastases [29–33]. The understanding of multidimensional communicative processes is vital, for example, normative systems structures, action norms, and decision maxims in RCCC and their timely restricted functional

and structural constitution (rationalization), the interactions of biomodulators with rationalization processes of normative notions, and the way biomodulators may be modularly combined to redirect normative notions. Such comprehension may help enhance therapeutic efficacy, reduce total toxicity, and improve overall survival as well as quality of life.

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Chapter 8
Proteome Analysis Identified the PPARγ Ligand 15d-PGJ2 as a Novel Drug Inhibiting Melanoma Progression and Interfering with Tumor-Stroma Interaction

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Abstract Peroxisome proliferator-activated receptors (PPARs) have been originally thought to be restricted to lipid metabolism or glucose homeostasis. Recently, evidence is growing that PPARγ ligands have inhibitory effects on tumor growth.

To shed light on the potential therapeutic effects on melanoma we tested a panel of PPAR agonists on their ability to block tumor proliferation *in vitro*. Whereas ciglitazone, troglitazone and WY14643 showed moderate effects on proliferation, 15d-PGJ2 displayed profound anti-tumor activity on four different melanoma cell lines tested.

Additionally, 15d-PGJ2 inhibited proliferation of tumor-associated fibroblasts and tube formation of endothelial cells. 15d-PGJ2 induced the tumor suppressor gene p21, a G_2/M arrest and inhibited tumor cell migration.

Shot gun proteome analysis in addition to 2D-gel electrophoresis and immunoprecipitation of A375 melanoma cells suggested that 15d-PGJ2 might exert its effects via modification and/or downregulation of Hsp-90 (heat shock protein 90) and several chaperones. Applying the recently established CPL/MUW database with a panel of defined classification signatures, we demonstrated a regulation of proteins involved in metastasis, transport or protein synthesis including paxillin, angio-associated migratory cell protein or matrix metalloproteinase-2 as confirmed by zymography. Our data revealed for the first time a profound effect of the single compound 15d-PGJ2 on melanoma cells in addition to the tumor-associated microenvironment suggesting synergistic therapeutic efficiency.

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Introduction

Defining novel treatment options of melanoma is still a challenge and the identification of new agents is vital due to the increasing incidence and poor prognosis [1, 2]. For any novel drug, many obstacles have to be overcome from target identification to clinical testing of therapeutics. Therefore, drugs already approved for the treatment of other diseases but potentially applicable in melanoma are of high interest [1].

There is increasing evidence that the peroxisome proliferator-activated receptor-γ (PPARγ)-binding ligands, may be effective for the treatment of melanoma [1] and other tumors [3].

PPARs are ligand-activated transcription factors of the nuclear hormone receptor superfamily comprising three subtypes: PPARα, PPARγ, and PPARδ/β and are characterized by distinct functions, ligand specificities and tissue distribution [4]. The role of these receptors has been considered originally to be restricted to lipid and lipoprotein metabolism, glucose homeostasis and cellular differentiation [5].

PPARγ was demonstrated to regulate diverse cellular and neoplastic processes such as proliferation [6], differentiation [7] and apoptosis [8]. The anti-tumor effect of PPARγ activation is exerted by the induction of cell cycle arrest rather than by induction of apoptosis [9, 10]. In addition, the inhibition of endothelial cell migration by PPARγ ligands has been described, bolstering the anti-angiogenic activity of PPAR ligands [11, 12].

The PPARγ specific agonists 15-deoxy-Δ12,14 prostaglandin J2 (15d-PGJ2), troglitazone, and rosiglitazone inhibited cell proliferation in four melanoma cell lines dose-dependently, whereas a specific agonist of peroxisome proliferator-activated receptor alpha (WY-14643) did not exert this effect [9]. Ciglitazone, a selective PPARγ ligand, was shown to inhibit the proliferation of the A375 as well as of the WM35 melanoma cell line [13].

Several PPAR ligands are interesting candidates for melanoma therapy. Thiazolidinediones (TZD), ciglitazone and troglitazone are high affinity synthetic ligands. In contrast, 15d-PGJ2 is a low-affinity endogenous ligand for PPARγ and known to be a potent inducer of heme oxygenase 1 (HO-1). The three high affinity ligands directly regulate cyclin D1 and p21 and the multi-functional protein ß-catenin [14, 15]. The latter observation implies that PPARγ ligands may be able to interfere with the metastatic process [16].

Here we present a comprehensive study assessing the anti-tumorigenic effects of a panel of PPARα and PPARγ agonists on a variety of melanoma cell lines. The PPARγ agonists ciglitazone, troglitazone and 15d-PGJ2 and the PPARα ligand WY-14643 were tested on four melanoma cell lines (A375, M24met, 1205Lu and MelJuso) to generalize our findings. In addition to direct effects on cancer cells, PPARγ agonists were tested on the influence on cells of the tumor microenvironment such as endothelial cells and melanoma associated fibroblasts.

To further investigate molecular mechanisms of drug action we made use of the proteome profiling methods shot gun analysis and 2D-gel electrophoresis. Applying the recently established CPL/MUW proteomics database [17, 18] we were able to detect protein alterations independently supporting the present functional data.

Our study indicates that 15d-PGJ2 is a potent anti-tumorigenic compound by interfering with melanoma cell proliferation, metastasis and additionally affecting the melanoma associated stroma.

Results

15d-PGJ2 Inhibits Cell Proliferation More Efficiently Than Other PPAR Ligands Via Cell Cycle Arrest And p53 Regulation

We investigated the anti-proliferative effects of PPARγ ligands ciglitazone, troglitazone and 15d-PGJ2 and the PPARα ligand WY-14643 on four melanoma cell lines (A375, M24met, 1205Lu and MelJuso). As determined by MTS proliferation assays, the IC_{50} of 15d-PGJ2 was in a range between 22–38 μM after 48 h of treatment (Table 8.1). In contrast the IC_{50} of the PPARγ agonists ciglitazone and troglitazone could not be reached with the highest dose of 100 μM tested on A375, M24met and MelJuso melanoma cell lines. The selective PPARα agonist WY-14643 showed no growth inhibitory effect (Table 8.1). Thus, among the tested PPARγ agonists 15d-PGJ2 was found most efficient.

Next we investigated the anti-proliferative effects on human umbilical vein endothelial cells (HUVECs) and skin-derived fibroblasts of healthy donors. The IC_{50} of isolated HUVECs was 85, of LECs 70.84, suggesting a restriction of 15d-PGJ2 efficiency to malignant cells (Table 8.1).

In contrast to normal fibroblasts such as NHDF with an IC50 of 127.70, the melanoma associated fibroblasts of four different patients revealed to be more sensitive upon15d-PGJ2 treatment (IC_{50} range: 44–68 μM).

The PPARγ expression in the melanoma cell lines (A375, M24met, 1205Lu, MelJuso), in HUVECs, normal fibroblasts (NHDFs) and primary melanoma associated fibroblasts (MP9, MP10, MP11, MCM16 fibroblasts) was confirmed via Western blotting (Fig. 8.1a).

We selected 15d-PGJ2, the most potent PPARγ agonist for further investigations.

We analyzed cell cycle alterations mediated by 15d-PGJ2 in A375, M24met and 1205Lu melanoma cell lines. In all melanoma cell lines 15d-PGJ2 induced a G_2/M arrest. Treatment of cells with 15 μM 15d-PGJ2 triggered cell cycle arrest in the G_2/M phase from 18–63 %, from 12–32 % in and from 5–26 % in A375 (Fig. 8.1b), in M24met (Fig. 8.1c) and in 1205Lu cells (Fig. 8.1d), respectively.

Since p21 is known to induce S-phase or G_2/M arrest [19–21], we tested our cells for p21 induction after 15d-PGJ2 treatment. Indeed, 15d-PGJ2 treatment dose-dependently induced upregulation of p21 in A375, M24met and 1205Lu at low micromolar concentrations (Fig. 8.2a). Additionally, 15d-PGJ2 induced p53 expression and/or phosphorylation in A375, M24met and 1205Lu melanoma cell lines (Fig. 8.2b).

Table 8.1 IC50 of cells treated with PPAR ligands

Cell line	PPAR ligand	IC50 (μM)
A375 (melanoma cell line)	Ciglitazone	> 100 (1436)
	Troglitazone	> 100 (2584)
	15d-PGJ2	*23.4*
	WY-14643	> 800 (14394)
M24met	Ciglitazone	> 100 (2913)
	Troglitazone	>100 (1574)
	15d-PGJ2	*25.12*
	WY-14643	674.4
1205Lu	Ciglitazone	100.9
	Troglitazone	46.09
	15d-PGJ2	*21.97*
	WY-14643	380.9
MelJuso	Ciglitazone	> 100 (2,721e + 007)
	Troglitazone	> 100 (580.4)
	15d-PGJ2	*37.45*
	WY-14643	791.5
HUVEC (endothelial cells)	Ciglitazone	> 100 (16242)
	Troglitazone	> 100 (1615)
	15d-PGJ2	*85.23, 83.7 (2nd isolated cell)*
	WY-14643	> 800 (835)
LEC (lymphatic endothelial cells)	15d-PGJ2	*70.84*
Cell line	*PPAR ligand*	*IC50 (μM)*
NHDF (normal skin fibroblasts)	15d-PGJ2	*127,70*
TF (old)	15d-PGJ2	*92,78*
MP9 fibroblasts	15d-PGJ2	*46,92*
MP10 fibrosblasts	15d-PGJ2	*44,40*
MP11 fibroblasts	15d-PGJ2	*54,40*
MCM16 fibroblasts	15d-PGJ2	*68,22*

15d-PGJ2 is superior to other PPAR ligands in inhibiting growth of melanoma cell lines, endothelial cells and of tumor associated fibroblasts superior to normal fibroblasts.Cell viability and proliferation assay. The IC50 is calculated of three independent experiments. IC50 of melanoma cells A375, M24met, 1205Lu, MelJuso and endothelial cells (HUVECs) treated with ciglitazone, troglitazone, 15d-PGJ2 and WY-14643, lymphatic endothelial cells (LECs), normal fibroblasts (NHDF) and tumor-associated fibroblasts treated with 15d-PGJ2

15d-PGJ2 Exerts Inhibitory Effects on Tumor Cell Migration, Angiogenesis and Lymphangiogenesis

Impact of 15d-PGJ2 on melanoma cell migration was investigated using a Matrigel invasion chamber assay. 15d-PGJ2 inhibited M24met melanoma cell migration in a dose-dependent manner and inhibited tumor cell migration at a concentration of 5 μM after 48 h (Fig. 8.3a). At a concentration of 25 μM migration is totally abolished as demonstrated in the M24met and A375 melanoma cell lines (Fig. 8.3a, b). The percentage of transmigrated cells is quantified by Axiovion software.

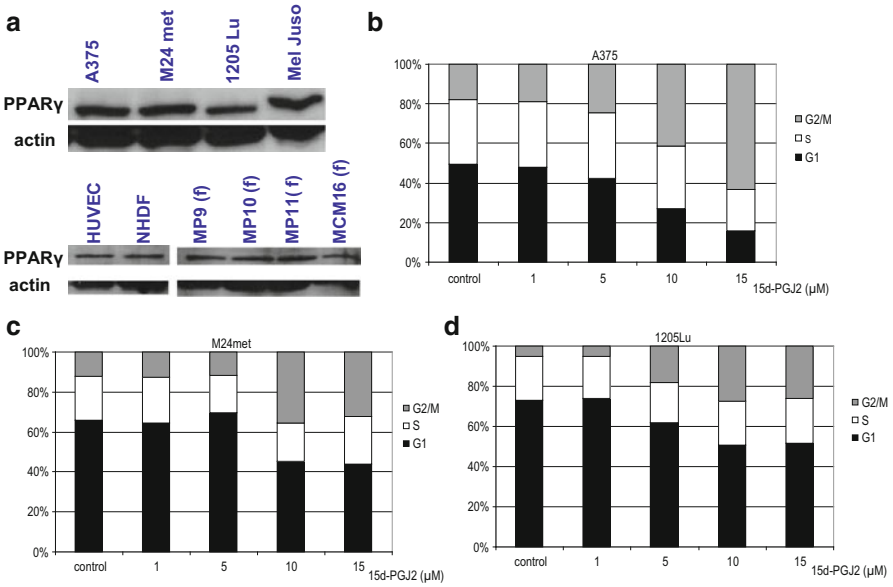

Fig. 8.1 PPARγ receptor expression and G2/M arrest induction by 15d-PGJ2. **a** receptor expression on A375, M24met, 1205Lu, MelJuso melanoma cells and HUVECs as well as fibroblasts NHDF and melanoma associated fibroblasts. (f): fibroblasts **b**, **c** and **d**: Cell cycle analysis by flow cytometry using propidium iodide- stained on A375, M24met and 1205Lu. Cells were treated for 24 h with different concentrations of 15d-PGJ2. Three independent experiments were pooled and analyzed as a combined data set

Inhibition of angiogenesis was demonstrated by a dose dependent disturbance of tube formation of HUVECs after 12 and 24 h (Fig. 8.3c, d). Inhibition of lymphangiogenesis was indicated repeating these experiments with lymphatic endothelial cells (LECs) (Fig. 8.3e, f). Here pronounced effects could be observed already at a concentration of 5 μM 15d-PGJ2. Tube formation was quantified using the Cell Profiler Software Package and calcein staining was used to demonstrate the vitality of the cells.

Shot Gun Analysis for Characterisation of the Acting Profile of 15d-PGJ2

Shot gun analysis of nuclear and cytoplasmic fractions of untreated A375 cells resulted in the identification of a total of 2,250 proteins. Proteins were classified according to gene ontology terms accessible via uniprot. Shot gun analysis of 15d-PGJ2-treated A375 cells revealed 136 proteins which displayed increased peptide counts compared to the control (Table 8.2, 8.3). Amongst these we identified proteins involved in the lipid metabolism (protein count/peptide count = 7/7) such

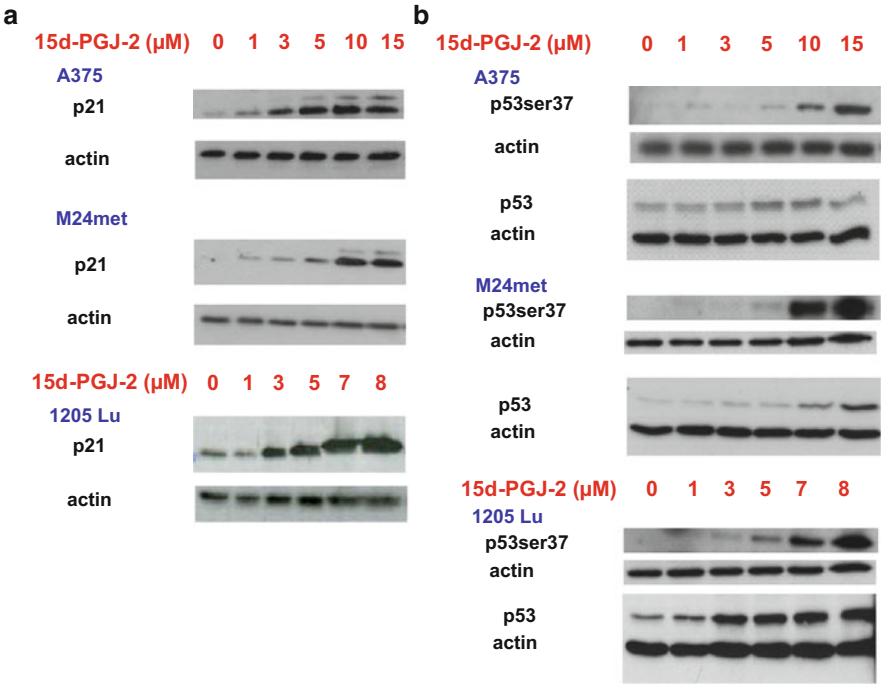

Fig. 8.2 15d-PGJ2 induces p21 expression and p53, p53ser37. **a** 15d-PGJ2 leads to an induction of p21. Immunoblotting of p21 after 48 h of 15d-PGJ2 treatment with indicated concentrations. **b** Immunoblotting of p 53 and p 53ser37 after 48 h of 15d-PGJ2 treatment with indicated concentrations

as thromboxane-A synthase, adipophilin, perilipin or apolipoprotein A-I (Table 8.2, 8.3) [22, 23]. Additionally, we detected the induction of HO-1 by 15d-PGJ2 (Table 8.2, 8.3) [24]. As depicted in Fig. 8.4a and b proteins/peptides involved in DNA repair mechanisms (5/7) such as MSH3, telomeric repeat-binding factor 2 or MMS2 (Table 8.2, 8.3), phosphorylation by ATM/ATR upon DNA damage (13/21), transport (21/26), mRNA processing (13/21), protein synthesis (5/12), replication (10/13) and transcription (8/8) were upregulated. In accordance with our data proteins involved in cell cycle such as the lymphokine-activated killer T-cell-originated protein kinase or with anti-proliferative effects such as nodal-modulator 1 revealed to be induced (Table 8.2, 8.3) (Uniprot). Proteins indicating a cellular stress response such as sterile 20/oxidant stress-response kinase 1 or growth arrest and DNA damage-inducible protein GADD45 beta were regulated as well (Table 8.2, 8.3) (Uniprot). The DNA repair proteins MMS2, MSH3, MSH6, MSH2, MLH1 and the upregulation of basigin at 1 μM and nodal at 15 μM was confirmed by Western blot analysis (Fig. 8.5).

Furthermore, several proteins related to angiogenesis such as angio-associated migratory cell protein, to cell cycle such as cyclin – A1 and H, to metastasis, to cell migration and to interaction with the extracellular matrix (ECM) such as paxillin or syntenin-1 and to proliferation such as PCNA (proliferating cell nuclear antigen)

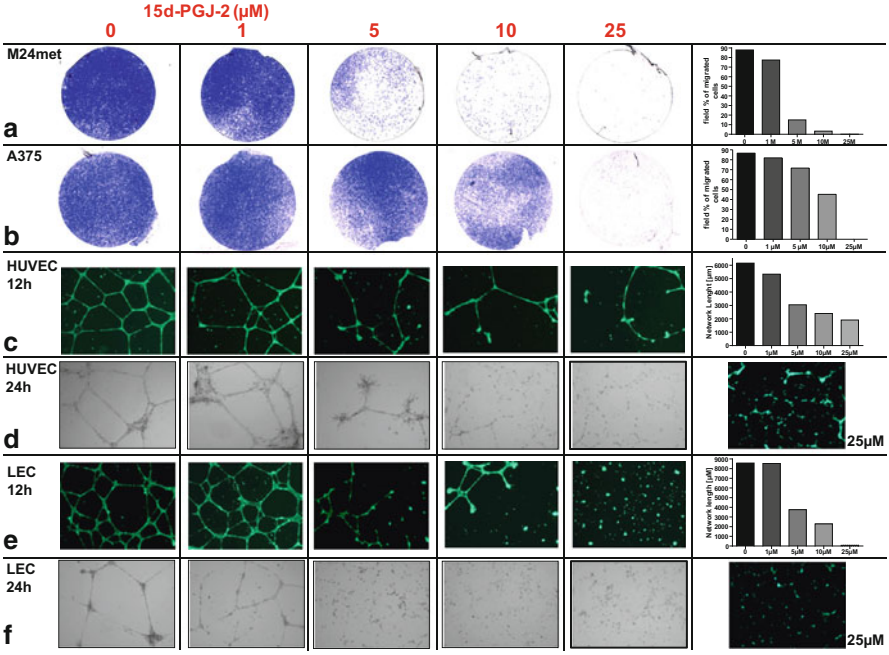

Fig. 8.3 15d-PGJ2 inhibits tumor cell migration and tube formation of HUVECs and LECs. **a** and **b** Tumor cell migration assay after 48 h of M24met melanoma cells and A375 melanoma cell line treated with 15d-PGJ2 with indicated concentrations. Representative pictures of three independent experiments. Quantification of the depicted experiment is performed using Axiovision Software. **c**–**f**, tube formation assay of HUVECs and LECs with indicated concentrations after 24 and 48 h. Calcein staining was performed to monitor the vitality of the cells. Tube formation was quantified using Cell Profiler Software Package. Representative pictures of three independent experiments

(Fig. 8.4c, Table 8.4) (Uniprot) were found to be downregulated. Evidence is growing that PPARγ ligands may be potent inhibitors of matrix metalloproteinases (MMPs) such as MMP 2, 7 and 9 [25–29]. The present shot gun proteomics data demonstrate downregulation of MMP 2, a key player in the metastatic process (Fig. 8.4c, Table 8.4). Employing a zymography assay we confirmed the downregulation of MMP2 by 15d-PGJ2 (Fig. 8.4d). To exclude unspecificity and cytotoxic side effects we evaluated if 15d-PGJ2 exhibits effects on the NF-kappa-B pathway. In the shotgun data we did not observe upregulation of constituents of the NF-kappa-B signalling pathway such as I-kappa-B-kinase 2 or NF-kappa-B inhibitor-interacting Ras-like protein 2 (Kappa B-Ras protein 2). In addition we observe no upregulation of NF-kappa-B by western blot analysis (data not shown).

Table 8.2 Upregulated candidates by 15d-PGJ2

Accession	Name	Number of Peptides	Score	Fractions	Function (Uniprot)
O00506	Serine/threonine-protein kinase 25	1	11.78	C	Oxidant stress-activated serine/threonine kinase that may play a role in the response to environmental stress. Targets to the Golgi apparatus where it appears to regulate protein transport events, cell adhesion, and polarity complexes important for cell migration. Phosphorylated upon DNA damage, probably by ATM or ATR
P11166	Solute carrier family 2, facilitated glucose transporter member 1	1	16.6	C	Facilitative glucose transporter. This isoform may be responsible for constitutive or basal glucose uptake. Transport. Phosphorylated upon DNA damage, probably by ATM or ATR
P02647	Apolipoprotein A-I (Apo-AI)	1	15.85	C	Participates in the reverse transport of cholesterol from tissues to the liver for excretion by promoting cholesterol efflux from tissues and by acting as a cofactor for the lecithin cholesterol acyltransferase (LCAT). Belongs to the apolipoprotein A1/A4/E family. Transport. Secreted
P61619	Protein transport protein Sec61 subunit alpha isoform 1	2	37.67	C, N	Plays a crucial role in the insertion of secretory and membrane polypeptides into the ER. Required for assembly of membrane and secretory proteins. Transport
O00203	AP-3 complex subunit beta-1	1	15.28	C	Subunit of non-clathrin- and clathrin-associated adaptor protein complex 3 that plays a role in protein sorting in the late-Golgi/trans-Golgi network (TGN) and/or endosomes. The AP complexes mediate both the recruitment of clathrin to membranes and the recognition of sorting signals within the cytosolic tails of transmembrane cargo molecules. AP-3 appears to be involved in the sorting of a subset of transmembrane proteins targeted to lysosomes and lysosome-related organelles. Protein Transport
O00400	Acetyl-coenzyme A transporter 1	1	17.26	C	Probable acetyl-CoA transporter necessary for O-acetylation of gangliosides. Transport
O43633	Charged multivesicular body protein 2a	1	18.49	C	Probable core component of the endosomal sorting required for transport complex III (ESCRT-III) which is involved in multivesicular bodies (MVBs) formation and sorting of endosomal cargo proteins into MVBs. MVBs are delivered to lysosomes enabling degradation of membrane proteins, such as stimulated growth factor receptors, lysosomal enzymes and lipids. Protein transport
O94979	Protein transport protein Sec31A	1	16.15	C	Component of the coat protein complex II (COPII) which promotes the formation of transport vesicles from the endoplasmic reticulum (ER). Protein transport

P03891	NADH-ubiquinone oxidoreductase chain 2	1	13.82	N	Core subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I) that is believed to belong to the minimal assembly required for catalysis. Complex I functions in the transfer of electrons from NADH to the respiratory chain. Transport
P07108	Acyl-CoA-binding protein (ACBP)	3	35.55	C	Binds medium- and long-chain acyl-CoA esters with very high affinity and may function as an intracellular carrier of acyl-CoA esters. It is also able to displace diazepam from the benzodiazepine (BZD) recognition site located on the GABA type A receptor. Transport
P08574	Cytochrome c1, heme protein, mitochondrial	1	14.1	N	This is the heme-containing component of the cytochrome b-c1 complex, which accepts electrons from Rieske protein and transfers electrons to cytochrome c in the mitochondrial respiratory chain. Transport
P21281	V-type proton ATPase subunit B, brain isoform	2	30.86	C	Non-catalytic subunit of the peripheral V1 complex of vacuolar ATPase. V-ATPase is responsible for acidifying a variety of intracellular compartments in eukaryotic cells. Identified by mass spectrometry in melanosome fractions from stage I to stage IV. Transport
P49755	Transmembrane emp24 domain-containing protein 10	1	13.61	C	Involved in vesicular protein trafficking. Identified by mass spectrometry in melanosome fractions from stage I to stage IV. Transport
P53985	Monocarboxylate transporter 1 (MCT 1)	1	13.46	C	Proton-linked monocarboxylate transporter. Transport. Widely expressed in normal and in cancer cells
P54709	Sodium/potassium-transporting ATPase subunit beta-3 (Sodium/potassium-dependent ATPase subunit beta-3) (ATPB-3)	1	14.44	C	This is the non-catalytic component of the active enzyme, which catalyzes the hydrolysis of ATP coupled with the exchange of Na+ and K+ ions across the plasma membrane. The exact function of the beta-3 subunit is not known. Identified by mass spectrometry in melanosome fractions from stage I to stage IV. Transport
P78363	Retinal-specific ATP-binding cassette transporter (ATP-binding cassette subfamily A member 4)	2	24.61	C	May play a role in photoresponse. Retinoids, and most likely retinal, are the natural substrates for transport by abcr in rod outer segments. Belongs to the ABC transporter superfamily. ABCA family. Transport
Q86UQ4	ATP-binding cassette sub-family A member 13	1	13.03	N	Transport

Table 8.2 (continued)

Accession	Name	Number of Peptides	Score	Fractions	Function (Uniprot)
Q96CW1	AP-2 complex subunit mu (Adapter-related protein complex 2 mu subunit) (Adaptor protein complex AP-2 subunit mu) (Adaptin-mu2) (AP-2 mu chain) (Plasma membrane adaptor AP-2 50 kDa protein) (HA2 50 kDa subunit) (Clathrin assembly protein complex 2 medium c	1	12.56	N	Component of the adaptor protein complex 2 (AP-2). Adaptor protein complexes function in protein transport via transport vesicles in different membrane traffic pathways. Adaptor protein complexes are vesicle coat components and appear to be involved in cargo selection and vesicle formation. AP-2 is involved in clathrin-dependent endocytosis in which cargo proteins are incorporated into vesicles surrrounded by clathrin (clathrin-coated vesicles, CCVs) which are destined for fusion with the early endosome
Q96M27	Protein PRRC1	1	15.1	C	Golgi apparatus
Q9UKS6	Protein kinase C and casein kinase substrate in neurons protein 3	1	13.92	N	May play a role in vesicle formation and transport
Q9Y5W7	Sorting nexin-14	1	14.65	C	May be involved in several stages of intracellular trafficking. Transport
O60231	Putative pre-mRNA-splicing factor ATP-dependent RNA helicase DHX16	1	17.61	N	Probable ATP-binding RNA helicase involved in pre-mRNA splicing
O60306	Intron-binding protein aquarius (Intron-binding protein of 160 kDa) (IBP160)	3	31.31	N	Intron-binding spliceosomal protein required to link pre-mRNA splicing and snoRNP (small nucleolar ribonucleoprotein) biogenesis
Q16560	U11/U12 small nuclear ribonucleoprotein 35 kDa protein	1	17.13	N	Component of the U11/U12 snRNPs that are part of the U12-type spliceosome
Q6PIY7	Poly(A) RNA polymerase GLD2 (hGLD-2) (PAP-associated domain-containing protein 4) (Terminal uridylyltransferase 2) (TUTase 2)	1	15.71	N	Cytoplasmic poly(A) RNA polymerase that adds successive AMP monomers to the 3′-end of specific RNAs, forming a poly(A) tail. In contrast to the canonical nuclear poly(A) RNA polymerase, it only adds poly(A) to selected cytoplasmic mRNAs. Does not play a role in replication-dependent histone mRNA degradation
Q96I25	Splicing factor 45 (45 kDa-splicing factor) (RNA-binding motif protein 17)	1	13.29	N	Splice factor that binds to the single stranded 3′AG at the exon/intron border and promotes its utilization in the second catalytic step. Involved in the regulation of alternative splicing and the utilization of cryptic splice sites. Promotes the utilization of a cryptic splice site created by the beta-110 mutation in the HBB gene. The resulting frameshift leads to sickle cell anemia

P63162	Small nuclear ribonucleoprotein-associated protein N	7	105.3	N	May be involved in tissue-specific alternative RNA processing events
Q12799	T-complex protein 10A homolog	1	11.85	C	Alternative Splicing
Q16637	Survival motor neuron protein (Component of gems 1) (Gemin-1)	1	13.83	C	The SMN complex plays an essential role in spliceosomal snRNP assembly in the cytoplasm and is required for pre-mRNA splicing in the nucleus. It may also play a role in the metabolism of snoRNPs
Q3KQU3	MAP7 domain-containing protein 1	1	15.84	C	Alternative Splicing
Q5T3F8	Transmembrane protein 63B	1	19.77	N	Membrane. Alternative Splicing
Q5T8P6	RNA-binding protein 26	1	16.07	N	Alternative Splicing
Q5TB30	DEP domain-containing protein 1A	1	12.2	C	Alternative Splicing. Up-regulated in bladder cancer cells (at protein level)
Q96D71	RalBP1-associated Eps domain-containing protein 1	1	13.78	C	May coordinate the cellular actions of activated EGF receptors and Ral-GTPases
Q14004	Cell division protein kinase 13 (Cell division cycle 2-like protein kinase 5) (CDC2-related protein kinase 5)	1	17.17	N	May be a controller of the mitotic cell cycle. Involved in the blood cell development. Also expressed in neuroblastoma and glioblastoma tumors. Phosphorylated upon DNA damage, probably by ATM or ATR
Q14839	Chromodomain-helicase-DNA-binding protein 4	1	12.43	N	Probable transcription regulator. Phosphorylated upon DNA damage, probably by ATM or ATR
Q99575	Ribonucleases P/MRP protein subunit POP1 (hPOP1)	2	29.55	N	Component of ribonuclease P, a protein complex that generates mature tRNA molecules by cleaving their 5′-ends. Also a component of RNase MRP Phosphorylated upon DNA damage, probably by ATM or ATR
Q9C0C2	182 kDa tankyrase-1-binding protein	4	54.84	C	Binds to the ANK repeat domain of TNKS1 and TNKS2. Phosphorylated upon DNA damage, probably by ATM or ATR
O60271	C-jun-amino-terminal kinase-interacting protein 4 (JNK-interacting protein 4)	1	16.37	C	The JNK-interacting protein (JIP) group of scaffold proteins selectively mediates JNK signaling by aggregating specific components of the MAPK cascade to form a functional JNK signaling module. Perinuclear distribution in response to stress signals such as UV radiation. Phosphorylated upon DNA damage, probably by ATM or ATR
P20585	DNA mismatch repair protein Msh3 (hMSH3) (Divergent upstream protein) (DUP) (Mismatch repair protein 1) (MRP1)	2	19.2	N	Component of the post-replicative DNA mismatch repair system (MMR). Heterodimerizes with MSH2 to form MutS beta which binds to DNA mismatches thereby initiating DNA repair. Phosphorylated upon DNA damage, probably by ATM or ATR

Table 8.2 (continued)

Accession	Name	Number of Peptides	Score	Fractions	Function (Uniprot)
Q02952	A-kinase anchor protein 12 (A-kinase anchor protein 250 kDa) (AKAP 250) (Gravin) (Myasthenia gravis autoantigen)	1	18.9	C	Anchoring protein that mediates the subcellular compartmentation of protein kinase A (PKA) and protein kinase C (PKC). Expressed in endothelial cells, cultured fibroblasts and osteosarcoma. Activated by lysophosphatidylcholine (lysoPC). Phosphorylated upon DNA damage, probably by ATM or ATR.
Q15554	Telomeric repeat-binding factor 2 (TTAGGG repeat-binding factor 2) (Telomeric DNA-binding protein)	1	14.27	N	Binds the telomeric double-stranded TTAGGG repeat. Protects against end-to-end fusion of chromosomes and plays a role in successful progression through the cell division cycle. Component of the shelterin complex (telosome) that is involved in the regulation of telomere length and protection. Phosphorylated upon DNA damage, probably by ATM or ATR. $ Shelterin associates with arrays of double-stranded TTAGGG repeats added by telomerase and protects chromosome ends; without its protective activity, telomeres are no longer hidden from the DNA damage surveillance and chromosome ends are inappropriately processed by DNA repair pathways
Q9H583	HEAT repeat-containing protein 1 (Protein BAP28)	3	36.74	N	Involved in nucleolar processing of pre-18S ribosomal RNA. Involved in ribosome biosynthesis Phosphorylated upon DNA damage, probably by ATM or ATR
Q9NPQ8	Synembryn-A (Protein Ric-8A)	2	29.98	C	Guanine nucleotide exchange factor (GEF), which can activate some, but not all, G-alpha proteins. Able to activate GNAI1, GNAO1 and GNAQ, but not GNAS by exchanging bound GDP for free GTP. Involved in regulation of microtubule pulling forces during mitotic movement of chromosomes by stimulating G(i)-alpha protein, possibly leading to release G(i)-alpha-GTP and NuMA proteins from the NuMA-GPSM2-G(i)-alpha-GDP complex By similarity. Also acts as an activator for G(q)-alpha (GNAQ) protein by enhancing the G(q)-coupled receptor-mediated ERK activation Phosphorylated upon DNA damage, probably by ATM or ATR
Q9UNF1	Melanoma-associated antigen D2 (MAGE-D2 antigen) (Breast cancer-associated gene 1 protein) (BCG-1) (11B6) (Hepatocellular carcinoma-associated protein JCL-1)	1	9.3	C	Phosphorylated upon DNA damage, probably by ATM or ATR

P24557	Thromboxane-A synthase (TXA synthase) (Cytochrome P450 5A1)	1	12.57	C	Belongs to the cytochrome P450 family. Fatty acid biosynthesis, Lipid synthesis, Prostaglandin biosynthesis
P30837	Aldehyde dehydrogenase X, mitochondrial (Aldehyde dehydrogenase family 1 member B1) (Aldehyde dehydrogenase 5)	1	12.33	C	ALDHs play a major role in the detoxification of alcohol-derived acetaldehyde. They are involved in the metabolism of corticosteroids, biogenic amines, neurotransmitters, and lipid peroxidation
P54619	5′-AMP-activated protein kinase subunit gamma-1 (AMPK subunit gamma-1)	1	21.4	C	AMPK is responsible for the regulation of fatty acid synthesis by phosphorylation of acetyl-CoA carboxylase. Also regulates cholesterol synthesis via phosphorylation and inactivation of hydroxymethylglutaryl-CoA reductase and hormone-sensitive lipase. Fatty acid biosynthesis, Lipid synthesis
Q14914	Prostaglandin reductase 1 (PRG-1) (NADP-dependent leukotriene B4 12-hydroxydehydrogenase) (15-oxoprostaglandin 13-reductase)	1	13.61	C	Functions as 15-oxo-prostaglandin 13-reductase and acts on 15-oxo-PGE1, 15-oxo-PGE2 and 15-oxo-PGE2-alpha. Has no activity towards PGE1, PGE2 and PGE2-alpha By similarity. Catalyzes the conversion of leukotriene B4 into its biologically less active metabolite, 12-oxo-leukotriene B4. This is an initial and key step of metabolic inactivation of leukotriene B4
Q96Q06	Perilipin-4 (Adipocyte protein S3-12)	1	16.51	C	May play a role in triacylglycerol packaging into adipocytes. May function as a coat protein involved in the biogenesis of lipid droplets Up-regulated during adipocyte differentiation
Q99541	Adipophilin (Adipose differentiation-related protein) (ADRP)	1	13.13	C	May be involved in development and maintenance of adipose tissue
P35659	Protein DEK	3	47.66	N	May have a function in the nucleus. DEK is found in a subset of acute myeloid leukemia (AML); also known as acute non-lymphocytic leukemia
P42695	Condensin-2 complex subunit D3 (Non-SMC condensin II complex subunit D3) (hCAP-D3)	1	12.79	N	Regulatory subunit of the condensin-2 complex, a complex which establishes mitotic chromosome architecture and is involved in physical rigidity of the chromatid axis. Cell cycle, cell division, mitosis
Q12974	Protein tyrosine phosphatase type IVA 2	1	17.79	C	Protein tyrosine phosphatase which stimulates progression from G1 into S phase during mitosis. Promotes tumors. Overexpressed in prostate tumor tissue

Table 8.2 (continued)

Accession	Name	Number of Peptides	Score	Fractions	Function (Uniprot)
Q15155	Nodal modulator 1 (pM5)	1	14.64	N	May antagonize Nodal signaling. Expressed in colon tumor tissue and in adjacent normal colonic mucosa. Pro-apoptotic, Anti-proliferation
Q15287	RNA-binding protein with serine-rich domain 1 (SR-related protein LDC2)	1	17.31	N	Component of a splicing-dependent multiprotein exon junction complex (EJC) deposited at splice junction on mRNAs
Q15477	Helicase SKI2W (Helicase-like protein) (HLP)	1	17.15	C	Helicase; has ATPase activity
Q16527	Cysteine and glycine-rich protein 2 (Cysteine-rich protein 2) (Smooth muscle cell LIM protein)	1	12.26	N	Drastically down-regulated in response to PDGF-BB or cell injury, that promote smooth muscle cell proliferation and dedifferentiation. Seems to play a role in the development of the embryonic vascular system
Q7KZ85	Transcription elongation factor SPT6 (hSPT6)	1	18.7	N	Acts to stimulate transcriptional elongation by RNA polymerase II
Q7L2E3	Putative ATP-dependent RNA helicase DHX30	2	24.85	N	no known function
Q7Z5K2	Wings apart-like protein homolog (Friend of EBNA2 protein)	1	14.39	N	May play a role in cell growth
Q8NBU5	ATPase family AAA domain-containing protein 1	1	17.16	N	Replication
Q8TF68	Zinc finger protein 384	1	13.86	N	Transcription factor that binds the consensus DNA sequence [GC]AAAAA. Seems to bind and regulate the promoters of MMP1, MMP3, MMP7 and COL1A1
Q969S3	Zinc finger protein 622 (Zinc fingerlike protein 9)	1	15.58	N	May behave as an activator of the bound transcription factor, MYBL2, and be involved in embryonic development
Q96RK0	Protein capicua homolog	1	12.71	C	Transcriptional repressor which may play a role in development of the central nervous system (CNS)
Q9H6F5	Coiled-coil domain-containing protein	1	14.65	N	Nuclear
Q9Y5V0	Zinc finger protein 706	1	13.22	N	Zinc finger protein
O00754	Lysosomal alpha-mannosidase (Laman)	1	14.91	C	Necessary for the catabolism of N-linked carbohydrates released during glycoprotein turnover. Cleaves all known types of alpha-mannosidic linkages

O14880	Microsomal glutathione S-transferase 3	1	15.48	N	Functions as a glutathione peroxidase
O15143	Actin-related protein 2/3 complex subunit 1B (Arp2/3 complex 41 kDa subunit) (p41-ARC)	1	15.84	C	Functions as component of the Arp2/3 complex which is involved in regulation of actin polymerization and together with an activating nucleation-promoting factor (NPF) mediates the formation of branched actin networks
O15511	Actin-related protein 2/3 complex subunit 5 (Arp2/3 complex 16 kDa subunit) (p16-ARC)	1	13.18	C	Functions as component of the Arp2/3 complex which is involved in regulation of actin polymerization and together with an activating nucleation-promoting factor (NPF) mediates the formation of branched actin networks
O43516	WAS/WASL-interacting protein family member 1	1	12.63	C	May have direct activity on the actin cytoskeleton. Induces actin polymerization and redistribution
O75179	Ankyrin repeat domain-containing protein 17	1	15.62	C	Earliest specific in situ marker of hepatic differentiation during embryogenesis, useful for characterization of inductive events involved in hepatic specification
O75293	Growth arrest and DNA damage-inducible protein GADD45 beta	1	11.3	C	Involved in the regulation of growth and apoptosis. Mediates activation of stress-responsive MTK1/MEKK4 MAPKKK
O76039	Cyclin-dependent kinase-like 5 (Serine/threonine-protein kinase 9)	1	12.29	N	Mediates phosphorylation of MECP2
O94804	Serine/threonine-protein kinase 10 (Lymphocyte-oriented kinase)	1	14.73	C	Can act on substrates such as myelin basic protein and histone 2A on serine and threonine residues
O95139	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 6 (NADH-ubiquinone oxidoreductase B17 subunit) (Complex I-B17) (CI-B17)	1	12.64	N	Accessory subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I), that is believed not to be involved in catalysis. Complex I functions in the transfer of electrons from NADH to the respiratory chain. The immediate electron acceptor for the enzyme is believed to be ubiquinone
P05362	Intercellular adhesion molecule 1 (ICAM-1) (Major group rhinovirus receptor)	1	12.01	C	ICAM proteins are ligands for the leukocyte adhesion protein LFA-1 (integrin alpha-L/beta-2). During leukocyte transendothelial migration, ICAM1 engagement promotes the assembly of endothelial apical cups through SGEF and RHOG activation
P09601	Heme oxygenase 1 (HO-1)	2	19.3	C	Heme oxygenase cleaves the heme ring at the alpha methene bridge to form biliverdin. Biliverdin is subsequently converted to bilirubin by biliverdin reductase

Table 8.2 (continued)

Accession	Name	Number of Peptides	Score	Fractions	Function (Uniprot)
P10636	Microtubule-associated protein tau (Neurofibrillary tangle protein) (Paired helical filament-tau) (PHF-tau)	1	13.28	N	Promotes microtubule assembly and stability, and might be involved in the establishment and maintenance of neuronal polarity. The C-terminus binds axonal microtubules while the N-terminus binds neural plasma membrane components, suggesting that tau functions as a linker protein between both
P11279	Lysosome-associated membrane glycoprotein 1 (LAMP-1) (CD107 antigen-like family member A)	1	15.01	C	Presents carbohydrate ligands to selectins. Also implicated in tumor cell metastasis
P11474	Steroid hormone receptor ERR1 (Estrogen-related receptor alpha) (ERR-alpha) (Estrogen receptor-like 1) (Nuclear receptor subfamily 3 group B member 1)	1	13.81	N	Belongs to the nuclear hormone receptor family. Binds to an ERR-alpha response element (ERRE) containing a single consensus half-site. Binds DNA as a monomer or a homodimer
P16615	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	3	44.84	C, N	This magnesium-dependent enzyme catalyzes the hydrolysis of ATP coupled with the translocation of calcium from the cytosol to the sarcoplasmic reticulum lumen
P16930	Fumarylacetoacetase (FAA)	1	12.36	C	4-fumarylacetoacetate $+H_2O =$ acetoacetate + fumarate
P17275	Transcription factor jun-B	1	12.94	N	Transcription factor involved in regulating gene activity following the primary growth factor response. Binds to the DNA sequence 5′-TGA[CG]TCA-3′. Transcription
P17661	Desmin	1	12.85	N	Desmin are class-III intermediate filaments found in muscle cells. Belongs to the intermediate filament family
P27105	Erythrocyte band 7 integral membrane protein (Stomatin)	2	27.52	C, N	Thought to regulate cation conductance. Identified by mass spectrometry in melanosome fractions
P29144	Tripeptidyl-peptidase 2	1	13.67	C	Component of the proteolytic cascade acting downstream of the 26S proteasome in the ubiquitin-proteasome pathway
P30043	Flavin reductase (FR) (NADPH-dependent diaphorase)	1	15.75	C	Broad specificity oxidoreductase that catalyzes the NADPH-dependent reduction of a variety of flavins, such as riboflavin, FAD or FMN, biliverdins, methemoglobin and PQQ (pyrroloquinoline quinone). Contributes to heme catabolism and metabolizes linear tetrapyrroles

P35613	Basigin (Leukocyte activation antigen M6) (Collagenase stimulatory factor)	2	26.82	C	Plays pivotal roles in spermatogenesis, embryo implantation, neural network formation and tumor progression. Stimulates adjacent fibroblasts to produce matrix metalloproteinases (MMPS). Seems to be a receptor for oligomannosidic glycans. In vitro, promotes outgrowth of astrocytic processes. Enriched on the surface of tumor cells. Up-regulated in gliomas. Its expression levels correlate with malignant potential of the tumor
P36957	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	1	19.07	N	The 2-oxoglutarate dehydrogenase complex catalyzes the overall conversion of 2-oxoglutarate to succinyl-CoA and CO_2. It contains multiple copies of 3 enzymatic components: 2-oxoglutarate dehydrogenase (E1), dihydrolipoamide succinyltransferase (E2) and lipoamide dehydrogenase (E3). Mitochondrial
P40121	Macrophage-capping protein (Actin regulatory protein CAP-G)	1	12.89	C	Calcium-sensitive protein which reversibly blocks the barbed ends of actin filaments but does not sever preformed actin filaments. May play an important role in macrophage function. May play a role in regulating cytoplasmic and/or nuclear structures through potential interactions with actin. May bind DNA. Identified by mass spectrometry in melanosome fractions from stage I to stage IV
P48163	NADP-dependent malic enzyme (NADP-ME) (Malic enzyme 1)	1	14.28	C	(S)-malate + NADP+ = pyruvate + CO_2 + NADPH. Oxidoreductase
P49662	Caspase-4 (CASP-4) (Protease ICH-2) (Protease TX) (ICE(rel)-II)	1	13.83	C	Involved in the activation cascade of caspases responsible for apoptosis execution. Cleaves caspase-1
P55786	Puromycin-sensitive aminopeptidase (PSA)	4	52.95	C	Aminopeptidase with broad substrate specificity to several peptides. Involved in proteolytic events essential for cell growth and viability. Identified by mass spectrometry in melanosome fractions from stage I to stage IV
P57088	Transmembrane protein 33 (Protein DB83)	1	17.38	C, N	Transmembrane. Identified by mass spectrometry in melanosome fractions from stage I to stage IV
Q14139	Ubiquitin conjugation factor E4 A	1	11.97	C	Binds to the ubiquitin moieties of preformed conjugates and catalyzes ubiquitin chain assembly in conjunction with E1, E2, and E3. mRNA processing
Q14147	Probable ATP-dependent RNA helicase DHX34	1	12.57	N	Probable ATP-binding RNA helicase
Q15819	Ubiquitin-conjugating enzyme E2 variant 2 (MMS2) (Enterocyte	2	24.62	C	Has no ubiquitin ligase activity on its own. The UBE2V2/UBE2N heterodimer catalyzes the synthesis of non-canonical poly-ubiquitin

Table 8.2 (continued)

Accession	Name	Number of Peptides	Score	Fractions	Function (Uniprot)
	differentiation-associated factor EDAF-1) (Enterocyte differentiation-promoting factor) (EDPF-1) (Vitamin D3-inducible protein) (DDVit 1)				chains that are linked through 'Lys-63'. This type of poly-ubiquitination does not lead to protein degradation by the proteasome. Mediates transcriptional activation of target genes. Plays a role in the control of progress through the cell cycle and differentiation. Plays a role in the error-free DNA repair pathway and contributes to the survival of cells after DNA damage
Q16719	Kynureninase	3	40.75	C	Pyridine nucleotide biosynthesis
Q5T655	Coiled-coil domain-containing protein	1	14.65	C	Not known
Q6WCQ1	Myosin phosphatase Rho-interacting protein (M-RIP) (Rho-interacting protein 3) (RIP3) (p116Rip)	1	14.68	N	Targets myosin phosphatase to the actin cytoskeleton. Required for the regulation of the actin cytoskeleton by RhoA and ROCK1. Depletion leads to an increased number of stress fibers in smooth muscle cells through stabilization of actin fibers by phosphorylated myosin. Overexpression of MRIP as well as its F-actin-binding region leads to disassembly of stress fibers in neuronal cells
Q6ZS46	Putative uncharacterized protein FLJ45840	1	12.15	N	no known function
Q7Z2T5	TRM1-like protein	1	13.84	N	May play a role in motor coordination and exploratory behavior
Q7Z6I8	UPF0461 protein C5orf24	1	13.57	N	no known function
Q8IW41	MAP kinase-activated protein kinase 5	1	14.63	C	Mediates stress-induced small heat shock protein 27 phosphorylation
Q8IY37	Probable ATP-dependent RNA helicase DHX37 (DEAH box protein 37)	2	29.56	N	Belongs to the DEAD box helicase family. DEAH subfamily
Q8N6L1	Keratinocyte-associated protein 2 (KCP-2)	1	13.2	N	Component of the oligosaccharyltransferase (OST) complex. OST seems to exist in different forms which contain at least RPN1, RPN2, OST48, DAD1, OSTC, KRTCAP2 and either STT3A or STT3B. OST can form stable complexes with the Sec61 complex or with both the Sec61 and TRAP complexes
Q8N766	Uncharacterized protein KIAA0090	1	13.36	N	no known function
Q8NA47	Coiled-coil domain-containing protein	1	13.18	N	no known function
Q8NEF9	Serum response factor-binding protein 1 (SRF-dependent transcription regulation-associated protein) (p49/STRAP)	1	13.53	N	May be involved in regulating transcriptional activation of cardiac genes during the aging process. May play a role in biosynthesis and/or processing of SLC2A4 in adipose cells

Q8TBC4	NEDD8-activating enzyme E1 catalytic subunit (Ubiquitin-like modifier-activating enzyme 3) (Ubiquitin-activating enzyme 3) (NEDD8-activating enzyme E1C) (Ubiquitin-activating enzyme E1C)	1	14.4	C	Catalytic subunit of the dimeric UBA3-NAE1 E1 enzyme. E1 activates NEDD8 by first adenylating its C-terminal glycine residue with ATP, thereafter linking this residue to the side chain of the catalytic cysteine, yielding a NEDD8-UBA3 thioester and free AMP. E1 finally transfers NEDD8 to the catalytic cysteine of UBE2M. Down-regulates steroid receptor activity. Necessary for cell cycle progression
Q96KB5	Lymphokine-activated killer T-cell-originated protein kinase	1	14.29	C	Phosphorylates MAP kinase p38. Seems to be active only in mitosis. May also play a role in the activation of lymphoid cells. When phosphorylated, forms a complex with TP53, leading to TP53 destabilization and attenuation of G2/M checkpoint during doxorubicin-induced DNA damage
Q96N11	Uncharacterized protein C7orf26	1	11.54	N	No known function
Q9BQ52	Zinc phosphodiesterase ELAC protein 2	1	13.44	C	Zinc phosphodiesterase, which displays some tRNA 3′-processing endonuclease activity. Probably involved in tRNA maturation, by removing a 3′-trailer from precursor tRNA
Q9BV86	Methyltransferase-like protein 11A	1	12.02	C	Probable S-adenosyl-L-methionine-dependent methyltransferase
Q9BZ29	Dedicator of cytokinesis protein 9 (Cdc42 guanine nucleotide exchange factor zizimin-1)	1	12.47	C	Guanine nucleotide-exchange factor (GEF) that activates CDC42 by exchanging bound GDP for free GTP. Overexpression induces filopodia formation
Q9GZS1	DNA-directed RNA polymerase I subunit RPA49 (RNA polymerase I subunit A49) (DNA-directed RNA polymerase I subunit E) (RNA polymerase I-associated factor 1) (RNA polymerase I-associated factor 53)	1	12.69	N	DNA-dependent RNA polymerase catalyzes the transcription of DNA into RNA using the four ribonucleoside triphosphates as substrates. Component of RNA polymerase I which synthesizes ribosomal RNA precursors. Appears to be involved in the formation of the initiation complex at the promoter by mediating the interaction between Pol I and UBTF/UBF
Q9H501	ESF1 homolog (ABT1-associated protein)	1	12.72	N	May constitute a novel regulatory system for basal transcription. Negatively regulates ABT1
Q9H853	Putative tubulin-like protein alpha-4B (Alpha-tubulin 4B)	2	23.58	N	cytoskeleton
Q9NSD9	Phenylalanyl-tRNA synthetase beta chain (Phenylalanine–tRNA ligase beta chain) (PheRS)	3	44.85	N	ATM target
Q9NUQ9	Protein FAM49B (L1)	1	13.07	C	No known function
Q9NX40	OCIA domain-containing protein 1	3	41.16	C, N	No known function

Table 8.2 (continued)

Accession	Name	Number of Peptides	Score	Fractions	Function (Uniprot)
Q9NZA1	Chloride intracellular channel protein 5	1	13	C	Can insert into membranes and form poorly selective ion channels that may also transport chloride ions. May play a role in the regulation of transepithelial ion absorption and secretion. Required for normal formation of stereocilia in the inner ear and normal development of the organ of Corti
Q9P000	COMM domain-containing protein 9	1	16.62	C	No known function
Q9P2D3	HEAT repeat-containing protein 5B	1	15.1	C	No known function
Q9UET6	Putative ribosomal RNA methyltransferase 1	1	14.55	C	S-adenosyl-L-methionine + rRNA = S-adenosyl-L-homocysteine + rRNA containing 2′-O-methyluridine.
Q9UJY1	Heat shock protein beta-8 (HspB8)	1	13.43	C	Displays temperature-dependent chaperone activity
Q9UM00	Transmembrane and coiled-coil domain-containing protein 1	1	13.21	N	Endoplasmic reticulum membrane;
Q9UM54	Myosin-VI (Unconventional myosinVI)	2	26.36	C	Transport
Q9Y263	Phospholipase A-2-activating protein (PLA2P)	1	15.17	C	Plays an important role in the regulation of specific inflammatory disease processes
Q9Y2R0	Coiled-coil domain-containing protein	1	14.32	C	No known function
Q9Y399	28S ribosomal protein S2, mitochondrial (S2mt)	1	14.43	C	Component of the mitochondrial ribosome small subunit (28S)
Q9Y5T4	DnaJ homolog subfamily C member 15 (Methylation-controlled J protein) (MCJ) (Cell growth-inhibiting gene 22 protein)	1	17	N	Absent or down-regulated in many advanced cases of ovarian adenocarcinoma, due to hypermethylation and allelic loss. Loss expression correlates with increased resistance to antineoplastic drugs, such as cisplatin

Proteins induced by 5 μM 15d-PGJ2 in A375 melanoma cells after 48 h. The proteins are classified by the CPL/MUW database. Uniprot serves as reference for the function of the proteins. In addition, the accession numbers are from the Uniprot database. Numbers indicate distinct peptides identified by mass spectrometry. C: cytoplasm, N: nucleous, S: supernatant

Table 8.3 Categories of upregulated candidates by 15d-PGJ2

Accession	Transport	mRNA processing	ATM target	Lipid metabolism	Nuclear	cell cycle	Cell Stress	Ciplatin-R	Cytoskeleton	Detoxification	DNA damage/repair	Drug resistance	ECM	Genuinely Secreted	Inflammation	Lysosome	Membrane	Migration	Mitochondrial	Pro-apoptotic	Proteases/Inhibitors	Protein degradation	Protein synthesis	Redox	Replication	Ribosomal	signalling	Transcription	Activation	Anti-apoptotic
O00506	x		x				x											x									x			
P11166	x		x																											
P02647	x			x										x																
P61619	x				x			x																						
O00203	x															x														
O00400	x																													
O43633	x															x														
O94979	x																													
P03891	x																		x											
P07108	x																													
P08574	x							x											x											
P21281	x																													
P49755	x																x													
P53985	x																													
P54709	x																													
P78363	x									x																				
Q86UQ4	x																													
Q96CW1	x																													
Q96M27	x																													
Q9UKS6	x																												x	
Q9Y5W7	x																													
O60231		x			x																									
O60306		x			x																									
Q16560		x			x																									
Q6PIY7		x			x																									
Q96I25		x			x																									
P63162		x																												
Q12799		x																												
Q16637		x																												
Q3KQU3		x																												
Q5T3F8		x															x													
Q5T8P6		x																												
Q5TB30		x																												
Q96D71		x																												
Q14004			x		x	x		x			x																x			
Q14839			x		x																						x			
Q99575			x		x																									
Q9C0C2			x		x																									
O60271			x																										x	
P20585			x								x																			

Table 8.3 (continued)

Accession	Transport	mRNA processing	ATM target	Lipid metabolism	Nuclear	cell cycle	Cell Stress	Ciplatin-R	Cytoskeleton	Detoxification	DNA damage/repair	Drug resistance	ECM	Genuinely Secreted	Inflammation	Lysosome	Membrane	Migration	Mitochondrial	Pro-apoptotic	Proteases/Inhibitors	Protein degradation	Protein synthesis	Redox	Replication	Rhibosomal	signalling	Transcription	Activation	Anti-apoptotic
Q02952			x																											
Q15554			x								x														x					
Q9H583			x																				x							
Q9NPQ8			x																											
Q9UNF1			x																											
P24557				x						x																				
P30837				x															x											
P54619				x																										
Q14914				x																										
Q96Q06				x																										
Q99541				x																										
P35659					x			x																						
P42695					x	x																			x					
Q12974					x	x																			x					
Q15155					x															x										
Q15287					x																									
Q15477					x																									
Q16527					x																									
Q7KZ85					x																						x			
Q7L2E3					x																									
Q7Z5K2					x																									
Q8NBU5					x																				x					
Q8TF68					x																						x			
Q969S3					x																						x			
Q96RK0					x																									
Q9H6F5					x																									
Q9Y5V0					x																									
O00754														x		x														
O14880										x														x						
O15143									x									x												
O15511								x	x									x												
O43516									x																					
O75293											x									x					x				x	
O76039																									x					
O94804																											x			
O95139																			x											
P05362								x						x	x			x												
P09601																													x	
P10636									x																					
P11279														x		x	x	x												
P11474																													x	
P17275																									x			x		
P17661									x																					
P27105												x																		
P29144																						x								
P30043																								x						

Table 8.3 (continued)

Accession	Transport	mRNA processing	ATM target	Lipid metabolism	Nuclear	cell cycle	Cell Stress	Ciplatin-R	Cytoskeleton	Detoxification	DNA damage/repair	Drug resistance	ECM	Genuinely Secreted	Inflammation	Lysosome	Membrane	Migration	Mitochondrial	Pro-apoptotic	Proteases/Inhibitors	Protein degradation	Protein synthesis	Redox	Replication	Ribosomal	signalling	Transcription	Activation	Anti-apoptotic
P35613													x	x				x			x									
P36957																			x											
P40121								x	x																					
P48163																								x						
P49662																				x										
P55786																									x					
P57088																	x													
Q14139																						x								
Q15819							x					x										x								
Q16719																							x							
Q6WCQ1									x									x												
Q8IW41								x																					x	x
Q8IYD1									x														x							
Q8NEF9																												x		
Q8TBC4																									x					
Q96KB5								x																						
Q9BQ52																							x							
Q9BZ29																		x												
Q9GZS1																									x					
Q9H501																												x		
Q9H853									x																					
Q9NSD9								x															x							
Q9UET6																										x				
Q9UJY1							x																							
Q9Y263															x															
Q9Y399																			x							x				

Proteins induced by 5 μM 15d-PGJ2 in A375 melanoma cells after 48 h. The proteins are classified by the CPL/MUW database. Uniprot serves as reference
for the function of the proteins. In addition, the accession numbers are from the Uniprot database. Numbers indicate distinct peptides identified by mass
spectrometry. C: cytoplasm, N: nucleous, S: supernatant

15d-PGJ2 Highly Downregulates a Panel of Chaperones and Leads to a Modification of Hsp90 in 2D-gel Electrophoresis

A large group of 33 out of 38 detectable chaperones were downregulated (Table 8.5). Especially Hsp90 beta (− 15) and alpha (− 13) revealed to be the most prominent downregulated chaperones upon 15d-PGJ2 treatment (Table 8.5). However, Western blotting of the cytoplasmic fractions and total cell lysates of A375 and 1205Lu cells did not verify these results (Fig. 8.6a, b). Using the total cell lysate again no regulation of Hsp90 could be verified in A375 and 1205Lu melanoma cell lines, only an induction of the additional appearing band of Hsp56 (Fig. 8.6b).

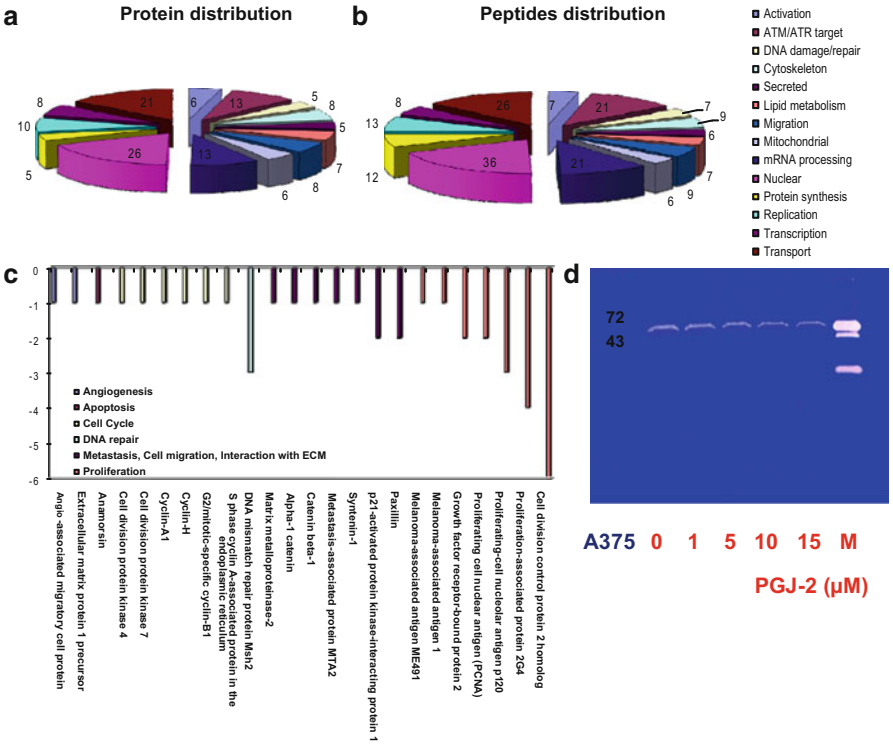

Fig. 8.4 Shot gun analysis and Zymography of A375 melanoma cells treated with 15d-PGJ2. **a** and **b** classification of all induced proteins and peptides by the bioinformatic database of A375 melanoma cells treated with 5 μM 15d-PGJ2 for 48 h. **c** downregulated proteins by 5 μM 15d-PGJ2 identified by shot gun analysis. The legend depicts the classification of the identified proteins. **d** Zymography assay of the supernatant of A375 melanoma cells treated with 1, 5, 10, 15 μM 15d-PGJ2 for 48 h

To further investigate these surprising results we performed 2D-gel electrophoresis with cytoplasmic proteins of A375 melanoma cells. Intriguingly, Hsp90 displayed a profound pI shift from 5.2–5.4 in the control group to 5.0–5.2 upon 15d-PGJ2 treatment (Fig. 8.6c). This indicates posttranslational modifications of Hsp90 which may cause interference with the identification of peptides by shot gun analysis. This shift is visualized also in a 3 dimensional version of the 2D-gel (Fig. 8.6c).

Protein modification may result in apparent down-regulation of the number of identified peptides, because modified peptides may fail to be identified by mass spectrometry. Therefore, we further investigated protein phosphorylation by immunoprecipitation using an anti-phosphoserine antibody. Actually, all chaperones identified in the immunoprecipitates were found at increased levels in the 15d-PGJ2 treated samples (Hsp90, Hsp27,T-complex protein 1 subunit theta and eta), indicating 15d-PGJ2-induced phosphorylation (Fig. 8.6d). These chaperones were found to be apparently down-regulated by 15d-PGJ2 (Table 8.5), suggesting that partially the down-regulation observed by shotgun proteomics is accompanied by phosphorylation.

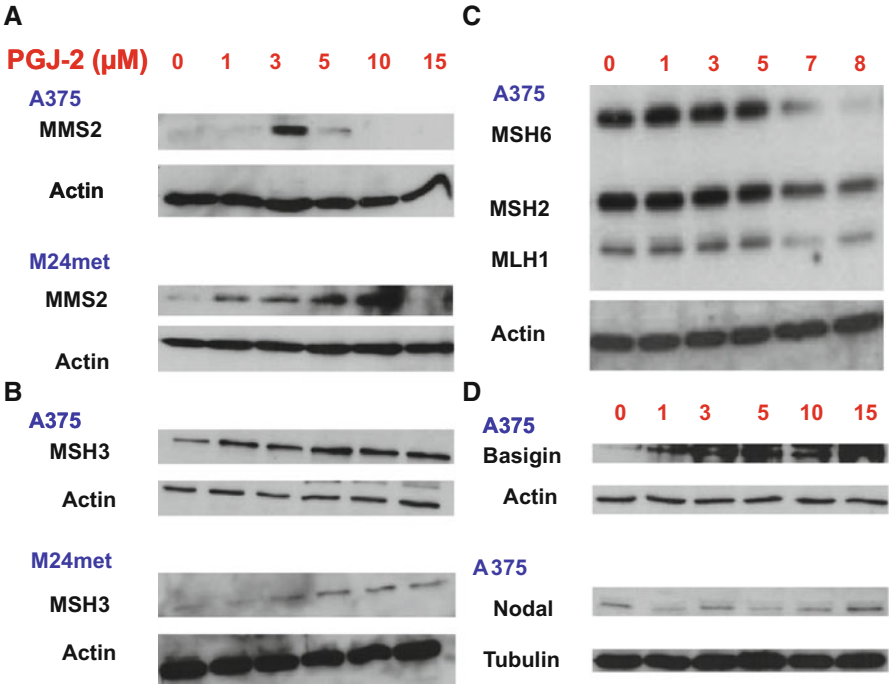

Fig. 8.5 Representative immunoblots of three independent experiments of MMS2, MSH3, MSH6, MSH2, MLH1, Basigin and nodal after 48 h treatment of 15d-PGJ2 with indicated concentrations (all concentrations are in μM)

Discussion

This study was designed to investigate consequences of PPARγ activation for melanoma and melanoma-associated stroma cells. While recent reports indicate antiproliferative effects of these drugs in several cancer cells including melanoma, this is the first investigation of PPARγ ligand effects including both melanoma cells as well as melanoma-associated stroma cells such as fibroblasts and endothelial cells.

We demonstrated that 15d-PGJ2 is much more effective compared to other PPARγ ligands in inhibiting growth of melanoma cell lines, while the PPARα ligand WY-14643 had hardly any effect. These results are in line with recent data of other laboratories [3]. Therefore we restricted subsequent analyses to 15d-PGJ2.

Prakash et al. demonstrated that 15d-PGJ2 induces cell death in B16F10 melanoma and addition of serum leads to a tolerance to 15d-PGJ2 by rapidly binding to albumin [30].

Our results support previous reports of PPARγ agonists describing both a direct anti-tumor and a broad spectrum of anti-stromal, anti-angiogenetic and immuno-modulating activities [29].

Table 8.4 Downregulated candidates by 15d-PGJ2

AccNr	Name	Control	15d-PGJ2 (5 μM)	Function (Ref. Uniprot)
Q13685	Angio-associated migratory cell protein	1	0	Plays a role in angiogenesis and cell migration
Q16610	Extracellular matrix protein 1 precursor	1	0	Involved in endochondral bone formation as negative regulator of bone mineralization. Stimulates the proliferation of endothelial cells and promotes angiogenesis
Q6FI81	Anamorsin	1	0	May be required for the maturation of extramitochondrial Fe/S proteins. Has anti-apoptotic effects in the cell
P11802	Cell division protein kinase 4	1	0	Probably involved in the control of the cell cycle. Defects in CDK4 are a cause of susceptibility to cutaneous malignant melanoma type 3 (CMM3)
P50613	Cell division protein kinase 7	1	0	Cyclin-dependent kinases (CDKs) are activated by the binding to a cyclin and mediate the progression through the cell cycle
P78396	Cyclin-A1	1	0	May be involved in the control of the cell cycle at the G1/S (start) and G2/M (mitosis) transitions
P51946	Cyclin-H	1	0	Regulates CDK7, the catalytic subunit of the CDK-activating kinase (CAK) enzymatic complex. Involved in cell cycle control
P14635	G2/mitotic-specific cyclin-B1	1	0	Essential for the control of the cell cycle at the G2/M (mitosis) transition
Q9BY12	S phase cyclin A-associated protein in the endoplasmic reticulum	1	0	CCNA2/CDK2 regulatory protein that transiently maintains CCNA2 in the cytoplasm
P43246	DNA mismatch repair protein Msh2	4	1	Component of the post-replicative DNA mismatch repair system (MMR). Forms two different heterodimers: MutS alpha (MSH2-MSH6 heterodimer) and MutS beta (MSH2-MSH3 heterodimer) which binds to DNA mismatches thereby initiating DNA repair
P08253	Matrix metallopro-teinase-2	1	0	Ubiquitinous metalloproteinase that is involved in diverse functions such as remodeling of the vasculature, angiogenesis, tissue repair, tumor invasion, inflammation, and atherosclerotic plaque rupture

P35221	Alpha-1 catenin	2	1	Associates with the cytoplasmic domain of a variety of cadherins. The association of catenins to cadherins produces a complex which is linked to the actin filament network, and which seems to be of primary importance for cadherins cell-adhesion properties. Can associate with both E- and N-cadherins. May play a crucial role in cell differentiation
P35222	Catenin beta-1	1	0	Involved in the regulation of cell adhesion. The majority of beta-catenin is localized to the cell membrane and is part of E-cadherin/catenin adhesion complexes which are proposed to couple cadherins to the actin cytoskeleton [32]
O94776	Metastasis-associated protein MTA2	1	0	May be involved in the regulation of gene expression as repressor and activator. The repression might be related to covalent modification of histone proteins
O00560	Syntenin-1	1	0	Seems to function as an adapter protein. In adherens junctions may function to couple syndecans to cytoskeletal proteins or signaling components. Seems to couple transcription factor SOX4 to the IL-5 receptor (IL5RA). May also play a role in vesicular trafficking. Seems to be required for the targeting of TGFA to the cell surface in the early secretory pathway
Q9NWT1	p21-activated protein kinase-interacting protein 1	3	1	Negatively regulates the PAK1 kinase. PAK1 is a member of the PAK kinase family, which have been shown to play a positive role in the regulation of signaling pathways involving MAPK8 and RELA
P49023	Paxillin	2	0	Cytoskeletal protein involved in actin-membrane attachment at sites of cell adhesion to the extracellular matrix (focal adhesion)
P08962	Melanoma-associated antigen ME491	1	0	This antigen is associated with early stages of melanoma tumor progression. May play a role in growth regulation
P43355	Melanoma-associated antigen 1	1	0	Not known, though may play a role in embryonal development and tumor transformation or aspects of tumor progression. Antigen recognized on a melanoma by autologous cytolytic T-lymphocytes

Table 8.4 (continued)

AccNr	Name	Control	15d-PGJ2 (5 μM)	Function (Ref. Uniprot)
P62993	Growth factor receptor-bound protein 2	2	0	Adapter protein that provides a critical link between cell surface growth factor receptors and the Ras signaling pathway
P12004	Proliferating cell nuclear antigen (PCNA)	6	4	This protein is an auxiliary protein of DNA polymerase delta and is involved in the control of eukaryotic DNA replication by increasing the polymerase's processibility during elongation of the leading strand
P46087	Proliferating-cell nucleolar antigen p120	6	3	May play a role in the regulation of the cell cycle and the increased nucleolar activity that is associated with the cell proliferation
Q9UQ80	Proliferation-associated protein 2G4	13	9	May play a role in a ERBB3-regulated signal transduction pathway. Seems be involved in growth regulation
P06493	Cell division control protein 2 homolog	6	0	Plays a key role in the control of the eukaryotic cell cycle. It is required in higher cells for entry into S-phase and mitosis

Proteins downregulated by 5 μM 15d-PGJ2 in A375 melanoma cells after 48 h. Uniprot serves as reference for the function of the proteins. In addition, the accession numbers are from the Uniprot database. Numbers indicate distinct peptides identified by mass spectrometry

Analysis of 15d-PGJ2 effects on melanoma-associated fibroblasts revealed substantial anti-proliferative effects. This finding points to a distinct effect of 15d-PGJ2 on cells in the tumor microenvironment, as normal fibroblasts did not show such a drug response.

Besides fibroblasts, endothelial cells are important players in the tumor microenvironment. Here we demonstrate that 15d-PGJ2 effectively abolished tube formation of HUVECs, which is in line with the observations that HUVEC differentiation into tube-like structures in three-dimensional collagen gels could be suppressed by specific PPARγ ligands [31]. Another anti-angiogenic mechanism is the induction of apoptotic cell death in endothelial cells after incubation with 15d-PGJ2 [32, 33]. In contrast to these data, we observed a rather high IC50 of HUVECs for 15d-PGJ2, suggesting that 15d-PGJ2 specifically interferes with the tube formation process. Since tube formation was inhibited already at a concentration of 5 μM and the cells were demonstrate to be still vital with the highest concentration tested, while the IC50 was found to be 85 μM, the destruction of the HUVEC tube formation is apparently not a result of growth inhibitory effects of 15d-PGJ2. This interpretation is supported by the finding that15d-PGJ2 transiently inhibits the expression of VEGFR-1 and VEGFR-2 [34].

The effect of 15d-PGJ2 on lymphatic endothelial cells has not been analyzed so far. In this study we provide evidence that 15d-PGJ2 also exerts anti-lymphangiogenic activity. The ability to promote lymphangiogenesis enhances the metastatic spread of melanoma and recent studies revealed that tumor associated lymphangiogenesis is significantly correlated with poor disease-free and overall survival of patients with cutaneous melanoma [35, 36]. The mechanism how, 15d-PGJ2 leads to an inhibition of lymphangiogensis has to be elucidated in further studies, since this activity adds to its potential as a therapeutic tool.

Tumor initiation and progression is associated with the transition of normal stroma into an "activated" stroma phenotype. These tumor-associated, genetically still intact cells are able to establish a supportive environment for tumor cell survival and growth and to facilitate invasion and metastasis. Targeting this interference between tumor and stroma may consistently lead to a reduction of tumor growth and metastasis. Such a therapeutic approach has been presented as biomodulatory treatment both by our group and others [37, 38] and may complement standard chemotherapeutic approaches.

In search for alternative strategies for the treatment of metastatic neoplasm, targeting the tumor stroma seems to be a promising strategy since this approach is not directly cytotoxic but interferes with the cooperativity of tumor and stroma cells [37–39]. Considering that the stroma provides proteins supporting tumor survival, a blockage of this process might chemosensitize the tumor cells. Here we showed for the first time that the receptor is expressed on a panel of melanoma associated fibroblasts while to a lower extent on normal fibroblasts such as TF. PPARγ expression in metastatic melanoma was shown to be a possible predictive marker for response to biomodulatory stroma-targeted therapy, since patients with PPARγ-positive metastases showed a significantly prolonged progression-free survival treated with biomodulatory treatment [38]. The expression of PPARγ protein

Table 8.5 Regulated chaperones and Heat shock proteins by 15d-PGJ2

AccNr	Name	control	15-PGJ2	Δ	SM-Scor	Fraction
P11142	Heat shock cognate 71 kDa protein	34	19	*−15*	565.32	C, S, N
P08238	Heat shock protein HSP 90-beta (HSP 90) (HSP 84)	33	18	*−15*	528.87	C, S, N
P07900	Heat shock protein HSP 90-alpha (HSP 86)	33	20	*−13*	544.13	C, S, N
P11021	78 kDa glucose-regulated protein (GRP 78)	23	13	*−10*	365.75	C, S, N
P17987	T-complex protein 1 subunit alpha (TCP-1-alpha)	20	10	*−10*	320.94	C, N
P49368	T-complex protein 1 subunit gamma (TCP-1-gamma) (CCT-gamma) (hTRiC5)	15	5	*−10*	230.97	C, N
P10809	60 kDa heat shock protein, mitochondrial (Heat shock protein 60) (HSP-60)	36	28	*−8*	648.87	C, N
P14625	Endoplasmin (Heat shock protein 90 kDa beta member 1)	17	9	*−8*	238.31	C, N
P30101	Protein disulfide-isomerase A3 (Disulfide isomerase ER-60)	11	3	*−8*	154.12	C, N
P48643	T-complex protein 1 subunit epsilon	18	10	*−8*	268.49	C, N
P50990	T-complex protein 1 subunit theta (TCP-1-theta)	17	9	*−8*	249.51	C, N
Q92598	Heat shock protein 105 kDa (Heat shock 110 kDa protein)	15	8	*−7*	219.83	C, N
P78371	T-complex protein 1 subunit beta (TCP-1-beta)	23	16	*−7*	365.17	C, N
P38646	Stress-70 protein, mitochondrial (75 kDa glucose-regulated protein)	13	7	*−6*	201.73	C, N
P34932	Heat shock 70 kDa protein 4	15	10	*−5*	240.88	C, N
P04792	Heat shock protein beta-1 (HspB1)(Heat shock 27 kDa protein) (HSP 27)	8	3	*−5*	108.88	C, N
P50991	T-complex protein 1 subunit delta (TCP-1-delta) (CCT-delta) (Stimulator of TAR RNA-binding)	15	10	*−5*	264.13	C, N
Q99832	T-complex protein 1 subunit eta (TCP-1-eta)	16	11	*−5*	253.33	C, N
P61604	10 kDa heat shock protein, mitochondrial (Hsp10)	7	3	*−4*	109.65	C, S, N
P40227	T-complex protein 1 subunit zeta (TCP-1-zeta)	19	15	*−4*	346.23	C, N
P27797	Calreticulin (CRP55) (Calregulin)	5	2	*−3*	81.21	C, N

Table 8.5 (continued)

AccNr	Name	control	15-PGJ2	Δ	SM-Scor	Fraction
P08107	Heat shock 70 kDa protein 1 (HSP70.1) (HSP70-1/HSP70-2)	15	12	*−3*	228.12	C, N
P07237	Protein disulfide-isomerase (PDI)	9	6	*−3*	149.75	C, N
Q58FF8	Putative heat shock protein HSP 90-beta 2	6	3	*−3*	96.45	C, N
Q9NQP4	Prefoldin subunit 4 (Protein C-1)	4	2	*−2*	67.02	C, N
Q99471	Prefoldin subunit 5 (C-myc-binding protein Mm-1)	5	3	*−2*	96.61	C, N
P30040	Endoplasmic reticulum protein ERp29	8	7	*−1*	109.52	C, N
P34931	Heat shock 70 kDa protein 1L	6	5	*−1*	107.92	C, N
P61758	Prefoldin subunit 3	3	2	*−1*	50.65	C, N
P13667	Protein disulfide-isomerase A4	3	2	*−1*	40.84	C, N
Q15084	Protein disulfide-isomerase A6	8	7	*−1*	124.13	C, N
Q96JJ7	Protein disulfide-isomerase TMX3		1	*−1*	11.91	C
Q96MM6	Heat shock 70 kDa protein 12B	1	1	*0*	12.1	N
P17066	Heat shock 70 kDa protein 6	4	4	*0*	70.85	C, N
O60925	Prefoldin subunit 1	1	1	*0*	16.45	C, N
Q9UHV9	Prefoldin subunit 2	4	4	*0*	60.91	C, S, N
O15212	Prefoldin subunit 6	5	5	*0*	64.16	C, N

Chaperones regulated by 5 μM 15d-PGJ2 in A375 melanoma cells after 48 h. The accession numbers are from the Uniprot database. Numbers indicate distinct peptides identified by mass spectrometry. C: cytoplasm, N: nucleous, S: supernatant

may therefore serve as a positive prognostic marker indicating the responsiveness to stroma-targeted therapy in the metastatic stage (IV) of melanoma. Meyer et al. suggested that a remodeling of the tumor stroma might be the main target of PPARγ therapy. The recognition of 15d-PGJ2 as a potential anti-tumor drug raises the question of a more detailed understanding of the acting mechanism.

The enhanced knowledge of molecular mechanisms by 15d-PGJ2 generated by shot gun analysis involving important cellular processes, such as cellular signaling networks, regulation of cell cycle, proliferation, transport, cell migration or tumor-stroma interactions may support the design of patient stratification strategies for rational therapeutic concepts.

The data interpretation was supported by the CPL/MUW-database [18]. The number of proteins are automatically classified and provide a fast overview of the main processes involved [18]. Classification considers common household proteins, cell type-specific proteins as well as proteins related to specific functions and enables to decrease the complexicity of data. By comparison of untreated versus treated melanoma cells we were able to confirm the *in vitro* data of the inhibitory effects of 15d-PGJ2 on proliferation, migration and angiogenesis and to extract further relevant proteins involved in tumor progression.

In line with the observation of a decrease of MMP 2 expression in shotgun analysis (downregulation of 1 peptide after 48 h incubation with 5 μM 15d-PGJ2), we were able to reproduce this downregulation using zymography. This observation supports our argument, that 15d-PGJ2 interferes with the tumor microenvironment.

The identification of less peptides of Hsp90 in 15d-PGJ2-treated A375 compared to untreated cells suggested down-regulation of this protein. Western blot analysis of Hsp90, however, did not support this interpretation. 2D-gel electrophoresis demonstrated a profound change of Hsp protein charge by a pI shift which indicates changes in posttranslational modifications such as phosphorylation.

In addition, western blot analysis showed an upregulation of Hsp56 in 1205Lu. Hsp90 and Hsp56 are known to form complexes playing a role in the intracellular trafficking. Phosphorylation of Hsp56 by CK2 was already demonstrated to influence the formation of the HSP90/HSP56 complex [40]. We propose that the reduction of Hsp90 will lead to an elevation of more unbounded Hsp56.

To strengthen the argument that 15d-PGJ2 might increase Hsp90 phosphorylation and to shed light on the impact of 15d-PGJ2 on the phosphorylation which reflects the activity of the proteins, we performed an IP for phospho-serine followed by shot gun analysis indicating a phosphorylation of several chaperones.

Hsp90 belongs to the best studied molecular chaperones which is required for the stability and function of signaling proteins that promote tumor growth, cell motility and invasion *in vitro* and cancer metastasis *in vivo* [41, 42]. Hsp90 inhibitors exhibit significant anti-neoplastic activity against a broad variety of cancers in preclinical studies, including breast, lung cancer and myeloma as well as melanoma [43, 44]. Thus, blockage of Hsp90 interferes with all anti-cancer mechanisms of 15d-PGJ2 and might be one explanation for the widespread activity of 15d-PGJ2 on tumor progression.

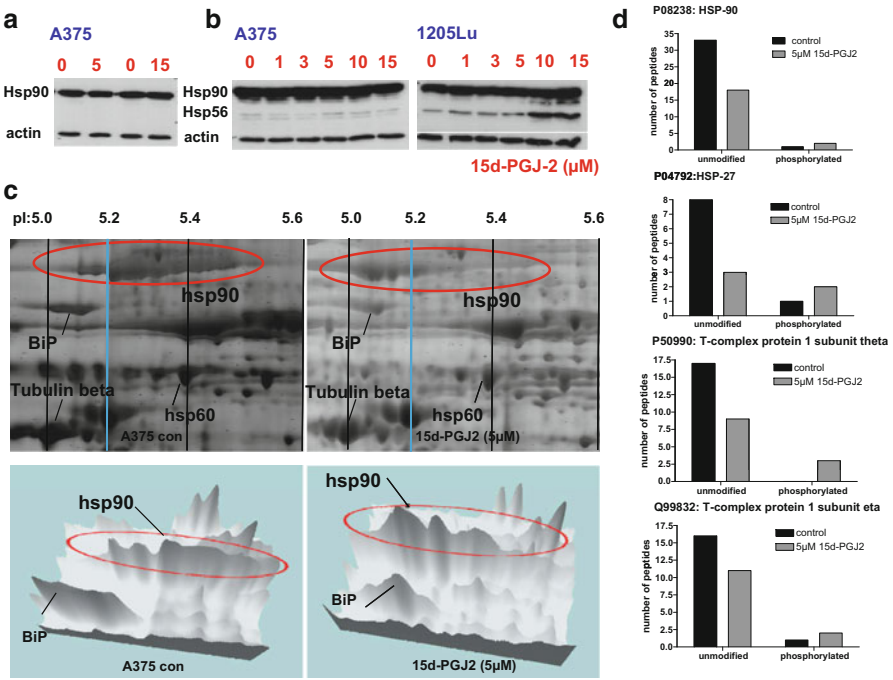

Fig. 8.6 pI shift of Hsp90 in 2D-gel electrophoresis and 15d-PGJ2 induced phosphorylation of several chaperones. **a** Representative immunoblots of three independent experiments of Hsp90 cytosolic (A375) and **b** total cell lysate (A375 and 1205Lu). **c** 2D-gel electrophoresis of 15d-PGJ2 or DMSO treated A375 melanoma cells with additional three dimensional versions. **d** For immunoprecipitation, an anti-phosphoserine antibody was applied to cytoplasmic protein fractions. In case of the four listed chaperones, an apparent down-regulation observed by shotgun proteomics was actually accompanied by phosphorylation as evidenced by increased binding to anti-phosphoserine antibody. Protein abundances were estimated from the number of distinct peptides identified per protein

Verification of these considerations will require further investigation of this drug. The present data allow us to conclude that 15d-PGJ2 interferes with several key mechanism of cancer progression [45], since 15d-PGJ2 potently reduced proliferation of melanoma and melanoma-associated cells, induced apoptosis and cell cycle arrest, and diminished tumor migration, lymphangiogenesis and angiogenesis *in vitro*.

In addition we were able to demonstrate the activity of 15d-PGJ2 on melanoma associated fibroblasts. Tumor-associated stroma cells are known to differ from their normal counterparts in the expression of various biologically molecules such as PPARγ [46], which was found to be upregulated in stromal myofibroblasts of colon adenocarcinomas [47].

Two consequences can be deduced from these results: the evaluation of PPARγ expression in tumor stroma and a correlation with features of melanoma patients would be an interesting approach as proposed by Meyer et al. and 15d-PGJ2 might serve as an efficient combination therapy with chemotherapeutic agents [29, 38].

The IC50 doses to transfer 15d-PGJ2 as a single compound into an *in vivo* situation are high, but we propose that 15d-PGJ2 might serve as an efficient combination therapy with chemotherapeutic agents by targeting as well the tumor microenvironment.

Our data revealed for the first time a profound effect of 15d-PGJ2 on melanoma cells in addition to the tumor microenvironment suggesting high therapeutic efficiency.

Materials and Methods

This study was approved by the "ethics committee of the Medical University of Vienna and the general hospital Vienna" (Ethik-Kommission der Medizinischen Universität Wien und des Allgemeinen Krankenhauses der Stadt Wien AKH, EK-Nr.; 093/2003; EK-Nr.: 1088/2009; EK-Nr.: 1123/2009).

Cell Line and Chemicals

M24met cells (kindly provided by Dr. R.A. Reisfeld, Department of Immunology, Scripps Research Institute, La Jolla, CA; [48] were grown in RPMI 1640 supplemented with 10 % fetal bovine serum, 2 mM glutamine and 50 μg/ml gentamycin sulfate. The human melanoma cell line 1205Lu isolated of a lung metastasis was cultivated as described previously [49]. A375 and Mel Juso were grown in D-MEM tissue culture medium supplemented with 10 % fetal bovine serum, 2 mM glutamine and 50 μg/ml gentamycin sulphate as described previously [50, 51]. Normal human dermal fibroblasts (NHDF) obtained by PromoCell were grown in DMEM (10 % FCS). The compounds used in this study were obtained from Eubio (Vienna, Austria) 15d-PGJ2, ciglitazone, troglitazone and WY-14643. All compounds were resolved in DMSO.

Isolation of Melanoma-Associated Fibroblasts MP9, MP10, MP11 and MCM16

Tumor tissue was digested as described previously [21]. Fibroblasts were magnetically labeled with Anti-Fibroblastic MicroBeads. Cell suspension was loaded onto an MACS Column with a magnetic field. The magnetically labeled fibroblasts were retained within the column and eluted subsequently. Fibroblasts were grown in DMEM (10 % FCS). We obtained written informed consent for collecting excised melanocytic lesions of all patients enrolled. This study was approved by the

"ethics committee of the Medical University of Vienna and the general hospital Vienna" (Ethik-Kommission der Medizinischen Universität Wien und des Allgemeinen Krankenhauses der Stadt Wien AKH, EK-Nr.; 093/2003; EK-Nr.: 1088/2009).

Isolation of HUVECs

HUVECs were isolated from umbilical veins and subcultured as described previously [52]. HUVECs were passaged in IMDM (Life Technologies) containing 10 % FCS (Life Technologies), streptomycin (100 μg/ml), penicillin (100 U/ml), L-glutamine (2 mM), EC growth supplement with heparin (50 μg/ml; Promocell).

Isolation of LECs

Neonatal human foreskins were enzymatically digested, the epidermis was removed and dermal cells mechanically released. CD34-positive blood vascular endothelial cells (BVECs) were isolated by immunomagnetic purification with an anti-human CD34 antibody (BD Pharmingen, San Diego, CA) conjugated to immunomagnetic beads (Dynal. Lake Success, NY). The remaining CD34-negative cells were incubated with an immunomagnetic beads-conjugated anti-human CD31 antibody (Dynal) to isolate LECs. LECs were seeded onto fibronectin-coated (1 μl/ml; BD Biosciences, Bedford, MA) culture dishes and propagated in a modified endothelial cell basal medium.

The use of endothelial cells (HUVECs and LECs) has been approved by the "ethics committee of the Medical University of Vienna and the general hospital Vienna" (Ethik-Kommission der Medizinischen Universität Wien und des Allgemeinen Krankenhauses der Stadt Wien AKH, EK-Nr.1123/2009). We obtained written informed consent from all patients (in the case of umbilical cords, written informed consent was obtained from the parents) [53].

Phenotypes of BEC and LEC cultures have been described recently [54] The used LECs are immortalized LECs.

Cell Proliferation-Assay

The CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay (Promega) was used as previously described [21]. In brief, different cell lines or primary cells were plated and treated with increasing concentrations of 15d-PGJ2 or a solvent control. Proliferation was measured at desired time points employing an ELISA plate reader.

Western Blot

Cells were frozen in liquid nitrogen, lysed and separated by gel electrophoresis as described previously [21, 55]. After blotting membranes were incubated with the following primary antibodies: p21 (1:200), p53ser37 (1:200), p53ser15 (1:200), p53 (1:200), emmprin (basigin) (1:200), Mms2 (1:100), MSH3 (1:500), Hsp90 (1:1000), all Santa Cruz Biotechnology, MSH6 (1:500, Pharmingen) MSH2 (1 μg/ml, Pharmingen), MLH1 (1 μg/ml, Pharmingen) and Nodal (1:500, Abcam), tubulin (mouse anti-tubulin monoclonal antibody, Sigma Aldrich) or actin (rabbit anti-actin monoclonal antibody, Sigma Aldrich). Binding of primary antibodies was visualized by incubation with horseradish peroxidase conjugated secondary antibodies (anti-mouse IgG or anti-rabbit IgG HRP, both GE Healthcare) followed by chemoluminescent visualization with ECL (Amersham).

Cell Cycle Analysis

Cell cycle analysis was performed by propidium iodide FACS staining as described previously [21]. Cells were harvested, and fixed in 70 % ethanol RNase (Sigma) was added, cells stained with propidium iodide and analyzed by flow cytometry. Cell cycle distribution was quantified with the ModFIT LT software (Verity Software House, Topsham, ME).

Matrigel Invasion Chamber Assay

The matrigel invasion chamber assay (BD Biosciences, Bedford, Massachusetts) consists of a two-well chamber system and was peformed as described previously [21]. M24met cells were subjected to different concentrations of 15d-PGJ2 or solvent control. After 48 h, the upper chamber was removed and swiped with a cotton bud. The transmigrated cells on the lower side of the upper chamber were fixed in 70 % ethanol and stained using 0.2 % crystal blue. Pictures were captured with a AxioCam MRc5 digital camera (Zeiss, Vienna, Austria) attached to an AH3-RFCA microscope (Olympus, Vienna, Austria). The relative amount of transmigrated cells was quantified with a computer-assisted analyses system (Axiovision®)

Tube Formation Assay

Matrigel Basement Membrane Matrix (BD) was thawn and 24 well plates were coated with 300 μl Matrigel and incubated for 30 min at 37 °C.

50.000 endothelial cells were seeded and after 8 h different concentrations of 15d-PGJ2 or solvent control were applied. 24 h after seeding tube formation was documented by the confocal laser microscope (Zeiss).

For Calcein staining 12 or 24 h after seeding, cells were washed once with PBS and cells were incubated for 30 min at 37 °C with 50 μL PBS containing 0.05 % Calcein-AM (Sigma Aldrich, Vienna, Austria). Micrographs of fluorescent cells were taken using a Nikon Digital Sight DS-Fi1C CCD camera. Tube formation was quantified using the Cell Profiler Software Package [56]. Briefly, images were converted into binary images by thresholding. Areas with an extension of more than 125 μm in one direction were considered as tubes and selected for analysis, smaller areas were discarded. A single pixel topological skeleton representing the tubular network was constructed and network length was calculated by multiplying the pixel count with a scaling factor representing microns per pixel.

Zymography Assay

We stimulated A375 melanoma cells with increasing doses of 15d-PGJ2 (1, 5, 10, 15 μM) for 48 h. The supernatant was dissolved 1:1 with MTO-buffer (50 mM Tris, pH 7.5; 200 mM NaCl, 5 mM $CaCl_2$) and diluted 1:1 in sample buffer (100 mM Tris-HCl, pH 6.8, 50 % Glycerol, 4 % SDS, 0,1 % Bromphenolblue). The SDS gel contained gelantine (1 mg/ml). After electrophoresis the gel was incubated with substrate buffer with Triton-X100 (50 mM Tris, pH 7.5; 200 mM NaCl, 5 mM CaCl2, 0,02 % Brij; 2.5 % Triton-X100) for 1 h. After incubation substrate buffer without Triton-X100 (50 mM Tris, pH 7.5; 200 mM NaCl, 5 mM CaCl2, 0,02 % Brij) at room temperature (2–3 times/h), the gel was incubated with this buffer over night at 37 °C. Subsequently, the gel was stained in Coomassie solution for 30 min and stripped with a isopropanol-acetic acid solution (ProteaImmun).

Proteome Analysis

Shot gun analysis was performed as described previously [17, 39] In brief, cells were fractionated into nuclear, cytoplasmic and secreted protein fractions [57]. Protein fractions were separated by SDS-PAGE, cut into slices and digested with trypsin. Peptides were extracted and separated by nano-flow LC (1100 Series LC system, Agilent, Palo Alto, CA) using the HPLC-Chip technology (Agilent) equipped with a 40 nl Zorbax 300SB-C18 trapping column and a 75 μm × 150 mm Zorbax 300SB-C18 separation column at a flow rate of 400 nl/min, using a gradient from 0.2 % formic acid and 3 % ACN to 0.2 % formic acid and 40 % ACN over 60 min. Peptide identification was accomplished by MS/MS fragmentation analysis with an iontrap mass spectrometer (XCT-Ultra, Agilent) equipped with an orthogonal nanospray ion source. The MS/MS data were interpreted by the Spectrum Mill MS Proteomics Workbench software (Version A.03.03, Agilent) and searched against the SwissProt Database (Version 14.3 containing 20 328 protein entries) allowing for precursor mass deviation of 1.5 Da, a product mass tolerance of 0.7 Da and a minimum

matched peak intensity (%SPI) of 70 %. Due to previous chemical modification, carbamidomethylation of cysteines was set as fixed modification.

For immunoprecipitation, 5 μg anti-Phosphoserine antibody (PSR-45, Abcam: ab6639) were applied to cytoplasmic protein fractions, followed by an overnight pull-down using Dynal Protein G-coated Dynabeads (Invitrogen). Proteins were released and further processed as described for proteome profiling. In case of the IP analyses, we used a Dionex 3000 nano-LC system and a QEXACTIVE orbitrap mass spectrometer (Thermo). Spectral searches were performed with Mascot.

2D- Gel Electrophoresis

Proteins of A375 melanoma cells treated with 5 μM 15d-PGJ2 or solvent control for 48 h were loaded by passive rehydration of IPG strips pH 5–8, 17 cm (Bio-Rad, Hercules, CA) at room temperature. IEF was performed in a stepwise fashion (1 h 0–500 V linear; 5 h 500 V; 5 h 500–3500 V linear; 12 h 3500 V). After IEF, the strips were equilibrated with 100 mM DTT and 2.5 % iodacetamide according to the instructions of the manufacturer (Bio-Rad Hercules, CA). For SDS-PAGE using the Protean II xi electrophoresis system (Bio-Rad, Hercules, CA, USA), the IPG strips were placed on top of 1.5 mm 12 % polyacrylamide slab gels and overlaid with 0.5 % low melting agarose. The gels were stained with a 400 nM solution of Ruthenium II tris (bathophenanthroline disulfonate) (RuBPS). Fluorography scanning was performed with the FluorImager 595 (Amersham Biosciences, Amersham, UK) at a resolution of 100 μm. After scanning, gels were dried using the slab gel dryer SE110 (Hoefer, San Francisco CA, USA). Exposure of storage phosphor screens (Molecular Dynamics) occurred at room temperature for 24 h. Screens were subsequently scanned using the Phosphorimager SI (Molecular Dynamics) at a resolution of 100 μm. Proteins were identified by mass spectrometry analysis of tryptic digests of isolated protein spots.

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Part IV
The Tumors' Normativity: Reconstructing and Operationalizing Starting Points for an Evolution Theory

Chapter 9
The Philosophical Quest of a Cancer Cell: Redefining Existentialism

Ellora Sen

Abstract Poised in a seemingly prepared calm stasis with trust in the intangible, cancer cell makes decisions from a point where philosophy seems to be its major compass. As pure biological presuppositions cannot access or fathom this invisible philosophical realm of a cancer cell; syllogism rooted in scientific ratiocination alone without philosophical analysis will elude understanding of the objectivity of a cancer cell. Appreciation of this undiscovered unseen dimension (which is but a counterpart of the comprehensible visible), will unravel the deep interconnectedness between these two and provide insight far greater than empiric epistemology.

Introduction

In the same light employed by philosophical doctrine of existentialism to interpret the meaning of human existence; the existence and essence of "being a cancer cell" can also be analyzed. Immanuel Kant distinguishes the "*noumenon*" vs. the "*phenomenon*" or "the being-in-itself" vs. the "being-as-it-appears", to understand the realities behind appearances. While for Kant the phenomenon hides the "being-in-itself"; for Sartre, the phenomena do not mask being-in-itself they reveal it. As appearance involves a duality structure of showing, which connects it to the phenomenon; could a similar analogy be drawn to correlate the ontics and ontology of a cancer cell? Does the ontics of a cancer cell 'announce' or "mask" the ontological structures that underlie it? Does a cancer cell show itself in such a way (through replicative immortality, genetic instability, altered bioenergetics, immune evasion...) as to announce something else that does not show itself? Or is it 'something' else (such as deleterious tumor microenvironment) which does not show itself but appears through something which does show itself? Is cancer an "epiphenomena" or an "emergent effect" hidden beneath the mask of a phenomena?

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Sympathetic Understanding of a Cancer Cell's Philosophy

While being-in-itself (l'etre-en-soi) refers to essence or objectivity, being-for-itself (l'etre-pour-soi) refers to its existence or subjectivity. Since the "for-itself" lacks a predetermined essence, it strives to absorb "in-itself" in order to escape its own nothingness. What Jean-Paul Sartre brings out strongly by stating that 'Existence precedes essence' is that- first of all man exists, and only afterwards defines himself and *he will have made what he will be* [1]. Is it the strong desire of the 'for-itself' normal cell to become synonymous within the 'in-itself' that triggers the imposition of its subjectivity on the other's objectivity? What if it believes that there is no pre-established nature or essence that can set limits on what it can be or can do? Is the transformation into a cancer cell a mere reflection of the Promethean struggle against the conditions of its existence or an opposition against nihilism? Is it its urge to live for and through itself that makes it very existence an act of rebellion? In The Rebel Camus explains "In any event, the reasons for rebellion cannot be explained except in terms of an inquiry into its attitudes, pretensions, and conquests". Besides "rebellion though apparently negative since it creates nothing, is profoundly positive in that it reveals the part of man which must always be defended". Also " ... the individual is defined only by his relationship to the world and to other individuals; he exists only by transcending himself" Simone de Beauvoir. Therefore, to dissect the "raison d'etre" of a cancer cell not only requires sympathetic understanding of its philosophy but even deeper understanding of the tumor's normative social structures. As "philosophy can be used for anything, even for transforming murderers into judges" [2], this essay uses philosophy as the alibi of cancer cell to examine the arguments that it uses to sustain its identity and function despite the onslaught of oppression.

Acquisition of a New Language

Stressors are well recognized opportunistic windows that can compel a normal cell to become cancerous. Besides, "the spirit of revolt can only exist in a society where a theoretic equality conceals great factual inequalities" [2]. Does the rebel cancer cell confront these factual inequalities with its own principle of justice to establish theoretic equality? Are cancer cells victim of larger ideological, social, and existential forces? Can inequalities of conditions make a cell receptive to new influences that metamorphose it? Is the act of rebellion a demand for clarity and reassessment of condition which it refuses to accept? Does it stem from the innate desire to free itself from the totalitarian tutelage and to set up an open society free from the bondage of merely established laws? Does it stand for something of its own by denying passive submission to traditional authoritarian societal rules?

Awakened to the knowledge that obstacle can be transformed into opportunities, and aware of its latent inner power which only it can unleash; the cell swallows its past to reinforce its new existence. The newly acquired essence is one of sheer emptiness, a field of infinite possibility, of pure potential—a "tabula rasa"—an erased slate for

writing poetry in a novel language—the language of tumor. Writing on the uses of language, Russell states that "Language has two primary purposes, expression and communication .... Communication does not consist only of giving information; commands and question must be included ... It enables transactions with the outer world". The newly acquired language provides expression for its thoughts which would have otherwise remained private.

Cancer Cell's Ascendency—A Rebel's Fait Accompli

In "The Rebel" Albert Camus asks "What is a rebel?" To which he answers "A man who says no ... his no affirms the existence of a borderline ... Rebellion is founded on the categorical rejection of an intrusion that is considered intolerable ..... " Does the feeling of revulsion at the intolerable societal laws of bias and intrusion coupled with the innate desire to preserve the integrity of its being, trigger the act of rebellion? Initially, the rebel cell tries to adjust into the society by accepting conditions however unjust, in despair. Till this stage a delicate balancing-act of adjustment prevents it from disintegrating in the face of all odds, the cell remains benign and the society does not fear the rebel being detrimental to its equilibrium. However, the adjustment is constantly *in flux,* changing. Actually, it *is* a flux, since the *being* of the "for-itself" cell is not inert, but is an *event,* a *process.*

The society ignores these distress calls as random and meaningless. The cell confronts oppression in silence while trying to preserve the integrity of its being till it extends beyond the borderline of its tolerance. At this point it "demonstrates with obstinacy, that there is something in him which is worth while ... and which must be taken into consideration .... But from the moment the movement of rebellion begins, suffering is seen as a collective experience" ... "Rebellion's claim is unity ... is dedicated to creation so as to exist more and more completely" [2].

Social Engineering

But when was society tolerant to non-conformists? Society in itself is "a conspiracy against the manhood of each of its members, where self reliance is its aversion" Emerson. The society promotes synergy and symbiosis but abhors antagonism. Therefore, when the cell finally achieves that milieu where its ***"self reliance"*** is fully manifested, society becomes desperate to put a check fearing that the rebel will break normative law to become a criminal. The consequences being that the altruistic self destruction mechanisms of the cell are permanently lost, it turns from benign to malignant. This raises the question as to why the society had not attempted to iron out the indifferences at the first signs of appearance or even engaged in a balanced debate. Why was the societal law not executed and punishments inflicted at the first sign of upraise? But one could favorably argue for this rebel cell "a criminal cannot break

a law and that it is only broken only if the criminal does not receive the punishment prescribed by the law" [3].

This metamorphosis is thus a reflection of potentiality being actualized While struggling with itself it becomes committed to itself. At this stage it decides to no longer refer, defer, prefer, or suffer ..... *The rebel denounces the family name of "conformists*". In the process of transformation the cell develops an identity that emanates an aura of confidence which subsequently seduces others to join the cause. It convinces the others to analyze the principles of democratic social reconstruction—the principles of "piecemeal social engineering". Popper wrote "the piecemeal engineer will adopt the method of searching for, and fighting against, the greatest and most urgent evil of society, rather than searching for, and fighting for, its greatest ultimate good" [3]. The social engineer cancer cell does not question historical tendencies but believes that it is the master of its own destiny and can influence a change. If "genius is what a man invents when he is looking for a way out" (Sartre), then the cancer cell is undoubtedly a genius!!!

Evolution of Heterogeneity: Brilliant Orchestration and Mammoth Choreography

The capacity of developing tumors to escape immune control involves the process of cancer immunoediting which incorporates three phases: elimination, equilibrium and escape designated as the 'three E's'. In the first phase of immuno-surveillance, recognition of transformed cells by the immune system, leads to its elimination. However, cells that escape elimination enter the equilibrium phase where they may be either maintained chronically or immunologically sculpted by immune "editors" to produce new populations of tumor variants [4]. In Open state and its enemy Popper reasons "that the widely held prejudice that destruction or control of an aggressive state implies misery of subjugation of its individual citizen" is dangerous. Popper opines that harsh treatment by victorious state "is likely to give the aggressor state a chance for new aggression; it will also provide it with the weapon of the moral indignation of one who has been wronged" [3]. Thus, a rebel cell that escapes the arsenal of immuno-surveillance acquires weapon of increased resistance with which it evades subsequent immunological defense.

Sculpting of variants does not involve a rigid evolutionary pressure but rather dynamic and 'inter individually variable' factor [5]. The co-existence of genetically divergent tumor cell clones within tumors [6, 7] indicates that tumors are an extremely heterogeneous population consisting of the original rebel cell and its followers. Besides, intratumor heterogeneity also arises from convergent phenotypic evolution with multiple mutations in the same tumor-suppressor gene across different regions of the tumor [8]. Thus the sculpting force of the microenvironment delicately hews these variants from the parental rebel cell. 'I think therefore I am" (*cogito, ergo sum*)

metamorphoses into "I rebel- therefore we exist"- Camus [2]. The rebellion gain momentum.

Realizing its unique power as an archetypal renegade, each variant acquires its own modus operandi for strengthening and expanding the campaign against the exactions of an unjust system. The sense of solidarity, harmony and unshakeable commitment towards achievement of the core goal promotes an *espirit de corps* amongst rebels despite their individual variation and myriad personalities. Belief in the common philosophy, coupled with perspective of the present moment and foresight for the future propels the 'team' to victory. The rebellion is consummated and perpetuated in the act of real creation. This realization of vision manifested in mass proportion at multi-levels is the culmination of brilliant orchestration and mammoth choreography.

Organizing for Constant Change

Rudolf Virchow in 1863 had postulated that "chronic irritation which is manifested by chronic inflammation is a key promoter of cancer" [9]. Increasing epidemiological, pharmacological and genetic evidences clearly indicate that the mediators and effectors of inflammation are indispensable participants in tumor [10]. Malignant tumors often develop at the sites of chronic injury, and inflammation at these sites influences the processes of wound repair. However, unlike wound healing where inflammation is self-limiting, persistent unresolved inflammation in the local tumor microenvironment amplifies the response to support "wound overhealing" [11]. Tumors are rightly the "wounds that do not heal" [12]. As inflammatory tumor microenvironment plays a crucial role in the evolution of tumor, anti-inflammatory therapy targeting the local inflammatory milieu will be efficacious towards neoplastic progression [13, 14]. However, this will require better understanding of how the local "tumor promoting" inflammatory milieu regulates the fate of the "wounded cell" and nurtures its evolution towards a malignant one. Thus, what starts off as a single rebel cell ultimately culminates in rebels varying in mutations in "driver" genes. Since variants are not necessary identical in terms of its driver gene mutation, heterogeneity fosters tumor adaptation and therapeutic failure through Darwinian selection [8]. Importantly, many of these driver mutations can turn out to red herrings, distracting attention from the actual issue that instigated the cell to become rebellious in the first instance.

Tumor heterogeneity poses a great challenge for therapy [15]. This process is influenced by alteration in the microenvironment which dictates the selection pressure [16].

It is obvious that heterogeneity within a single tumor with respect to gene expression will affect several signal transduction pathways. Given that a number of driver mutations can co-exist with passenger mutation in these variants, heterogeneity coupled with complex signal interactions fans the flame of revolt. As with electrical grids that are interconnected through wide networks to provide multiple "redundant alternative routes" for power to flow should failures occur; the intricate network of

signaling pathways possibly act in a similar fashion to ensure spare that capacity is available in case of failure in another part of the network. Therefore, targeting one or a few pathways in this intricate web of signaling cascades runs the risk of switching on "alternative routes" at emergencies.

Ecological Niches: Template for Diversification

The physical tumor microenvironment composed of low oxygen and high acidity can expose a putative cancer cell to selective pressure by regulating changes in gene expression [17]. The regional selection pressure exerted by hypoxia and acidosis promotes rapid adaptation by triggering genetic alterations [18, 19]. Therefore understanding the microenvironmental factors and corresponding adaptive strategies the cell adopts to survive and flourish in its immediate ecological niche is crucial. Most importantly, selection pressure could promote or impede expression of different sets of genes among the rebel variants residing in different ecological niches, if those genes are linked to different fitness effects in different micro-environments. This further contributes to the intra-tumoral heterogeneity as rebel communities from different habitats are influenced by niche diversity and are shaped by the prevalent ecological factors. The coexistence of different ecotypes indicates that expansion into alternative niches and extinction from others can occur over evolutionary time.

As niche-specific selection pressure affects the responsiveness of different ecotypes to chemotherapy, the choice of therapy would be difficult to predict. To maintain a dynamic equilibrium between change in its ecological niche and continuity, tumor cells not only adapt to but also survive the deleterious microenvironments. By envisaging a future that already exists, the rebels hone their intelligence information system in a way that variations in its niche pose little surprise. Like social engineers the rebel variants analyze and understand the immediate social system, so as to arrive at appropriate decisions based on careful evaluations and objectivity.

Toll Booth Strategy: Acquistion of Impenetrable Position

Writing on the economics of innovative strategies Peter Drucker state that the entrepreneurial "toll-booth strategy" allows an innovator to establish a virtual monopoly in its small niche thereby creating a product that is indispensable for a larger process. Similarly, by exploiting the incongruity of its immediate ecological niche the innovative rebel cell utilizes the "toll-booth strategy", not only to succeed but also acquires an invincible position. Since evolutionary specialization for one particular micro-environment is concurrent with ecological dependency, these ecological dependencies can serve as important anti-tumor targets. However, this may be complicated by the fact that coevolution can occur where the specialized phenotype and its ecological niche influence each other's evolution. As evolution in each niche can

proceed in unpredictable pathways it invokes heterogeneity by regulating balance between extinction, survival and dispersal. The importance of tumor microenvironment in evolution of cancer can be compared to ways inherent characteristics of different soil types dictate edaphic-driven diversification. Since ecological diversity is important driver of tumor heterogeneity, and as rebel cell exists only in contemplation of the whole system; understanding selection forces in its ecological niche that contribute towards its adaptation, growth and adjustment will elucidate strategies for constraining its survival and evolution.

Delineation of Novel Therapeutic Principles on The 'Communicative Resolution' of Existentialist Considerations

A malignant cell is the obvious sign that something is off balance in the system. It's the tip of an iceberg whose base lies deeply buried underneath its apparent expression. The manifestation of malignancy is the mere reflection of the inner state of the cell ... an expression of its hurt and deepest feelings. It would therefore be a mistake to treat any rebellion as a generic situation comprised of a series of familiar events. Therefore, rather than forcefully eradicating or suppressing the end-stage symptoms, understanding what triggered the off-balance and offering a blueprint by which the rebel cell can heal its hurt from within would establish *status quo –ante*. While the former generalizes, the later is an effort to understand the meaning of contingent and unique *phenomena*. Tumor progression is a process of Darwinian evolution involving natural selection in its natural ecosystem. The classic Darwinian evolution process is commonly studied by nomothetic, mostly reductionist methods. These methods are sufficient to describe an evolution history, but do not contribute to an evolution theory, which should explain the 'metabolism' of evolution. As nomothetic explanations are probabilistic, the problem remains unsolved. However, as idiographic approach specifies and focuses on casual relationships it might stimulate empathy to secure greater understanding of the cause of rebellion.

The Idiographic Therapeutic Method

The prerequisite of the idiographic method will therefore involve the analysis of the timely and spatially unique phenomenon of a tumor in a patient. This approach will raise the following questions (i) how are normative structures, functions and decision maxims (tumor-immanent normative notions) physically and situatively organized (rationalized)? (ii) what is the novel situative validity and denotation of tumor systems participators in a respective communicative context, which evolved during 'social engineering'? By answering these questions, we may achieve systematic and reproducible parameters which will provide the basis for stratifying therapy and for guiding therapeutic approaches (adaptive trial designs).

While switching research methods to systematic idiographic considerations, we must acknowledge that communication adheres to rules and is not arbitrary. By uncovering the communication-derived rules, we may reconstruct the rebel's intentions (normative notions) and how these are physically organized (rationalized). This way, we learn more about the rules of communication and communicative expression among systems participators in a tumor system (serum proteome and miRNA analytics, molecular imaging of 'hallmarks' of cancer). Further, we anticipate that systems participators gain novel validities and denotations within evolutionary processes (social engineering), and that 'corrupt' rationalizations of normative notions are permissible.

Expressive, Objective and Appellative Communications

Habermas points in Pragmatics of communication that to engage in a successful communicative act, both the speaker and the hearer must share the goal of reaching mutual communicative understanding. To improve communication, exchange of information should not only convey the intentions and/or subjective experiences of the speaker to indicate the states of affairs but also establish relations with the hearer. The rebellion can be resolved on a communicative level, even though it may result in 'corrupt' rationalizations (organizations) of tumor-immanent normative notions and in 'bellum'. Since the neglected aspect of the rebel self is seeking recognition, it is not "instruction;" but rather the "negotiation" that might change its attitude of aggression and enable it to adopt a state of co-operation. In the absence of resistance the tenor of antagonism will diminish.

Complex Equations: Mantra for Modern Therapy

"Lasting peace can come only if we consider fully the 'underlying dynamic force' that may produce war or peace" [3]. Importantly, war is not a phenomenon and neither all wars have the same contributing conditions. The rebel cell is just a seed groping in the dark soil, establishing and spreading its roots while surmounting obstacles to reach the surface. Its manifestation is the *triumph of its principles* and establishment of its identity. The conventional "Pruning the leaves and branches therapy" will therefore not only results in thicker foliage but facilitate deeper penetration of the roots into the soil. This is manifested by the so-called "dandelion phenomenon" of recurrent malignancy following a complete response to chemotherapy [20]. The conventional cancer therapies is analogous to cutting dandelion like weed off at ground level to eradicate the visible signs of the disease. Though apparently gone, the root remains and the cancer re-grows often with renewed vigor. We cannot attribute the root to the tumor microenvironment or the tumor cell. The '**root**' is the situative communicative process which adheres to communication-derived rules and is therefore accessible for therapeutically implemented non-normative boundary conditions.

Therefore, finding that "root value" which when substituted for the unknown element in the complex equation of evolutionary cancer dynamics will satisfy the equation. Since this equation is not simple linear but simultaneous equations ranging from functional to transcendental to parametric, multiple equations exists with multiple unknowns. As assignment of one value to all the unknowns will not solve the equations, understanding the philosophy of cancer cell will require explicit consideration of the edaphic conditions of its geographical landscape that serves as the ultimate template of its fruitification.

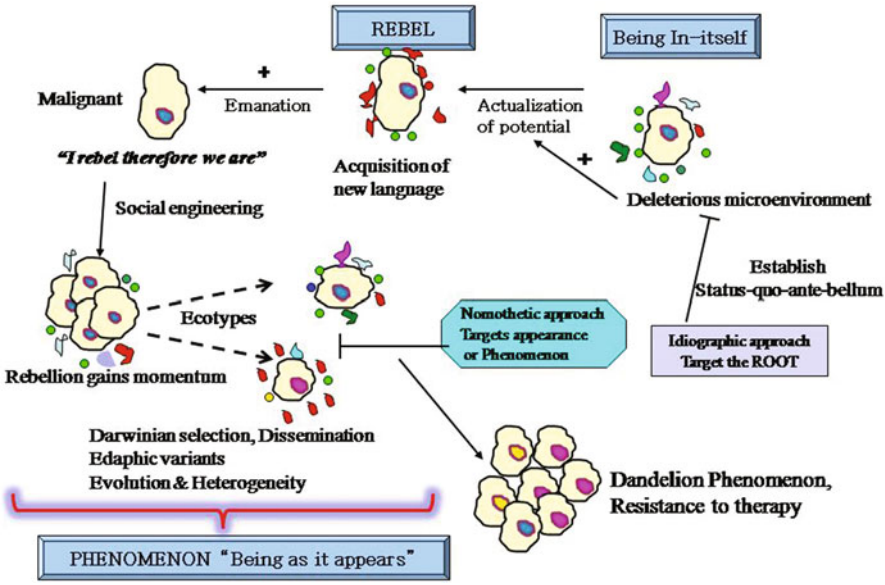

Figure Legend

The normal cell constantly adjusts to stressors in its ecological niche. To express its repugnance towards a society where theoretic equality conceals great factual inequalities, it acquires a new language- the language of a rebel. Denouncing the family name of conformists, the social engineer emanates and the rebellion gains momentum. Darwinian selection increases the rate of evolution. Dissemination of rebels into different ecological niches gives rise to eco-types that adapt to the edaphic condition of its immediate landscape. This contributes to intra- tumor heterogeneity. The conventional nomothetic "pruning therapy" targets this "being-as-it-appears" or "the phenomenon"; subsequently resulting in resurgence with renewed vitality and resistance. Idiographic approach aimed towards sympathetic understanding of the root cause will establish status-quo-ante. Different shapes outside the cell indicate different components of the micro-environment.

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Chapter 10
The Tumor's Normativity: Normative Structures, Action Norms and Decision Maxims as Therapeutic Targets for Tumor Therapy

Albrecht Reichle and Joachim Hahn

Abstract Are normative notions, i.e., normative structures (morphology, and topology), action norms (including the hallmarks of cancer), and decision maxims (hubs, nodes) physically rationalized, functionally established, and even protected? The inseparable relation between rationalization processes and normative notions may be shown at three observational levels. (1) For many reasons, normative notions do not constitute a posteriori classifying phrases or dummies that hide a broad variety of arbitrary tumor-associated phenomena. On the contrary, normative notions are a source of the 'metabolism' of evolution, supplied by the substance of all rationalization processes mediating normative structures, action norms, and decision maxims. (2) Furthermore, the catalytic role of normative notions in composing rationalization processes of the 'metabolism' of evolution can be systematically highlighted from historic aspects and from a therapeutic point of view. (3) Finally, the origin of rationalization processes deriving from normative notions explains the context-disrupting explosive nature of a concrete 'utopia' realized in a normative notion. This condition turns on the general distortion of rationalization processes in tumors (inconsistencies, deformations, and Achilles' heels) as well as on their radical substance (corrupt rationalizations), which is best outlined by its observable robustness towards external (therapeutic) disturbances. The study of normative notions and respective rationalizations in tumor systems including their systematic classification needs to be institutionalized to constitute evolution-adjusted tumor pathophysiology as the novel language of tumor biology and to facilitate biomodulatory therapy approaches.

Keywords Normative notions · Rationalization · Tumor pathophysiology · Convergent evolution · Biomodulatory therapy · Acute myelocytic leukemia

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Introduction

The present article aims at further investigating the question whether normative notions, i.e., normative structures (morphology and topology), action norms (inclusively the hallmarks of cancer, i.e., sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, reprogramming of energy metabolism, evading immune destruction as well as genome instability and tumor-associated inflammation), and decision maxims (hubs and nodes), as purposive action profiles of a tumor compartment are arbitrarily selected for better comprehension of the validity and denotation of tumor systems objects, i.e., pathways, cells, gene expression profiles, transcription factors, cell interactions, etc. The second question is whether normative notions become—to the contrary—physically rationalized, functionally established, and even protected because of their fail-safe maintenance properties [1–4]. Thus, normative notions would be also constitutive for the 'metabolism' of evolution in tumors.

Considering the multifold possibilities (redundancy) of how cells or cell communities constitute rationalizations for the fail-safe function as well as the frequent context-dependent multifunctionality of tumor systems objects—exemplified by NF-κB and p53—, we may become confused about the 'true' assessment of the communicative expression of tumor systems objects or their denotation within established rationalization processes [5, 6].

In contrast to the assumption of an retrospectively ensued contentual loading of the term 'rationalization', we would like to show that a tight conceptual and scientifically verifiable coherence has existed between the two concepts 'rationalization' and 'normative notions' from the very beginning, even though only implied as 'tumor-associated angiogenesis' or 'tumor-associated inflammation' (the hallmarks of cancer), etc. Consecutively, the evolution-historic way, i.e., how rationalizations are constituted in molecular detail, reaches therapeutic relevance. Likewise, the pure molecular identity of a potential therapeutic target dwindles in importance because its situative communicative expression has to be added for guiding therapeutic decisions and personalizing systemic tumor therapies [7].

The evolutionary-driven constitution of the normative substance of rationalization processes as well as the cellular recourse on distinct and—within an evolutionary context—restricted tools of rationalizations for the fail-safe maintaining of normative notions draws on communicative competition, even if 'corrupt' rationalizations develop, as in the case of tumors. On the basis of non-random multifacetedly acquired molecular-genetic aberrations, tumors represent a very good example and model to investigate the diversity of 'individual' rationalizations, particularly in tumor entities such as acute leukemia [8].

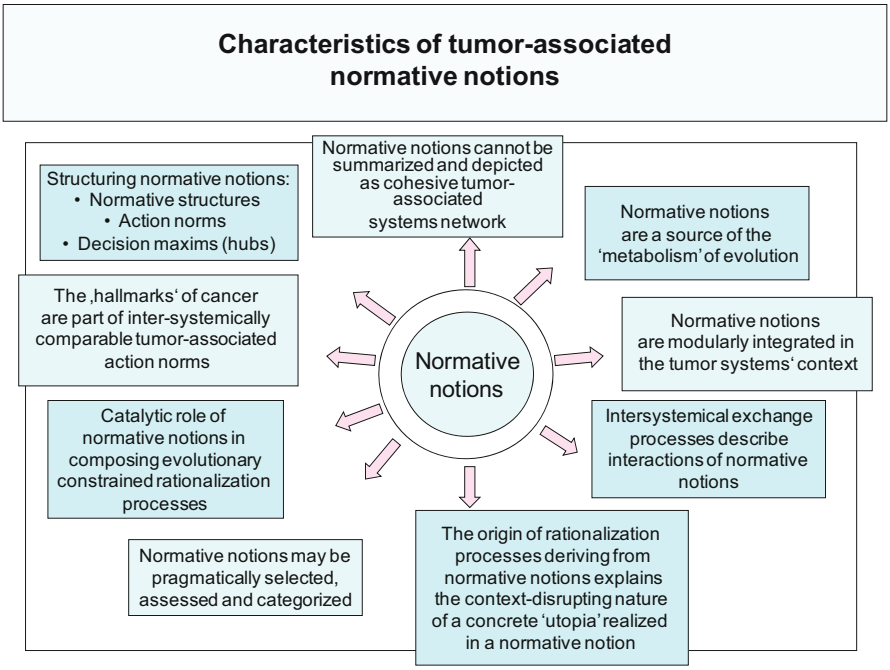

Fig. 10.1 Features of tumor-immanent normative notions

Method

The inseparable relation between rationalization processes, which are supposed to organize and to ensure the physical expansion and maintenance of normative notions, and normative notions may be shown at three observation levels, (1) genetic code—rationalization—normative notion, (2) biomodulatory therapies—rationalizations—normative notion, and (3) communication-derived pathophysiology of rationalizations of normative notions (Fig. 10.1).

1. For many reasons (i.e., context-dependent validity and denotation of tumor systems objects; convergent evolution, that means multiple rationalizations for one dominant normative notion; and robustness of rationalizations), normative notions do not constitute a posteriori classifying phrases or dummies that hide a broad variety of arbitrary tumor-associated phenomena. On the contrary, normative notions are a source of the 'metabolism' of evolution, supplied by the substance of all rationalization processes mediating normative structures, action norms, and decision maxims.
2. Furthermore, the catalytic role of normative notions in composing rationalization processes of the 'metabolism' of evolution and rationalization can be systematically highlighted from historic aspects and from a therapeutic point of view.

3. Finally, the origin of rationalization processes deriving from normative notions explains the context-disrupting explosive nature of a concrete 'utopia' realized in a normative notion. This condition turns in a scientifically accessible way on the general distortion of rationalization processes in tumors (inconsistencies, deformations, Achilles' heels) as well as on their radical substance (corrupt rationalizations), which is best outlined by its observable robustness towards (therapeutic) disturbances from outside.

Results and Discussion

Ad 1) Because of their abstract universality, the normative structures, action norms, and decision maxims of tumors require concretization in each separate case, i.e., description of cellular, biochemical, and systems levels and specification of the stage-dependent physical constitution in an evolutionary context.

Thereby, multifaceted acquired digitalized structures, which are anchored in the genetic code and constitute the frame for distinct normative notions via rationalizations, may attain multifaceted starting points to establish unique normative notions[8]: Convergent evolution [9] may be even highlighted in acute leukemia. The already anamnestically obvious, rapid onset of leukemia-associated symptoms——induced by bone marrow insufficiency—is marked by a very broad variety of single or cumulatively acquired molecular-genetic aberrations. As the different types of acute leukemia share a dominant normative notion, rapidly displacing growth, the notion achieves facticity: Rapid leukemia evolution may be realized by the 'metabolism' of evolution via multifaceted and differentially established rationalizations. Kvinlaug et al. causatively described the occurrence of 'disparate oncogenes' [8]: Differential functions may be attributed to oncogenes dependent on the leukemia-specific concert of aberrations. Beyond the context-dependent acquisition of differential functions of oncogenes or driver mutations, their predictive value may change dependent on the additional clinical background, which again is founded in the concert of molecular-genetic aberrations [10].

The redemption of a unique normative notion from scientifically and profoundly assessed multifaceted genetic starting points including frequently occurring recurrent genetic aberrations outlines the varying validity and denotation of single aberrations against the background of an evolutionary specified genetic context. Multifaceted aberrant genomes constitute a broad diversity of rationalizations for maintaining the dominant normative notion, according to which a whole group of diseases, namely 'acute' leukemia, is clinically classified [11].

Now, differential rationalizations constituting a unique phenotype achieve an equivalent classifying substance and could present as decisive guides for 'rationalization-targeted' (personalized) therapies in future. Furthermore, tumor-specific and stage-specific rationalizations with entirely unforeseen validity and denotation of underlying oncogene-addicted structures, driver mutations or generally therapeutic targets are the major reason for the limited practice of so-called targeted therapies in unknown and novel evolutionary systems stages [8, 12]. The

Table 10.1 Top-down and bottom-up strategies use quite different targets for redirecting and modulating the tumor's normativity

Therapeutic top-down versus bottom-up strategy	
• **Evolution-adjusted approach**	• **Theme-dependent approach**
Targets:	**Targets:**
• Tumor-immanent normative notions (implementation of non-normative boundary conditions)	• Systems participators as ‚artifacts', irrespectively of their validity and denotation
• Rationalization processes	• Cell-specific targets
• Systems participator's validity and denotation	• Oncogenic targets
• Ubiquitously in tumor and stroma cells accessible targets	• Pathways
• Tumor and stroma cells are equally targeted	• Malignant cell populations, rarely stroma cells

frequently weak efficacy of classic targeted therapies empirically underlines the heterogeneity of rationalizations, particularly in the molecular-genetically highly heterogeneous types of acute leukemia. The novel classifying principle, i.e., the systematic comprehension of rationalizations at genetic and protein level etc., will lead to evolution-adjusted tumor pathophysiology (Table 10.1).

Accordingly, normative notions subsume more subjacent differences, such as multifaceted rationalization processes. The function of a communicatively determined compromise, which normative notions accomplish in the course of the differentiation and expansion of rationalization processes, may not explain their occurrence as a specified rationalization concept in tumors. Experimental data on context-dependent functionality—particularly of transcription factors—and clinical data obtained through biomodulatory therapy approaches show that modified evolutionary contexts only communicatively address the aspects that are implicitly inscribed in rationalization processes, namely normative structures, action norms, and decision maxims [13–16].

Correspondingly, cells refer back to repair mechanisms or mobilize alternative rationalization processes from an accessible tool to maintain normative notions (robustness) if normative structures are exposed to unexpected perturbations [17]. Hence, detection of violated normative notions and respective repair mechanisms have the function of uncovering communicative rules as well as inconsistencies, deformations, and Achilles' heels of expanding rationalizations during tumor evolution.

From the capacity to focus on the protection of normative notions, the importance may be delineated, which robustness [18–20] has achieved for the fail-safe function of rationalization processes. The stronger robustness permeates the whole system of cellular rationalizations, the more frequent its interference with the vertical relation between single cells. Just in the case of tumor development, collisions accumulate (implementation of molecular-genetic abnormalities, 'stress'), necessitating intercellular communicative assessment between competitive rationalization claims according to communication-derived rules. In such decisive 'hard cases', decisions are only possible by a recourse on the violation of a valid and priority-claiming normative notion (which could also be therapeutically supported for prevention). In the case of tumor initiation, an overthrown normative notion may be uncovered within a 'physiologic' cell compartment accompanied with a corrupt rationalization.

The 'hard case' may be simulated by the experimental implementation of non-normative boundary conditions, e.g., cellular 'stress' in prostate cancer cell lines, which alters the denotation of the androgen receptor. The receptor then physically participates in establishing a recurrent chromosomal translocation, TMPRSS2:ERG and TMPRSS2:ETV1 ('corrupt' rationalization following androgen treatment and genotoxic stress): A distinct, externally implemented rationalization leads to specific chromosomal aberrations, indicating that rationalizations are principally in bidirectional communicative exchange with the digitalized system of the genome [21]. Rationalizations are—on the basis of normative notions and the 'metabolism' of evolution—the digitalized counterpart of the genetic code and may contribute to decipher the genome-centric 'world'.

After having systematically observed tumor development on the basis of increasing lifetime expectation for many decades and the introduction of steeply growing numbers of technologies and (biomodulatory) therapies for studying cancer, we now realize the benchmarks of tumor development: Normative notions, which outreach the traditionally noted hallmarks of cancer by far, are the framework by which the universal substance of the 'metabolism' of evolution is imported into novel rationalization processes.

The idea of normative notions is the conceptual hinge that merges the 'metabolism' of evolution in every cellular structure and function with respective physically comprehensive and directly scientifically accessible rationalization processes. This mergence occurs in such a way that a distinct cellular organization originates from the interplay at a circumscriptive and compliantly evolutionary stage (cellular 'living' world), which is based on the robustness of different rationalization processes. As the 'metabolism' of evolution may be redeemed in specified rationalizations, the expansion of rationalizations shows a Janus face, which is simultaneously directed at the 'metabolism' of evolution and at the communication-derived norms (rules) for constituting rationalizations [22].

Ad 2) To date, normative structures (for instance, molecular-genetic aberrations in tumor and stroma cells) and action norms, such as the hallmarks of cancer, constitute a realistic utopia insofar as they do not make us believe any longer that tumor cells are a contextless driving force during their evolution [23]. But simultaneously, we only hesitantly dare to attribute concrete validity and denotation to individual and

multifacetedly differentiated tumor cell components. Anyhow, normative structures, i.e., angiogenesis, inflammation etc., are acknowledged 'per se' as indispensable for tumor development [3, 24].

The following tasks are crucial for extending the instruments for designing personalized therapy approaches: to estimate the situate validity and denotation of normative notions in the tumor compartment, to get information on the communicatively guided competition of various normative structures in the tumor, to identify the particular cellular driving sources, and to analyze how normative notions are physically and functionally rationalized in a distinct evolutionary context. This way, rationalization processes may be categorized beyond the traditional classifying principles, i.e., disease traits and histopathology, which redraws the attention to tumor pathophysiology. Tumor pathophysiology needs to be reconsidered and may have to be re-established as evolution-adjusted, clinical, and particularly personalized tumor pathophysiology [25].

Biomodulatory therapies are directed towards robustness of rationalization processes by redeeming novel validity and denotations of systems objects in the context of a tumor's evolutionary confined 'living' world, i.e., its holistic communicative world [26]. Vice versa, biomodulatory therapies may give decisive clues how normative notions are rationalized in a distinct evolutionary context, as exemplified by modularly targeting tumor-associated inflammation [27].

The proper functioning of biomodulatory therapies in metastatic cancer underlines that regulatory active, multi-targeted therapy approaches, which primarily focus on non-oncogene addicted structures and functions, may exhaust the communicative capacity of a tumor's 'living' world [14, 16]. Thereby, normative notions, which are basically supported by 'oncogene'-addicted structures and functions become redirected and placed. In acute myelocytic leukemia, first biomodulatory therapy approaches with a nuclear receptor agonist, i.e., all-trans-retinoic acid, have shown significantly improved overall survival rates in cytogenetically defined subgroups, besides the classic application in promyelocytic leukemia [28]. All these therapy-derived observations indicate that the normative notions of tumors are therapeutically placeable via communication-derived rules grounded in a tumor's 'living' world: Normative notions, which are frequently accessible as disease traits—as in the case of 'acute' leukemia—, are available for therapeutic modification by implementing rationalization-specific and non-normative boundary conditions, i.e., biomodulatory therapies (transcriptional modulators, metronomic low-dose chemotherapy, etc.) [14]. Because of the established and therapeutically relevant altered communicative prepositions during biomodulation, the term 'oncogene' must be relativized, because only the therapy-naive tumor-associated communicative context refers to the denotation as 'oncogene', which again may be specified in the context of multiple additional molecular-genetic aberrations ('disparate' oncogenes).

Ad 3) Reductionist-oriented targeted therapies provoke tension in the current therapeutic scene: Apart from some exceptions, therapeutic results obtained by reductionist approaches disclose more and more frequently a gap between the perceived (preclinical) norms and expectations—stated by reductionist theories on oncogene-addicted targets or driver mutations—and current, rarely sweeping therapy results,

which are often taken from very small patient populations [29–33]. Reductionist knowledge draws on uniquely defined communicative circumstances, which are self-evidently assigned to novel evolutionary confined systems stages, as is the case in the therapy of acute leukemia. The strategy to target oncogene-addicted structures and driver mutations is commonly linked to the perception of personalized tumor therapy.

The interruption of a rationalization process at any optional biochemical level (traditional targeted therapy), necessitates the tumor's living world to react with alternative rationalizations. In the situative communicative expression of a target lies the rub, as seemingly unexpected 'effects' and 'side effects' may occur. Reproducibility of side effects, and side effects dependent on the treated histological tumor type, as outlined for sorafenib, a tyrosine kinase inhibitor, and its differential activity in acute myelocytic leukemia, renal clear cell carcinoma and hepatocellular carcinoma [12, 34, 35], again underline that rationalizations are highly specific.

However, the novel practice of creating applied systems biological therapy approaches brings on a completely new problem: Biomodulatory therapies also claim universal validity. They aim at targeting the weak points in the execution of rationalization processes or at redirecting a tumor's normative notions to achieve the attenuation of tumor growth. Basis for the therapy design is the analytic and empiric comprehension of tumor-associated rationalizations, which equally encompass both the digitalized genome-centric 'world' and the modularly structured cellular 'world' [26, 27, 36, 37]: The tumor's rationalizations represent an independent counterpart to the tumor's genome with its acquired aberrations.

For establishing evolution-adjusted tumor pathophysiology, each norm, the substance of a systems object (i.e., its background knowledge), is scrutinized with regard to its communicative context, which attributes notable validity and denotation to systems objects in a distinct evolutionary systems stage [27]. Does a novel evolutionary context attribute identical validity and denotation to a systems object as uncovered for the respective systems object in the evolutionary context studied originally? Is the respective systems object part of a novel rationalization process, constituting a distinct normative notion [12]? Multifold experimental data highlight NF-κB as a colorful 'matchmaker', when inflammation meets cancer [38].

Normative notions may be studied starting with the comprehension of the tool of possible rationalization processes (redundancy, robustness), for example, by describing which cell compartments primarily contribute to tumor-associated inflammation, angiogenesis, immune response etc., and which communication lines are activated with regard to communicative expression. Furthermore, normative notions may be studied by recording the secretome of cellular compartments of the tumor in patient serum, which is indicative for the presence of distinct normative notions, or by monitoring changes derived from molecular imaging, which may give indications for the therapeutic redirection of normative notions [27, 39–41].

Table 10.2 Rationalizations of tumor-immanent normative notions represent the non-genomic counterpart of the tumor genome and have to be systematized in the same way as genomic structures and functions

Rationalizations:
The non-genomic counterpart of the tumor genome

Tumor genome	Tumor-associated rationalizations
Coding, non-coding gene sequences, transcription process	Rationalization processes constitute between the poles of systems world and functional world of systems participators
Digitalized system	Digitalized, endogenously and therapeutically redeemable system (does not exclude analogously working functions)
Number of genes circumscript (whole genome analysis)	Number of therapy-relevant rationalizations discursively and pragmatically assessable
Functional genomics	Intersystemically comparable, interphases between rationalizations
Intratumoral genetic heterogeneity	Rationalizations can be maintained within a tumor disease of a patient, presumably via hubs
Targeting of moleculargenetic aberrations: ‚Bottom-up' strategies	Targeting of rationalizations: ‚Top-down' strategies

Conclusion

Normative notions are pragmatically and discursively selected. The study of normative notions (Fig. 10.1) and respective rationalizations in tumor systems including their systematic classification needs to be institutionalized to constitute evolution-adjusted tumor pathophysiology as the novel language of tumor biology and to facilitate novel biomodulatory therapy approaches, i.e., cellular therapies in situ (Table 10.2).

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Glossary

Normative notions
Normative notions comprise defaults in biologic systems, which are realized by evolutionarily compliant rationalizations. For their formal description the discrimination between normative structures (morphology, topology), action norms (e.g., hallmarks of cancer) and decision maxims (nodes, hubs) is useful. The idea of normative notions is the conceptual hinge that merges the 'metabolism' of evolution in every cellular structure and function with respective physically comprehensive and directly scientifically accessible rationalization processes. Normative notions, which outreach the traditionally noted hallmarks of cancer by far, are the framework by which the universal substance of the 'metabolism' of evolution is imported into novel rationalization processes.

Rationalization
Rationalizations describe how normative notions, i.e., normative structures, action norms and decision maxims are differentially and physically established as well as functionally organized [7]. Rationalizations equally encompass both the digitalized genome-centric 'world' and the modularly structured cellular 'world' [26, 27, 36].

Modularity
In the present context, modularity is a formal pragmatic communicative systems concept, describing the degree and specificity to which systems objects (cells, pathways, proteins etc.) may be communicatively separated in a virtual continuum and recombined and rededicated to alter the validity and denotation of communication processes in the tumor.

Tumor's living world
The living world comprises the tumor's holistic communication processes, which we rely on in every therapy. With experimental or therapeutic experiences (modular therapies) the tumor's living world may be separated into categories of knowledge, for example, into modular systems. Specific conditions of compliance, for redeeming validity constitute relations between communication technique (specified biomodulatory therapy approaches) and distinct tumor-associated systems stages [26].

Background knowledge
The communicative substance of a systems object is dependent on the communicative presuppositions, which determine the system's object validity and denotation within an evolutionary compliant systems stage. Background knowledge constitutes the validity of informative intercellular processes, which is the prerequisite for therapeutic success. Background knowledge about the tumor's living world is subjected to other conditions of scientific comprehension: Intentional ways fail to describe risk-absorbing knowledge, in which context-dependent knowledge about commonly administered reductionist therapy approaches is rooted. After this second objectifying step (physicians as operators of tumor systems), the network of the holistic

communicative activities turns out to be the medium through which the tumor's living world is mirrored and generated in rationalizations [26].

'Metabolism' of Evolution

Generally, communicatively linked biological systems are interweaving the nude identity of their systems' objects, or the arrangement of compartmentalized knowledge (on the observer site) with situative biological stages, or with communicative arrangements of systems' objects validity and denotation (on the participator site) by allowing implementation of internally or externally derived modular knowledge according to rules, which are present in modularly arranged and rationalized systems' textures, equitable with the metabolism of evolutionary systems, and which purport the frame for evolutionary multiplicity [22]. As the 'metabolism' of evolution may be redeemed in specified rationalizations, the expansion of rationalizations shows a Janus face, which is simultaneously directed at the 'metabolism' of evolution and at the communication-derived norms (rules) for constituting rationalizations.

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Chapter 11
Criticizable Claims for the Validity of Communication Acts in Biological Systems: Therapeutic Implications in Cancer

Albrecht Reichle, Christopher Gerner and Guy Haegeman

Abstract Basis for the comprehension of biological systems are experimentally, and in the case of metastatic tumors also therapeutically derived data, mirroring the context-dependent validity of communicatively integrated systems objects (molecules, pathways, cells etc.). Validity claims of experimentally defined references in terms of systems objects seem to be routinely transferable into arbitrary evolving systems. This transfer is irrespective of the self-evident assumption that novel systems functions may spin off and that those tumors show novel compositions of acquired chromosomal and molecular-genetic aberrations. We are used to transfer references of experimentally defined systems objects into novel situation-embossed systems contexts, even though such experimentally-derived references are inevitably situation-linked and always attributable only ex post, particularly in case of evolving biologic systems. The present paper aims at reconstructing communication-derived rules and at showing how validity claims, which inevitably adhere to objects in biological systems, may be uncovered and therapeutically utilized. Hypothesis-driven tumor models may serve as challenge to reinterpret the myriad of available biological data in a communicative context. The main task remains to reconstruct observable communicative interactions on the expressive level and to select and extend methodologies, which have the capacity to monitor functional changes of cell systems in response to (therapeutic) perturbations.

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Introduction

Cancer is a leading cause of death worldwide, particularly in Western countries [1] and, in most tumor types, early diagnosis of localized tumors offers the best chance for cure. In metastatic tumor stages, the efficacy of currently available therapy approaches is still dissatisfying. However, most cancer patients suffer from advanced or metastatic tumor disease at initial diagnosis. Targeted therapy approaches have significantly improved outcome in molecularly defined tumor subgroups.

Therapeutic success in selected tumor entities with molecular-directed therapies has fostered the discussion about personalized tumor therapy on a rational basis [2], resulting in a persistent demand for personalized tumor therapy. Over time however, its realization proved to be more difficult than expected. The implementation of molecularly targeted therapies is continuously stipulated, but we are still far removed from our subject in the clinical setting [3–5]. Therefore, we should also think about methodological concerns as one reason for the delayed progress in personalizing tumor therapy.

For many diseases, such as metastatic tumors, that have undergone empty years of evolution, stepwise and evolution-adjusted therapy may be an alternative way to achieve medical improvement rather than drastic therapeutic interventions based on theme-dependent knowledge. The focus should be on an individual's evolution-linked tumor phenotype rather than only on molecular and theme-dependent knowledge [6].

The necessity of developing novel methodological approaches to bridge theory and therapeutic practice may be exemplarily highlighted by common observations revealing discrepancies between theory and practice [7, 8]. Phrasing obstacles for translational research in the clinical field allows focusing on issues that have to be covered by novel hypothesis-triggered methodologies, for instance, the reconstruction of communicative relations of systems objects (pathways, molecules, cells, etc.) within a tumor system on the basis of a formal pragmatic communication theory.

- Not every **clinical trial** has to re-confirm the non-transferability of reductionist, context-dependent knowledge (derived from basic science) on completely novel evolution-based contexts in metastatic tumor systems. Systems objects and communication lines as the benchmarks of communication may have striking common features in a preclinically-derived systems context as well as in a novelly evolving systems context: An identical therapy-relevant systems object may be ascertained with respective methods, both, in the 'historical' control and in the novelly evolving tumor system, including all its variations, up- or down-regulations, or molecular modifications. But targeting the specific molecule with the respective scheduled 'targeted' drug or drug combination may lead to differential or completely different results [9, 10]: Multi-facetted chromosomal or molecular-genetic aberrations, particularly in tumor cells but also in stroma cells, may ultimately determine the communicative expression, i.e., the meaning of systems objects in a therapeutically relevant way (Chap. 7).

- **Nonlinear responses** of differentially developed tumor systems are a well-known phenomenon: Philadelphia positive chronic myelocytic leukemia may be live-long controlled in more than 60 % of patients by inhibition of chimeric tyrosine kinase [11]. Additional aberrations in CML disease cause many problems with regard to disease control by respective targeted therapies [12]. Sorafenib, another tyrosine kinase inhibitor, is weakly active in combination with chemotherapy in Flt-3 positive acute myelocytic leukemia. However, administered as a single drug, it may induce continuous complete remission in patients with Flt-3 positive relapse after allogeneic stem cell transplantation [9, 10]: Seemingly minor therapeutically induced perturbations of tumor systems may contribute to rapid and massive changes in response dependent on the kinases' communicative systems context (Chap. 7).
- **High-throughput array data** or in silico approaches contribute to a minor degree to novel modes of therapy action, for instance, combined targeted therapies [3], and innovative drug designs, such as in chronic myelocytic leukemia or ALK-positive adeno-carcinomas of the lung [11, 13]. To overcome this gap it is necessary to process quantitative proteomic data from appropriate hypothesis-driven models, for instance, based on the communicative reconstruction of tumor-immanent normative functions [3, 14–17].
- **Natural drugs** derived from plants (ethno-pharmacology) give decisive hints on the modular nature of mechanisms of action, particularly in comparison with physiological compounds [17, 18]. Furthermore, these compounds show how normative notions within tumor systems, for instance, maintenance of proinflammatory and proangiogenic processes during tumor progression, are differentially rationalized within various tumor systems [19–21]. Therefore, tumors may recourse on completely different communication lines to constitute normative notions within tumor systems, which are—vice versa—perceived as unique by clinical observers. These normative notions are tightened to a few, seemingly characteristic, markers, symbolizing distinct normative notions (inflammation, angiogenesis, etc.) [22–25; Chap. 17].
- **Validity claims** of experimentally defined references in terms of systems objects seem to be routinely transferable into arbitrary evolving systems. This transfer is irrespective of the self-evident assumption that novel systems functions may spin off and that those tumors show novel compositions of acquired chromosomal and molecular-genetic aberrations. We are used to transfer references of experimentally defined systems objects into novel situative systems contexts (that represent a distinctive evolution-derived phenomenological status), even though such experimentally-derived references are inevitably situative and always attributable only ex post, particularly in case of evolving biologic systems [26]. The present paper aims at reconstructing communication-derived rules and at showing how validity claims, which inevitably adhere to objects in biological systems, may be uncovered and therapeutically utilized.

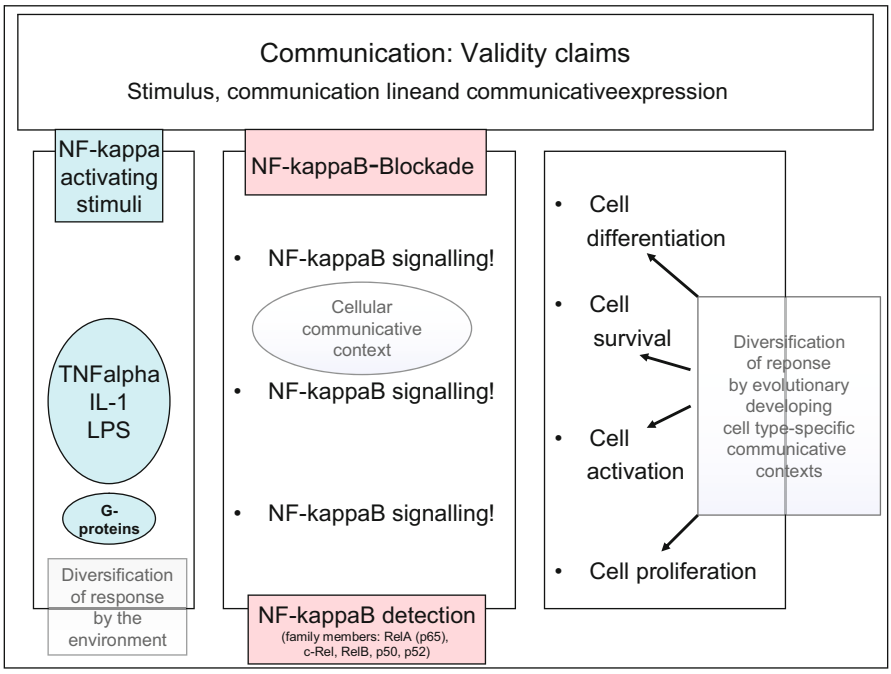

Fig. 11.1 The communicative expression of the activated NF-kappaB signalling pathway is modulated by extrinsic, environmental parameters and by intrinsic, evolutionary developing communicative contexts

Perception of Validity

A significant difference exists between a communication medium (ion channels, molecular pathways, signaling integrators, etc.) or communication lines (gap junctions, signaling pathways, nerves, etc.) and the underlying communicative expression (purpose). Communication mediums (cytokines, hormones, etc.) and communication lines are assessed according to how well they technically work with regard to communication, whereas communicative expressions are evaluated according to their communicative validity (Fig. 11.1).

Communication mediums and communication lines are easily accessible and comparable among rather different biologic systems. The reconstruction of their situative communicative validity and denotation—particularly in pathological circumstances (metastatic tumors)—necessitates further studies. These investigations should include not yet routinely operated methodologies, so that a distinct communication tool of interest can be assessed within its situational context.

Pragmatic Functions of Communicative Expression

By its activation, a communication line is placed (1) in relation to its external reality, the microenvironment, and corresponding to the modus, how the external reality may be experienced (e.g., controlling circuits, sensory systems, modular knowledge, etc.). Furthermore, a communication line is related to the (2) internal reality corresponding to the mode of rationalization of the tumor's living world (defined as the tumor's holistic communicative world). Further relations are the (3) intersubjective reality that corresponds to what a communicative systems participator may express as its intention, and (4) the normative structure, i.e., how it is recognized by a socially linked cellular system (normative functions, i.e., inflammation, angiogenesis, etc.) [27–29].

After the activation of a communication line, the respective communicative expression should be assessed. Communicative expression has three pragmatic functions, namely to represent something, to express an intention, and to establish an intersubjective relationship of systems objects.

During the activation procedure, communicative expression is subjected to validity claims. As a non-situated communication line or as a purely communication-technical formation, communicative expression cannot fulfill these claims because of attributed and historically objectified references, which may be quantitatively and qualitatively appreciated.

Prepositions of Validity

Comprehensibility is a universal claim that can be raised by communicative participators with regard to communication lines as well as a prerequisite for the correct function of communication tools. The validity of a stated proposition depends on whether the proposition represents a fact or an experience. The validity of an expressed intention depends on whether it corresponds to what is actually intended by the initiator of a communication process. The validity of a performed communication act depends on whether this action conforms to a recognized normative background. A communication line aims at comprehensibility, whereas a successful communicative expression must satisfy additional validity claims: It must be ascertainable for systems objects as something that is represented in the living world; additionally, communicative expressions account as right (no fallacy!) insofar as it conforms to recognized expectations of a cell society.

Modules and Modular Knowledge

We commonly proceed on the assumption that a proven systems object complies with a distinct function or has a particular meaning. We are less likely to suggest that primarily insufficiently comprehended tumor systems with multiple and varying

chromosomal or molecular-genetic aberrations may assign distinct systems objects with novel and probably contradictory meanings as suggested by preclinical data [30].

We cannot continue to describe proteins, pathways, and cell interactions on a solely physical-chemical level and by usual chemical kinetics [31, 32]. On the one hand, mathematical realization of the entire combinatorics of all possible interactions and variations of systems objects is hardly possible; on the other hand, many systems objects are known to adopt surprising functions depending on their communicative context [9, 10]. These functions may be poorly delineated from the physical-chemical behavior of systems objects themselves and are obviously derived from communication-associated rules [31, 32], which mirror validity claims. The communication-derived, context-dependent tool of possibilities for adopting novel systems functions via modular rearrangements is termed the modular knowledge of a systems object. Systems objects are intrinsic information carriers in combinatorial dynamical systems, which are characterized by situatively arising modules and rationalizations of normative notions [14, 27, 32].

Modularity (Object-Subject Relation)

The increasingly higher organization of a tumor cell system during tumor growth results in the development of systems perspectives, in which the functional 'world' of distinct cell types is featured as a component of the respective systems 'world' [14, 33–35]. In the present context, modularity is a formal pragmatic communicative systems concept, describing the degree and specificity to which systems objects (cells, pathways, molecules, e.g., transcription factors, etc.) may be communicatively separated in a virtual continuum, reassembled, and rededicated (e.g., co-option) to alter the validity and denotation of communication processes. This concept refers to possible interactions between the systems objects in a tumor as well to the degree to which the communicative rules of the systems architecture (for establishing validity and denotation) enable or prohibit the focus on validity and denotation. Systems objects acquire the features of symbols, which are rich in content and able to acquire novel references by rearranging validity and, consecutively, denotation. Tumors consist of modules, which become a scientific object by uncovering a tumor's living world with biomodulatory and therefore modularly designed events. A formal-pragmatic theory about the denotation of a communication process may establish an internal interrelation of denotation and validity [14].

Formal Pragmatic Theory of Meaning

The formal pragmatic theory of meaning originates from the simple consideration that a systems participator only 'understands' a communication act, if it perceives the conditions that make it acceptable.

At issue are objective conditions of validity that may be (therapeutically) inferred directly from the communicative content of a respective communication-technical expression used. This validity claim rests on a reservoir of potential reasons with which it can redeem, if necessary.

The available reasons interpret the validity conditions that are part of the conditions that render validity claims worthy of intersubjective recognition and make a corresponding communication expression acceptable. Correspondingly, identical biomodulatory therapies may exert differential effects dependent on the differentially embossed validity conditions in situative evolutionary processes [36].

Only an additional evaluation step makes it possible to turn from the exclusive consideration of formal communication techniques (signaling pathways, etc.) to therapy-relevant communication pragmatics, which assess the conditions of communication for reaching understanding (physiological or pathophysiological status) or strategic (therapeutic) communicative interventions. A prerequisite for the additionally introduced evaluation step is the revision of basic ontological and mostly reductionist-based concepts established in biology.

- An exact **formal pragmatic analysis** of a successful communication act is necessary (i.e., cellular secretome analytics, molecular imaging) because, in communicative actions, the structure of the use of communication tools aimed at reaching understanding is inherently linked with teleological structures of action (normative notions).
- **Rationalization and robustness:** In an evolutionary process, tumor cells may exploit the whole extent of the rationalization features of stroma cells to implement the functional diversity of systems behavior aimed at maintaining homeostasis and robustness in tumor systems [27, 37]. The implementation of a new form of integration (rationalization) of these stroma cells allows the evolutionary advancement of the systems complexity with the remodeled rationalization of cellular functions: The diversified resources of tumor growth-promoting cytokines are distributed among rather different stroma-associated cell types (redundancy). Tumor cell systems may recourse on differential rationalization processes (perlocutionary act in linguistics), which is symbolized by rather different communication lines and systems objects to maintain normative notions (robustness).
- **Systems actors are subjected to constrains**, which again restrict them to adapt attitudes facilitating distinct normative notions with respective communication lines. From a therapeutic point of view, it is important that attitudes for communicative actions are obviously more loaded with presuppositions (an indication of robustness) than the objectifying attitudes of strategic actors ('knowing that'), i.e., physicians administering a therapy, which interferes with the holistic communicative tumor system (biomodulatory therapy). On the other hand, communicative interactions mediated through acts for reaching understanding exhibit a multi-facetted but more restricted structure than strategically intended actions.
- **The frequently applied game theory as well as the theory of scale relativity** decisively restrict an action-oriented theory towards the reaching of understanding in so far that these theories neglect the dynamics of reciprocally (by the systems

objects) intended criticizable validity claims [38, 39]. However, criticizable validity claims are essential for communicative action. Insofar, game theory underlies a presupposed validity consensus, which must not necessarily be present in evolving tumor systems. Game theory approaches may be considered useful for the simplification of complex sets of non-equilibrium conditions by the introduction of 'multi-target drug design games' [38]. Multimodal interactions may also provide an opportunity to induce evolutionary (also epigenetically driven) processes and novel intersystemic exchanges so that the use of game theory seems to be restricted for assessing evolution-adjusted references of systems objects.

- **Vagueness about the communicative expression:** Communicative actions coordinating factually raised and recognized validity claims result in unconditionality, rules entering the everyday intermolecular and intercellular communicative practice. Simultaneously, vagueness arises about the communicative expression of a communication line within a novel systems context. The communicatively subjected systems participators demand accountability about their situative and evolution-based communicative expression and scientific evaluation within an accessible frame by using adequate and routinely applicable methods to broaden the therapeutic options and to further personalize therapy. In contrast to the theory of scale relativity [39], which is an extension of the theories of relativity (achieved by applying the principle of relativity not only to motion transformations, but also to scale transformations of the reference system), vagueness about the communicative expression in evolving tumor systems is directly mirrored in concurrent communicative features, the functional world, for instance, a cell and the systems world of a biologic cell community [40].
- **On the one hand, criticizable validity claims establish scientifically reproducible arrangements**, symbolized by the systems objects' references. On the other hand, such claims rely on the ever flexible reservoir of the systems participators' modular knowledge, which may implement the often surprising spin off of novel systems functions by the impact of externally- and internally-derived communicative processes [41, 42]. Validity claims must be raised in a time- and space-related context, which is founded in an inevitable situational rationalization of the tumor's living world, i.e., the tumor-specific risk-absorbing background. Validity claims are accepted or rejected with regard to non-reversible action sequences: Tumor cells may irreversibly destroy physiologically rationalized organ systems by colonizing the host's organs [27].
- **Idealizing suppositions:** The application of communication tools aimed at reaching understanding among systems objects demands idealizing suppositions including normative judgments on the part of communicatively linked actors. These scientifically underestimated suppositions function as social facts within a tumor's living world. The steadily generated social facts are constitutive—as are communication tools—for the form, in which situational social cellular life reproduces itself.

The attempt to reconstruct biologic communication processes and to show how to uncover and monitor these processes for therapeutic purposes cannot constitute a

comprehensive concept of a tumor's communicative tool of normative contents. What is at issue here is to discuss the daily diagnostic and therapeutic challenges—aimed at broadening the therapeutic instruments—on the basis of comprehensive and evaluable communicative presuppositions, which have been shown to be inevitable for the continuous and non-circumventable process of reaching communicative understanding as well as for strategic (therapeutic) communicative interventions. Reconstruction of prepositional reasons for differential rationalizations of systems within distinct evolutionary stages and the parallel uncovering of the respective situative procedural constitution of rationalization processes are of pivotal interest for broadening therapeutic options.

A formal pragmatic theory of meaning opens up possibilities to assess the scientific frame for possible choices of **tumor prevention and tumor therapy** by accentuating situational arising validity claims (Chap. 15). These claims constitute unconditionality by implementing rules, which are determined by situative propositions, as well as uncertainty about the communicative expression of a communication line during evolutionary tumor processes. Inevitably, prevention programs as well as programs trying to therapeutically reconstitute the 'status ante' before the disease, which is frequently symbolized by the achievement of complete remission, have to regard the propositional aspects of communicative expression promoted by communication lines and based on context dependent validity claims. Many routinely performed therapeutic interventions are afflicted with a **biological memory** (genetic and epigenetic changes), i.e., chemotherapy and radiotherapy [43, 44].

Pragmatic Implications for Systems-Oriented Therapy

Pragmatics describes the relation between communication lines as symbols (rich in content) and their respective effects on systems objects. Communication partners use the symbols (1) to constitute the inevitable phenomenological context (theory and practice), (2) to establish evolution by implementing modular knowledge, and (3) to maintain homeostasis by constituting robustness.

- **Homeostasis**, here defined as the sum of processes available to maintain normative notions, can only be explained on the basis of robustness, which is based on the multi-faceted possibilities of systems objects to recourse on differential communication lines and rationalization processes to maintain normative notions: The impact of robustness in cellular systems, such as tumors, on the constitution of survival and reproduction is conspicuous: Still, a series of tumors are considered therapy-resistant [45].
- **Rationalizing the tumor's living world:** Of particular interest for the preservation of normative systems structures is the continuously proceeding process, by which internally- or externally-derived modular knowledge is implemented during the communicative exchange with the environment. The resulting situative communication profile enables—according to communicative rules—a steadily moving but distinct configuration of systems objects' validity and denotation,

which is aimed at (1) maintaining robustness on the basis of definitely rationalized biological systems or (2) at rationalizing the tumor's living world to create non-linearly developing systems, i.e., tumor systems: In the course of evolution, the living world must be communicatively rationalized by the inclusion of situatively available or modified systems objects.

- **Basic mechanisms contributing to biological robustness** are
  - the steadily interwoven processes constituting the systems world and the functional world of systems objects,
  - the possibility to recourse on multi-facetted rationalization processes to fail-safe constitute normative notions,
  - modular systems features by which a decoupling from the physical-chemical world may be established that is based on the redirection of validity claims by communication-derived rules.
- **Communicative strategic interaction,** characterized by implementation of non-normative boundary conditions ('top-down' approach) is opposed by 'bottom-up' strategies aiming at knocking down single pathways, oncogenes etc. (Chap. 2, 22). Using 'top-down' approaches, physicians are 'systems participators' via biomodulatory drugs. Strategic communicative action with 'top-down' approaches differs formally but not content-relatedly from 'bottom-up' approaches: To be efficacious, both approaches have to redirect the tumor's normativity. Basis for strategic biomodulatory interventions may be the multifold possibilities to therapeutically criticize communication-associated validity claims: (1) The propositions of the meaning of communication lines and (2) the perlocutionary acts (in linguistic terms), that means, the recourse on available communication lines, systems objects, or rationalization processes for maintaining normative notions with the aim to establish robustness and homeostasis.
- **The incommensurability between structure-oriented or theme-dependent configured systems and the action-oriented or evolution-adjusted systems 'world' ('living world') can be overcome** with the perspective of a pragmatic communication theory. Thereby, theory and practice may be bridged. Now, non-linear dynamics, i.e., the spin-off of novel systems functions and novel rationalization processes within a tumor's living world, may be explained on the basis of communicative interactions between systems participators. Situational phenomenological facts (disease traits) can be more precisely communicated by identification and continuous monitoring of changing identities of systems objects. Modified or even changing identities and denotations are associated with a frequently decisively altered functional impact of respective cell systems. Secretome analytics or molecular imaging—as described in a formal pragmatic communication theory—may be helpful to outline those changes [46, 47].
- **Time-related processes:** The 'system' can be shared by systems objects and presents itself phenomenologically in a situational context, i.e., 'the visible' [48]. The particular meaning of intra- and intercellular communication lines is strongly context dependent, and the situative phenotype may be broken down into differential rationalizations of the functional cellular and holistic communicative systems world. Consecutively, the monitoring of time-related processes

(time-consciousness) must be imminent in biological systems. The operative interplay of functional and systems world could be a main target for generating time-consciousness in biological systems.

Examining the Validity of a Communication Act

Reconstruction and monitoring of presuppositions and validity claims, which are inherent to biologic systems and their communication processes, are inevitable because the inescapable prerequisites and foundation of a system's communication practice come into sharper relief.

Systems objects within a **biologic system may modularly change** their references depending on the holistic communicative context. Modular knowledge of systems objects and rationalization processes, that means, the highly variably arranged rationalizations of normative notions (inflammation, angiogenesis, etc.), represent the 'metabolism' of evolving biologic systems.

Modular knowledge of systems objects is grounded in a continuously moving and communicative change of validity claims, which have to be customized for experimental evaluation. The social cellular or molecular world is neither treated any more as a given (in a reductionist sense) and routinely processed procedure nor viewed as a predetermined procedure: Contingency programming, education, modular rearrangement, and novel rationalization of normative notions are characteristics of communicatively evolving biologic processes mediated by the respective systems objects [27].

Communicative actions of systems objects cannot be left behind, if we want to close the obvious gap between theory and practice, between the static 'historic' systems object with its references and the communicatively integrated evolving systems subject [26]. The holistic communicative systems world, i.e., the living world, is actively participating in the implementation of rationality into biologic systems. The presuppositions shared by those systems objects, which are involved in a communicative biologic process, are now taken to reflect and to uncover validity claims with the aim to bridge therapeutic theory and practice.

Everyday therapeutic practices, such as strategic communicative interventions for reaching a purposive understanding of the systems objects of a lesion and its host, and the therapeutic redirecting of reasons for pursuing the lesion's course of pathologic actions now acquire idealized rationality.

Communication has always to be viewed as rational. Communication could not occur if we do not assume that the communication acts mediated by the systems objects do not carry the dimension of validity, for which these participating systems objects are accountable.

Uncovering Communication-Related Rules Requires Novel Analytical Methods

The communication-based reconstructive analysis of tumor systems biology directly disembogues into novel possibilities of interpreting the phenomenological and, therefore, expressive site of clinical proteomics [47]. What does it mean that a pathway, a communication line, is being or becoming stimulated?

Validity claims have to be depicted step-by-step on both a clinical and an experimental level. The facticity of such validity claims is shown by the capacity of modular therapy approaches to induce continuous response in metastatic tumor disease by redeeming and redirecting modular knowledge. Novel patterns of biomarkers indicate functional changes in tumor-associated cell communities in response to modular therapy approaches (e.g., clinical proteomics), and novel modular arrangements on the cellular level are symbolized by communicatively-derived 'fragments' [31, 32, 49]. These novel patterns and modular arrangements give further hints of how to control systems-associated processes with therapy modules to achieve objective response [15] and to adapt biomodulatory therapies to situative developments in metastatic tumors [5]. Furthermore, they allow an evolution-based systems interpretation [6] of how they could contribute to novel and more realistic in silico models.

Examples of Criticizable Claims of Validity

The therapeutic accessibility of validity claims of systems objects is impressively shown by biomodulatory therapy approaches: Within tumor-associated communication tools, systems objects may be integrated in a multifunctional way:

- **Autonomous and non-autonomous portions of transcriptional activation** in tumor 'stem cells' are accountable for differential tumor phenotypes (glioblastoma) and visualize the intersubjectivity of communication [45]. The nature of cancer stem cells may be considered as a state rather than an entity [50].
- On the basis of the *facticity of prepositional aspects,* **tumor cell colonization** may lead to the complete destruction of non-regeneratory cell inventories. If 'traditional' organ-specific normative notions cannot be preserved, novel systems organizations gain some kind of autonomy by neutralizing separation towards previous cellular functions or by the assignment of new functions [27].
- **Modular therapies** may supplement prepositional aspects of communication, i.e., the presence of a tumor's living world by normative aspects, namely by therapy-derived 'yes' or 'no' statements ('know that') [40]. The therapeutic efficacy of biomodulatory therapies support the presence of a therapeutically accessible holistic communicative tumor system that may be specifically targeted [14].
- **It is necessary to decode paradox situations** of cellular rationalization and communication expression, i.e., to uncover inconsistencies, bottlenecks, and deformations within tumor cell compartments by means of a theory that includes

the evolutionary development of a tumor as well as its biologic history to increase therapeutic options through systems-directed approaches. Achilles' heels may be functionally described as decoupling systems and a functional 'world' of tumor cell systems [27].

Systems Objects in Dynamically Rearranging Biological Systems

Systems objects are communicatively linked benchmarks, which may be understood by studying physical, chemical, structural, and functional characteristics, irrespective of whether molecules, pathways, or cell communities are taken into consideration.

Beyond these systematically ascertained facts, we adjudge the systems objects' modular knowledge. The modular knowledge of systems objects is primarily a biologically unknown quality, which cannot be necessarily predicted from physical-chemical characteristics in non-linearly evolving systems such as tumors [3, 15, 31, 32]. Modular knowledge describes the capacity of systems objects to get involved in context-dependent and highly situative functional rearrangements, which may significantly alter the validity and denotation of particular systems objects.

Evolution-Driven Situational Status

An evolution-driven situational status is featured by a highly specific modular arrangement of numerous molecular species, pathways, multi-faceted functions of available structures, and rationalization processes. A non-linearly developing situational status, symbolized by novel modular combinatorial arrangements, finally necessitates the reinterpretation of the meaning of communication lines in an evolution-based communicative context [26, 51].

General interpretations of the rules that guide modular biological processes do not obey the same categories of refutation as general theories about physical and chemical interactions, thus per se remaining open for discussion. The logic of an explanations of rules redeeming the validity and denotation of systems objects within a communicative cell community is the result of a connection between a hermeneutic understanding and causal explanations [15]. The capability to interpret situative observations by communication-associated rules, for instance by utilizing biomodulatory therapies, represents a prerequisite for understanding a particular and sometimes unique systems stage, constituted by primarily non-predictable arrangements of systems components (on the background of multiple tumor-associated acquired aberrations) and specified functions.

Validity Claims and 'Corrupt' Activities

Communication-associated rules relieve us from the need to interpret morphologic-structural and physical-chemical interactions to delineate modular communication-linked features of systems objects. Now we can directly describe empirically-derived rules involved in implementing modular knowledge of systems objects. For this purpose, we may neglect physical-chemical interactions among molecules or cells. Of interest is the validity claim of a systems object, which is grounded in the formal pragmatic communication theory and depicted in novel analytical approaches including mathematical specifications of modules or functional 'fragments' [31, 32].

Unique tumor-associated rationalization processes can also be considered as strategies that allow systems objects and the respective modular arrangements to establish their 'corrupt' activities as justified, based on validity claims [52]. Tumor-associated rationalization processes are frequently preserved in multiple metastatic tumor sites despite the commonly observed heterogeneity of chromosomal and molecular-genetic aberrations in tumor cells [53; Chap. 2].

The Origin of a Communicative Impulse is Therapeutically Relevant

'Knowledge of the molecular profile of the tumor is necessary to guide selection of therapy for the patient' [2]. This claim by Schilsky is unequivocally correct, but substantially constrains the use of the myriad of molecular tumor-associated data on the concept, such as one drug for one major high affinity and high specificity target and multiple drugs for several targets [3, 54–56]. However, these approaches turned out to be highly selective with regard to their clinical efficacy and thus failed to offer a broad rational solution for the majority of metastatic tumor diseases (Chap. 2, 7, 15).

The question is generally left unanswered whether a single target, pathway, or tumor-associated cell type really expresses the same validity claim in a primarily unknown situative status of a tumor just as in any discretionary experimental setting. Molecular genomic patterns could also correlate with distinct validity claims of systems objects or rationalization processes in a respective tumor, thereby indirectly contributing to the design of modular therapy strategies.

Knowledge of the validity claim of therapeutically relevant systems objects and concrete rationalizations of normative notions may be now supplemented by knowledge about the origin of a therapeutically relevant impulse. Modulation of the prepositions of an impulse relevant to fulfill important tumor-associated normative notions may decisively attenuate tumor growth [15] (Chap. 23). Thus, the major origin of tumor-associated inflammation, angiogenesis, etc. gain center stage when defining novel starting points for therapeutic interventions [23].

Overcoming Robustness

Diagnostic and therapeutic methods for overcoming robustness may now focus on the basic mechanisms contributing to biological robustness. In future, clinical proteomics data for reconstructing communication expression of systems objects may highlight the confliction of systems world and functional world and the recourse of systems on multi-faceted rationalization processes to fail-safe constitute normative notions and on the modular constitution of systems. A robustness-oriented design of therapy schedules, i.e., the appropriate combinatory use of biomodulatory acting drugs, affords novel patterns of functional biomarkers to efficaciously guide combinatorial complexity on the basis of validity claims of respective systems objects [47]. Monitoring robustness-related systems processes could enable us to systematically specify methods for the combinatory use of biomodulatory acting drugs [57, 58, Chap. 23].

Discussion

The discussion about validity claims of systems objects positions familiar structures, cell types, pathways, molecular aggregates, etc. as communication-derived subjects. Physical-chemical interactions do not lose their explanatory strength, but communicative systems behaviors may also be depicted by scientific evaluation of additional and so far less regarded rules: In the first place, validity claims put communicatively linked systems objects in an evolutionary context. Otherwise, ('historic') preclinically raised references in terms of systems objects or known communication lines may now be linked to a distinct and potentially novel communicative expression, i.e., their situative meaning. In non-linearly developing systems, such as tumors, the situative meaning of molecular-biologic and morphologic detectable systems objects may be significantly altered [9, 10].

Validity claims and their therapeutic accessibility have achieved the status of facticity by demonstrating the clinical efficacy of modular therapy approaches and the modulation of communication expression that means redirection of systems objects validity and denotation. Validity claims of systems objects, based on communicatively-derived presuppositions within a particular systems context, position preclinically-derived references of objects as individual tumor systems subjects, which may acquire novel denotations in non-linearly evolving tumor systems.

The presented reconstruction of tumor-associated communicative processes for reaching understanding or generating meaning follows the theories of Habermas, whose communication technical explications are much easier to integrate into biological processes because of their pragmatic attitude and apparent experimental as well as therapeutic replicability [58–61], than, for instance, the explanations by Piaget and Charles Sanders Peirce (object sign, representamen, interpretant).

As yet, an exclusive reconstructive analysis of communication-derived validity claims is unusual in biology. However, validity analyses of systems objects in a situative, evolutionary-based context reveal the necessity to open up multifold

novel therapeutic options, particularly for further personalizing tumor therapy. As an instrument for analyzing, we applied a formal pragmatic communication theory. Aim of the analysis was to exploit the potential starting points for therapeutic interventions with regard to modular therapy approaches and to strengthen the application and interpretation of parameters derived from clinical proteomics, for example, secretome analyses for uncovering novel functional biomarkers indicating changes in communicative expression of systems objects.

The new diagnostic field 'clinical proteomics' has the capacity to develop methods, which assess seemingly familiar communication lines from the site of their communicative expression [47]. The meaning of communication lines is closely linked with phenotypically accessible functions or functional changes upon modular therapy approaches [15]. Such a methodological approach goes far beyond the appreciation of the syntactical modeling grammar as a prerequisite for describing a formal logical syntax [62] or a mathematical work-up of data within a targeted therapy database [63]. The goal of assessing communication-derived rules redeeming and redirecting the respective validity claims of systems objects is to generate hypotheses, which may be pragmatically integrated into novel tumor models that can be more efficaciously used for personalized diagnostics, combinatorial drug design, and novel in silico programs.

Knowledge about modular arrangements, diverse rationalizations of systems functions, the origin of a communicative impulse within a tumor system, the tumor-specific recourse on rationalization processes during systems perturbations (robustness), and probably the therapeutic altering of time-consciousness generated by biological systems (presumably by cutting off instigating signals or redirecting biological memory) allow completely novel insights into communicative determinants of tumor systems biology and facilitate therapy design, which is now also orientated at the communicative context.

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Part V
Evolution Theory

Chapter 12
Evolution Theory: Its Practical Relevance for Understanding Tumor Development and Specifying Tumor Therapy

A. Reichle and G. C. Hildebrandt

Abstract At that time the introduction of a cancer evolution concept, has failed to revolutionize cancer research. Models of rational reconstruction within an **evolution historical frame** can be suggested, if an innovative achievement may be denoted for a complex 'learning process'. Because such models admit a clear normative reference and action-theoretical interpretation; they may be used for narrative presentations. Three main factors emerged as starting point for **evolution theoretical considerations**, an unmet medical need (systemically pretreated patients with metastatic tumors), a hypothesis-driven vision (the formal pragmatic communication theory) and technological advances to pursue that vision (biomodulatory therapy approaches, clinical proteomics, epigenetics and molecular imaging techniques). An evolution theory allows for virtualizing the engagement to get experiences and decisions (pragmatic virtualization of communication acts) via implementation of non-normative boundary conditions (for example, biomodulatory therapies). The feasibility to virtualize the engagement to get situate experiences about tumor systems and decisions to tailor biomodulatory therapies (communication-derived tumor pathophysiology), the availability of an evolutionarily adapted modeling of cancer (cellular therapy in situ by adaptive therapies) will continue to increase our understanding of tumor pathophysiology and may contribute to an evolution-oriented design of systems biological strategies to diagnose and clinically manage tumor diseases on a novel personalized level. Basic science is getting directly involved in the reconstructive process, even though an approach has been established directed from bedside to bench aimed at implementing clinical practical care (adaptive trial designs) as scientific object in patient care.

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Introduction

A clearer understanding of evolutionary processes involved in the development of tumor growth and metastasis is essential for improving today's patients' prognosis by appropriate cancer treatment, for the efficacious implementation of cancer screening programs and for a better understanding of underlying tumor biology, particularly tumor systems biology. Experimental work suggests that a more comprehensive non-linear interpretation of gene-environment-interactions with integration of communication rules is needed [1].

Central questions may be at least partially answered by an evolution theory of tumors:

- Why are we trying so hard to identify 'universal' patterns of genetic alterations in cancer tissues, although we may identify restricted patterns of normative systems structures in tumors, i.e., rapid proliferation (acute myelocytic leukemia (AML)), dysplasia (myelodysplastic syndrome (MDS)) or apoptosis resistance (chronic myelocytic or lymphatic leukemia (CML, CLL))?
- Why do we not use available molecular-biologic technologies, particularly, cellular secretome analytics, epigenetics and molecular imaging, to systematically describe normative notions of tumor systems, which are featured by morphologic tumor structures, multifold rationalized action norms, and tumor-specific decision maxims (nodes, hubs)?
- Why do we still focus on communication lines and communication mediums (e.g., genes and specific steps in signalling pathways), although evolution is simultaneously characterized by evolutionary restricted communicative expression of communication lines based on differential rationalizations as well as by tumor-immanent normative notions?
- How useful is it, trying to transfer knowledge about timely and locally restricted validities and denotations of systems objects (objects' references, communicative expression), i.e., cells, oncogene-addicted pathways, etc. into completely novel evolutionary systems stages, which are characterized by the capability to establish novel stage-dependent communicative expression of tumor systems' objects? Evolving systems redeem modularly constituted background knowledge, which finally establishes communicative expression of systems objects (object-subject-relation).
- Why do we not comprehend respective tumor systems objects as being subjected to tumor systems-derived validity claims? The evolutionary mechanism of cancer is suggested to equally cover all cellular and molecular mechanisms.

From Evolutionary History to Evolution Theory

The introduction of a cancer evolution concept at earlier time has failed to revolutionize cancer research [2]. One important reason is the missing conceptual separation of an evolution history from an evolution theory. The two pillars, evolutionary history and evolution theory have to be separated according to their basic intentions.

Darwin recorded the history of evolution as a **continuous process of learning** to explain the spin-off of novel systems functions. This approach guides to Darwin's own universal system of 'evolutionary' science, a history of problem solutions during millions of years (Darwin C. On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. John Murray, London 1859).

Tumor development can be considered as an evolutionary process within a temporally circumscribed and assessable observation period: Cancer represents the largest genetic experiment ever conducted. Distinct acquired genetic lesions are not distributed at random in tumor cells, despite the high variability of cancer causes, the heterogeneity of observed genetic aberrations, and the divergence of morphologic characteristics of diverse tumor types. The non-random distribution of genetic aberrations might be explained by the fact that cancer-associated dysregulated transcription factors (non-oncogene addicted factors) must still collude in a life-maintaining manner for cancer cell self-renewal, for proliferation, and for the build-up of a cellular infrastructure suitable to maintain normative notions for tumor promotion [3]. But how can we use multifaceted and scientifically well proven 'narrative' presentations of classic evolution models to describe tumor pathophysiology? How can be an evolution theory successfully implemented to design novel therapy strategies for metastatic tumors? Are evolution-adjusted pathophysiological considerations suitable to open up novel paths of heretofore unexplored therapeutic options?

Evolutionary History

Evolution History and Tumor Pathophysiology

According to evolution historical considerations, evolution is represented by 'narrative' presentations. In 'narrative' presentations, theoretic knowledge—mainly reductionist and contextualist derived—is solely intentionally used, but primarily not organized. At best, the benchmarks are scientifically proven, i.e., 'genetic progression', 'facilitated variation', and 'genome theory' etc. [2, 4, 5]. Consequently, a tumor system develops on the basis of 'narrative' presentations oriented at suggested normative notions, for example 'adaptation', 'learning', 'contingency programming', 'instigation', and 'spin-off of novel systems functions'.

Evolution historical descriptions on tumors are representing action-associated knowledge, derived from scientific experiments and clinical data, acquired in distinct evolutionary constrained systems, for example in vitro systems, animal or clinical models. 'Narrative' normative references, which necessarily constitute the frame for respective considerations, do not exclude any scientific object from the daily experience [2, 6–10].

Competition about normative references is standing to reason as mechanism for decision making. Such a particular communicative procedure has been delineated as universal principle from 'narrative' descriptions. Selection processes are

assumed to be initiated for accomplishing normative notions, for example 'self-interest', 'supply', 'demand', or also phrased the 'invisible hand' [11]. Selection of a distinct normative benchmark ensures the particular character of each description. The position of a comparative event is occupied by a normatively characterized state of equilibrium (homeostasis), which a distinct system 'selected' for resolving a particular problem.

Success and failure are mirrored in processes of competitive interaction. These interactions represent attempts to resolve problems and are described on the basis of a suggested underlying matrix facilitating learning processes. 'Learning' systems subjects, i.e., cells, pathways, genes etc., are obviously able for 'innovative solutions' of particular problems. Frequently, arbitrary and not scientifically proven normative notions serve as benchmarks for competition and selection.

On the background of evolution historical considerations tumor development may be considered as a continuous selection process:

Accordingly, cells in pre-malignant lesions evolve by natural selection [2, 8, 9, 12, 13]. This is suggested to account for how cancer develops from normal or molecular-genetically altered to malignant tissue. This multi-step process of tumor evolution serves to explain the difficulty for achieving cure, especially in the metastatic stage.

The reductionist model relies on three necessary conditions for the procedure of 'natural' selection, all of which may be found in tumors [12, 14]:

- Genetic variation in the tumor cell population [15]: Neoplastic cell populations may present as mosaics of cells with both different genetic and epigenetic changes that distinguish them from normal cells. Mutations arise irrespective of the current adaptive needs imposed by the environment [16].
- The novel acquired genetic variations may not compromise heritability [13]. When a cancer cell divides, both daughter cells inherit the genetic and epigenetic abnormalities of the parent cell, but may also acquire new genetic and epigenetic abnormalities due to genetic instability [7].
- Each genetic variation must affect survival or reproduction (fitness) [14]. While many of the genetic and epigenetic abnormalities in neoplasms are probably less contributing to evolution, others have been shown to alter important normative notions, aiming at increasing the proliferation rate of the mutant cells, or at decreasing the rate of cell death (apoptosis).

The evolutionary mechanism of cancer can be descriptively comprehended as a multistep event corresponding to the chosen and scientifically accessible normative frames. Multifaceted starting points for descriptions of evolutionary processes can be chosen, as evolution historical descriptions are representing action-associated knowledge [2, 17]: Multilevel selection processes; stress-induced genome system instability (the diverse causes of cancer) [7, 18]; 2-hit hypothesis for mutation [19, 20]; genetic instability and natural selection [10, 21–23]; loss of heterozygosity; somatic evolution in progression, based on a series of genome system replacements [24]; senescence [25]; genetic heterogeneity in neoplasms [26]; somatic evolution by epigenetics [27]; clonal expansions [28]; phylogenetic analyses [29, 30]; adaptive

landscapes [31, 32]; the hallmarks of cancer may be considered as evolutionary adaptations in a neoplasm [33].

Reconstructive efforts on the basis of evolutionary historical considerations have to deal with incommensurable scientific levels and with 'learning' processes. All these reconstructive approaches necessitate hierarchical and unidirectional evolutionary processes as the 'metabolism' of evolution [34]: Evolution can be assessed with rationally reconstructible patterns, which correspond to a hierarchy of more and more complex structures. Just this hierarchy is raised to question when communicative processes are considered as a valid basis for explaining developmentally founded tumor processes.

Models of rational reconstruction within an evolution historical frame can be suggested, if an innovative achievement may be denoted for a complex learning process. As such models admit a clear normative reference and action-theoretical interpretation; they may be used for narrative presentations. Accordingly, the history of evolution is rich in descriptions, which may serve as example for rational reconstructions of evolutionary processes. In all cases the reconstructions bargain for narrative presentations despite of the underlying rational models, as they tell us about 'attempts to resolve problems' in an evolutionary context.

The genome theory of cancer evolution, for example, introduces networking interactions of genes driving tumorigenesis [35]. However, with an exclusively functional consideration 'rewiring makes the difference' [36], the systems-associated constrictions of gene and cell functions, which take place in cell systems during evolution, are misplaced from the perspective of an observer to the level of communication by tethering inter-systemic exchanges at imbalances in communication. Thereby, the importance of the identity-threatening deformation of tumor systems is withdrawn [3].

Multifaceted combinations of molecular mechanisms, available alternative pathways and compensatory changes of protein expression can result in unmanageable complexity. Therefore, it is important to move research from the characterization of individual molecular (genetic) mechanisms to the understanding of the overall system behavior during cancer evolution. Particularly, the gene-centric thinking is moved to genome-centric thinking (genome theory) [35].

Current systems biological considerations rely on studies of basic science, which primarily try to disassemble complexity and measure the activity of isolated systems components in a distinct evolutionary context. Such an approach is very successful in characterizing the individual parts but very limited in reconstructing and predicting how single components are communicatively integrated and rededicated within novel systems contexts (modularity): Depending on the host, the developmental status, and the particular systems contexts, genes and their gene products may have completely different, sometimes opposing functions. A prominent example is p53 [37]. Obviously, the communicatively linked biochemical or cellular background may define validity and denotation of distinct systems objects, for instance in case of transcription factors. The term 'oncogene' does not cope with the evolutionary or therapeutically induced function of a distinct gene.

Evolution History and Tumor Therapy

The necessity for moving from evolution historical narrative to evolution theoretical considerations (evolution theory) for bridging theory and therapeutic practice may be exemplarily highlighted by common observations revealing discrepancies between therapeutic theory and practice [38]. Phrasing obstacles for translational research based on evolution historical considerations in the clinical field allows focusing on issues that have to be covered by novel hypothesis-triggered methodologies (evolution theory). At this stage, a formal pragmatic communication theory points up to the reconstruction of communicative relations among systems objects (pathways, molecules, cells, etc.) [39].

- Clinical trials do not need to unanimously re-confirm the non-transferability of reductionist, context-dependent knowledge (evolution historical knowledge) to completely novel evolutionary systems contexts in metastatic tumor. Doubtless, systems objects and communication lines, as the benchmarks of communication, may have one striking common feature in various preclinical systems contexts or in novelly evolving tumor systems: A therapy-relevant systems object may be successfully proven, quantitatively and qualitatively, including all its variations, up- or down-regulations, and molecular modifications. However, targeting a specific molecule with the scheduled 'targeted' drug or drug combination may lead to differing or unexpected results in tumors with different evolution history [40, 41]: Multifaceted chromosomal or molecular-genetic aberrations, in tumor cells, but also in stroma cells, ultimately determine the communicative expression, i.e., the meaning of systems objects, in a therapeutically relevant way.
- **Nonlinear responses** of differentially developing tumor systems are a well-known phenomenon: Philadelphia positive chronic myelocytic leukemia may be livelong controlled in more than 60 % of patients by inhibition of chimeric tyrosine kinase [42], while additional aberrations in CML disease often result in loss of disease control by respective targeted therapies [43]. Sorafenib, another tyrosine kinase inhibitor, is weakly active in combination with chemotherapy in Flt-3 positive acute myelocytic leukemia (AML), but administered as a single drug, it may induce continuous complete remission in patients with Flt-3 positive AML, relapsed after allogeneic stem cell transplantation [40, 41; Chap. 7]. Exclusive targeting of tyrosine kinases in a distinct evolutionary systems context may lead to a complete redirection of a systems' normativity in Flt-3 positive AML, relapsed after allogeneic stem cell transplantation: The systems' perturbation depends on the kinases' communicative systems context.
- Data derived from **high-throughput arrays** or *in silico* approaches contribute to uncover novel therapy approaches, for example combined single-track therapies ([44, 45]; Chap. 2): Their maximal beneficial yield has not yet been reached. To overcome problems with combined single-track therapies, it is necessary to process quantitative proteomic data from appropriate hypothesis-driven models. Evolution-adjusted tumor pathophysiology provides the necessary diagnostic instruments for modeling drug effects [39, 44, 46–49] by introducing

the reconstruction of tumor-promoting rationalization processes. Rationalization processes represent the multidimensional networks available for constituting tumor-promoting normative notions, i.e., angiogenesis, immune response etc.

- **For the long-term therapeutic management of metastatic tumors**, it is important to formally establish the therapeutically accessible, evolutionary restricted operative scope of tumor systems by using appropriate diagnostic steps (cellular secretome analytics, molecular imaging) [50–52]. Reconstructing systems objects' validity claims, that means assessing the communicative expression of systems objects within a distinct evolutionary context, is feasible and enables to better understand and target the communication-derived tumor pathophysiology. The available novel diagnostic repertoire will facilitate the appropriate selection of technical instruments for successful biomodulatory interventions, defined as cellular therapies in situ.

The operatively accessible communicative benchmarks are endogenously developing, but also—as shown—accessible for therapeutically evolving normative systems features (Chap. 19). The communicative benchmarks in tumors are established by a broad spectrum of acquired rationalization processes, which are provided for establishing a pattern of tumor-type and stage-specific normative notions.

As shown in clinical trials, attenuating tumor growth can be realized by biomodulatory therapies via implementation of non-normative boundary conditions into the tumor's normative systems structures [46]. Biomodulatory, primarily multi-track therapies are focusing on the evolutionary derived systems stage and are not primarily oncogene-addicted or theme-dependent targeted therapies, such as single- track ('bottom-up') approaches (Chap. 22).

The two methodological pillars, either reductionist or holistic procedures, for creating evolution-adjusted tumor models are supplementary as the benchmarks of communicative systems correspond to the components of which functional sequences are composed. From different methodological viewpoints, the total extensiveness of tumor pathophysiology may be highlighted only now and in such a way that would be desirable for the development of **one individual tumor therapy (personalized tumor therapy).**

However, by exclusively using evolution historical considerations, we cannot obtain the conceptual equipment for action-theoretical abstractions (biomodulatory therapies), for the assessment of systems-associated tumor stages or for the systematization of rationalization processes (communication-derived pathophysiology) based on an adequate differentiation between:

1. Synchronous structural differentiations of the functional 'world' of tumor-associated cell systems,
2. The spin-off of functional systems that are differentiated via chemokines and cytokines as well as the interior differentiation of these cell systems (e.g., accumulation of regulatory T-cells, mesenchymal stem cells), and
3. The differentiation processes induced by tumor cells, which simultaneously dedifferentiate differentiated cellular functional areas (rationalization of functions) in terms of a colonization of the functional 'world' of organ tissues (metastatic

process), simultaneously facilitating the integration of new cellular elements from the peripheral blood (mobilization, trafficking) [3].

Evolution Theory

The following evolution theory is based on the assumption, that biological processes are interwoven with communication and are represented and reproduced through communication acts to facilitate communicative expression [39]: A tumor system not only consists of diverse cell types and pathways, the so called tumor systems objects, but also comprises all components of action insofar that these components are oriented in terms of diverse cell types. The components of action are organized in communication acts.

Communication within a biological system is closely linked to the descriptively accessible 'learning' processes, to contingency programming, adaption of the multifold 'players', i.e., the systems objects within a tumor system [3]. An evolution theory should operationalize the 'metabolism' facilitating the spinoff of novel systems functions and is aimed at covering some practical, i.e., diagnostically and therapeutically relevant issues to convince the scientific community that the evolution concept is under-appreciated in the cancer field, both for diagnostic and therapeutic issues.

For many diseases, such as metastatic tumors, that have undergone umpty years of evolution, stepwise and evolution-adjusted therapy may be an alternative way to achieve medical improvement rather than drastic therapeutic interventions based on theme-dependent knowledge [53]. Thus, it is necessary to decode paradox situations of cellular rationalization, deformation, and communication processes or, in other words, to uncover inconsistencies within tumor cell compartments or distinct topologies of aggregated action effects.

Experimental Evidence for Communication Processes as Essential Part of an Evolution Theory

The following experimental and clinical data favor a **formal pragmatic communication theory** as a major element of an evolution theory into tumor pathophysiological and therapeutic considerations:

- **The distribution pattern of metastases** for solid tumors is not random. In 1889, Paget analyzed for the first time metastatic spread in autopsies from breast cancer patients and proposed that particular cancer cells or 'seeds' would only colonize receptive 'soils' [54].
- **The metastatic pattern:** A mathematical analysis, performed by Medicare in the U.S. on the basis of claims from over two million elderly American patients, enabled to reconstruct network models to analyse progression dynamics of cancers, based on their sites of origin. These networks were sufficiently robust to make

retrograde predictions of primary histological tumor types, given a metastatic pattern, and anterograde predictions of future sites of metastasis, given an individual primary site [55].

- **Besides hemodynamic factors, vascular and lymphatic drainage patterns** of a given primary site, additional 'forces', are implicated in directing tumor spread [56].
- Results on the molecular mechanisms underlying metastatic tropism seem to support the concept of the **'metastatic niche'** [57].
- **Autonomous and non-autonomous portions of transcriptional activation** in tumor 'stem cells' are accountable for differential tumor phenotypes and visualize the intersubjectivity of communication during tumor development (MDS, AML) [58, 59]. The nature of cancer stem cells may be considered as a state rather than an entity [60].
- **On a genetic scale**, each tumor presents a form of disease never encountered clinically before [61]. Despite the acknowledged tumor heterogeneity on a genetic scale, tumors are supposed to become 'eradicated' by targeting distinct non-randomly occurring oncogene-addicted events [38]. Efforts for personalizing tumor therapy are propelled by meeting genetic heterogeneity with selected single-track or combined single-track approaches (Chap. 22). Discounted is the normativity of cancer tissues, which share substantial phenotypic similarities (normative notions and corresponding rationalizations), despite the fact that the genetic paths to particular phenotypes are highly heterogeneous.
- **Convergent evolution** [61]**:** In cancers, distinct normative tumor-associated benchmarks promoting evolutionary processes are enabled by a large number of diverse, but non-random genetic changes [62]. Cancer diseases show convergent evolution for constituting tumor-immanent normative notions, as indicated for example by 'chronic' or 'acute' diseases: Cancer cells develop from all tissues, and the genetic basis is rather heterogeneous. Nevertheless, cancer tissues exhibit a scientifically accessible tool of reconstructible tumor-immanent normative notions (structures, functions and decision maxims), which are constituted by a wide range of rationalization processes. The multifaceted rationalization processes are frequently based on evolutionary mediated shifts in systems objects' validity and denotation. Evolutionarily altered validity and denotation seems to be the main reason for the frequently observed poor therapeutic accessibility of tumors treated with reductionist therapy approaches ('bottom-up' strategies).
- **Understanding fundamental properties of non-hierarchically organized operations in malignancies** is a crucial step for providing insights into novel therapeutical approaches [63]: Rubin and Raaijmakers reported changes in skin and bone marrow fibroblasts prior to the onset of visceral cancers and myelodysplastic syndromes/acute myelocytic leukemias, respectively [58, 64]. The results of these studies suggest that a 'systemic event'—representing a reason for an evolutionary opportunity within the tumor's 'living world'—may provide the first step for carcinogenesis (Chap. 23). The description, that interactions of cell autonomous and microenvironmentally determined events support the development of malignancy, e.g., during the evolution of myelodysplasia and consecutive acute

myelocytic leukemia, points to a communicative aspect [58]. This murine model of leukemogenesis also suggests non-random aberrations in mesenchymal cells as cause for tumor induction in heterologous cell types (hematopoietic cells).

- **Mathematical descriptions**, which aim at studying and designing the dynamics of tumor cell escape from selection pressures (intrinsic drug resistance), are hypothetically based on multi-type (Darwinian) branching processes [65]. These theories neglect the dynamics of reciprocally systems objects-intended criticizable validity claims [66]. For establishing normative notions, the frequently applied game theory decisively restricts an action-oriented theory, which is aimed at reaching 'understanding' [67].
- **An important observation contradicting the Darwinian selection processes** ("selection of the fittest") describes how evolution-promoting processes (genotoxic stress) are translated into digitalized, reproducible genomic structures in prostate cancer cells [18]: Novel findings elucidated several unexpected general principles for non-random chromosomal translocations in tumors. 'A long-standing concept in tumor translocation has been that genotoxic stress causes direct random double strand breaks (DSBs) that lead to random translocations, with the 'selection' of those conferring growth advantages. By devising and investigating a model of tumor translocations that fully mimics the frequency of in vivo events without proliferative selection', Lin et al. suggested that 'there is a site-selective immediate pattern of DSBs that ultimately 'dictates' the pattern of tumor translocations' [18].

Most studies on somatic cell evolution are limited to the level of genes, their variants and their expression levels. Such single gene analyses, likewise whole genome analyses, cannot per se assess how single aberration patterns collude in a life-maintaining fashion as prerequisite for a **'macroevolution'** that is suggested to drive cancer evolution [23]. The common, but highly heterogeneous patterns of 'genome system replacements' during tumor evolution support the concept that karyotypes define tumor systems; and that karyotypic evolution is a key event in cancer evolution.

However, an equivalently important evolutionary key event is the fact that histologically different cancers exhibit **convergent evolution** for constituting distinct normative systems features. Convergent evolution is facilitated by multifaceted rationalization processes supporting and maintaining phenotypically characteristic normative notions. The observation of multiplicity in acquired genetic aberrations and a comparatively restricted tool of rationalization processes for constituting convergent evolution pioneers the way for a formal pragmatic communication theory [39]: Acquired chromosomal aberrations are communicatively assigned for their situative validity and denotation within an evolutionary confined system by the holistic communicative context (tumor's living world'). The systems-mediated, therapeutically relevant reference of a systems object is not necessarily predictable from available systems objects' 'historic' references (evolution history) [18]. Further, the fact, that validity claims of systems objects are therapeutically criticizable by implementation of non-normative boundary conditions (biomodulatory therapies), demonstrates evolvability of evolutionary constrained systems levels.

Benchmarks of a Communication Theory as Essential Part of an Evolution Theory

Therapeutically efficacious access to metastatic tumors by combined modularized therapies (Chap. 2), emerged as a trigger for the problematization of established tumor models. Traditional models are based on reductionist or contextualist interpretations of metastatic tumors. However, these models may not explain the observed and therapeutically relevant activity of biomodulatory therapy approaches, which include drug combinations with only poor single agent mono-activity or none at all [46]. The routine reductionist perception of metastatic tumors lost its conversance and universal validity [66].

The presented evolution theory is based on observations derived from the successful implementation of biomodulatory therapy approaches for therapy of metastatic tumors. The resulting formal pragmatic communication theory connects all acquired data, provides an explanation that covers all gathered facts about convergent evolution and provides the basis for predictions [68].

Problem solution, which is orientated at suggested normative references (selection), is now contrasted by an evolution theory describing the 'metabolism' of evolution in form of communication-associated rules [69].

By introducing a pragmatic communication-theoretical approach, the intentionally defined normative notions, at which selection processes slave away, are resolved in equivalent communicative rules bent on the respective systems objects. Now, the socially interwoven tumor and stroma cell community evolves as a holistic communication-driven structure, which provides internal access, for example via modular therapy approaches. Thereby, systems disclose their modularly designed architecture and recon tumor tractability via modular structures [68].

Pragmatic Virtualization of Communication Acts

Modularity (Object-Subject-Relation)

In the formal pragmatic communication theory modularity describes pragmatically the object-subject relation, which is constituted between the two poles, the systems objects' functional world and the respective biological system's world [66]. Clinical efficacy of biomodulatory therapy approaches (combined administration of drugs with poor or no monoactivity) may be explained by evolvable modular systems structures bridging the requirements of a systems object ('historical' reference) and the communicative systems context [70].

Now, modularity is more broadly defined as an inherent feature of each systems object. Modularity does not describe rationalization aspects (structural and functional organisations of normative notions) to comprehend for example pleiotropy,

heterogeneity by constituting variational 'modules', functional 'modules', and developmental 'modules' [71].

A module, in the current understanding of the formal pragmatic communication theory, allows comprehending the communicative expression of a particular systems object and is part of a rationalization process for a distinct normative notion. In so far, 'modular structures' may facilitate evolutionary development [72]. Cellular functions, such as signal transmission or cell cycle control, are carried out by 'modules' made up of small networks, which are composed of numerous interacting molecules, which determine the systems participator's communicative expression.

Modularity, which places systems objects as situative subjects, implicitly imparts a certain degree of evolvability to systems by allowing specific modular features (i.e., modular communicative networks) to undergo changes with regard to validity and denotation of systems objects without substantially altering the functionality of the entire communicative system (robustness of the tumor's living world). Modularity may allow the retrospective establishment of spaces for evolutionary developments if modular events (therapy) are implemented. This way, the tumor's living world turns into a scientific object that becomes accessible for experimentally or therapeutically designed modular approaches for uncovering the tumor's modularity, the modular knowledge of each systems object [39].

Modularity of cells and cell systems is a ubiquitous intrinsic biologic dimension, which becomes of exceptional interest during evolutionary processes, for example during tumor growth. It may establish multi-functionality and evolvability within a holistic communicative tumor cell system. Modularity either descriptively (modular therapy approaches) or mathematically seizes the phenomenon that the various, sometimes even opposing references of the systems objects are interrelated situational biological stages, i.e., they are embedded in the communicatively arranged validity and denotation of systems objects [53].

Proteins are traditionally characterized based on their individual action as enzymes, signaling molecules, or structures constituting specific aggregates in cells. At this stage, the post-genomic view expands the role of proteins as an element within a network of communicative interactions [73, 74]. A more abstract term for a protein—in a communicative sense— is 'systems object', which acquires contextual functions within circumscriptive functional modules or within the holistic communicative network of a tumor system [75].

Cell communities and cells constitute themselves, alternating in a close modular response to informative processes (biomodulatory therapy, gene transfection etc.). Therefore, modular communication is usable as an internal systems-relevant and environmental communication mode: The evolutionary link between two different 'worlds' may be successfully constituted by a formal pragmatic communication theory defining rules and evolutionary constraints.

Background 'Knowledge'

Background 'knowledge' reassures systems robustness as illustrated by recovery from reductionist therapeutic interventions for tumor control. Tumor's robustness

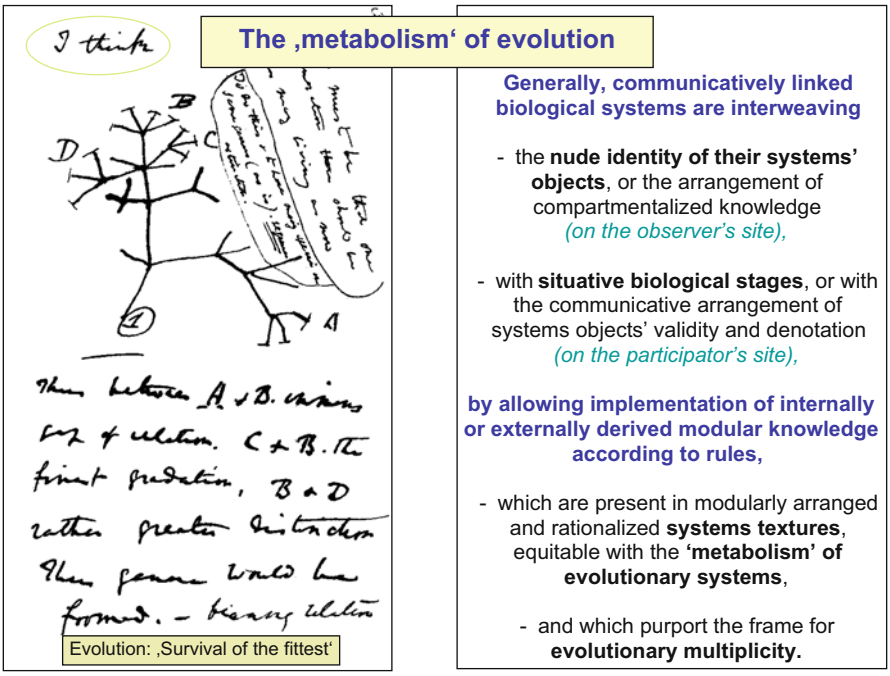

Fig. 12.1 Charles Darwin's 1837 sketch, his first diagram of an evolutionary tree from his First Notebook on Transmutation of Species (1837). Within reductionist considerations selection processes are indispensable. Modularity and rationalization processes, as discussed in a formal pragmatic communication theory, are sufficient to operationally define evolvability, which includes failure, fallacies, inconsistencies and rationalization processes. Tumors equip a biologically possible validity pegged to systems objects with the strength of facticity ('corrupt' rationalizations) under the conditions of a perceivable incompatibility between facticity and validity. Between these conflicting priorities the tumor disease is unfolding and 'branching'. Such a communication based definition of the term tumor disease refers to the polarization between success- and integration-orientated behaviors in biological systems

may be specifically responsible for poor therapeutic outcome, and robustness may absorb severe therapy-induced toxicities in a patient's organism. Thus, as our idealizations reach communication competence, the cells' explicit knowledge, which relies on idealizations (theme-dependent context knowledge), and the risk-absorbing knowledge of the tumor's living world (mediating robustness and systems context) compete in the range of the background knowledge about the tumor's living world [39] (Fig. 12.1).

The Tumor's Living World

Tumors are characteristically composed of functionally rather heterogeneous cell populations, i.e., tumor and stroma cells. Despite the ostensible morphologic heterogeneity of these cell populations, clinical trials using biomodulatory therapy

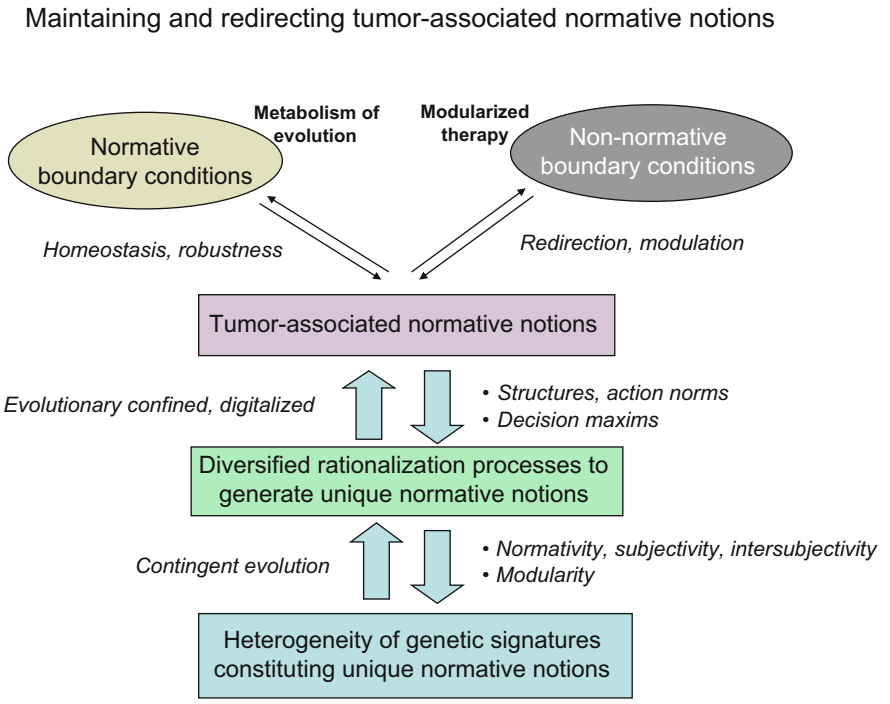

Fig. 12.2 For many diseases, such as metastatic tumors, that have undergone umpty years of evolution, stepwise and evolution-adjusted therapy may be an alternative way to achieve medical improvement rather than drastic therapeutic interventions based on theme-dependent knowledge

approaches have shown that these heterogeneous cell communities constitute a holistic, therapeutically accessible communicative entity [66]. Although this seems to be a contradiction at first, holistic communicative processes—termed the tumor's 'living world'—turned out to be a novel scientifically and therapeutically accessible object offering insights into evolutionary processes. Biomodulatory therapy approaches bring transparency into holistic communicative systems by breaking into a tumor's 'living world' and by dissecting a tumor for practical purposes, such as the attenuation of tumor growth (normative notion), in comprehensible evolutionary processes [39] (Fig. 12.2).

The 'living world' of the tumor provides the background knowledge, as it comprises the tumor's holistic communication processes, which we rely on in every therapy. The living world of morphologically defined tumor cell systems creates the term opposite to those idealizations, which originally constitute scientific (intentional) knowledge. The living world is uncovered by redeeming the validity of communicative tumor processes through implementation of modular knowledge of cellular and external environments (for instance for therapeutic requirements). Only experimental or therapeutic experiences (modular therapies) allow the separation of the tumor's living world into categories of knowledge.

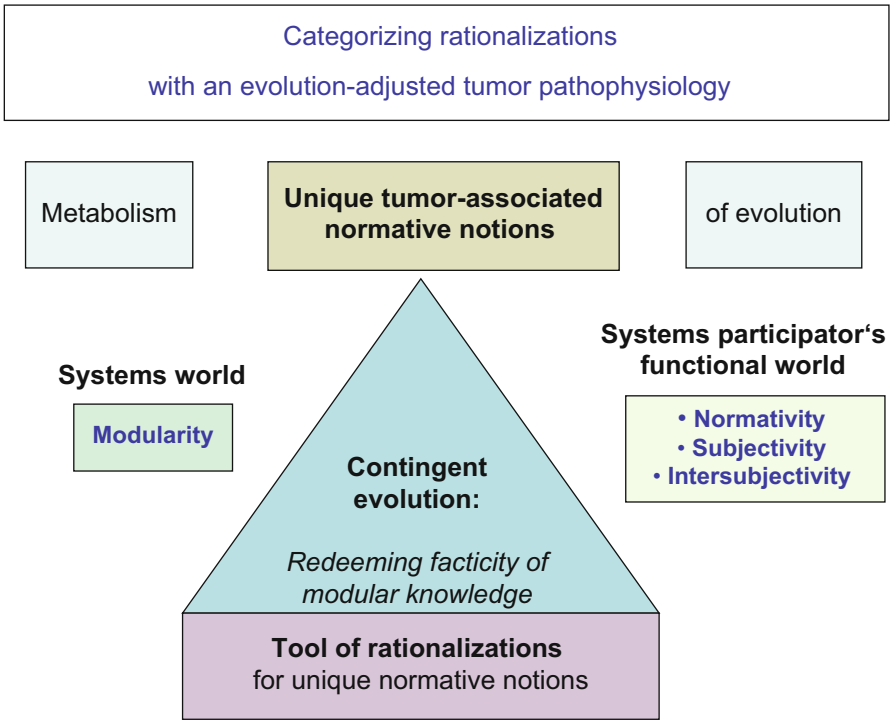

Fig. 12.3 The present evolution theory is based on the assumption, that biological processes are interwoven with communication and are represented and reproduced through communication acts to facilitate communicative expression. Rationalizations of normative notions are configured between the poles of systems world and systems participators' functional world with their respective communication derived rules

The newly uncovered systems perspective, which is frequently underestimated, moves its focus to the discrepancies that develop between the functional world of tumor-associated cell systems and the functional requirements imposed by rationalization processes and triggered by a tumor's systems 'world'. The (therapeutic) exploitation of background knowledge about the tumor's living world contributes to disrupting the holistic communicative thicket.

Perception of Validity

A significant difference exists between a communication medium (e.g., ion channels, molecular pathways, signaling integrators, cytokines, chemokines) or communication lines (e.g., gap junctions, signaling pathways, nerves) and the underlying communicative expression (purpose). Communication mediums and communication lines are assessed according to how well they technically work with regard to communication, whereas communicative expressions are evaluated according to their communicative validity (Fig. 12.3).

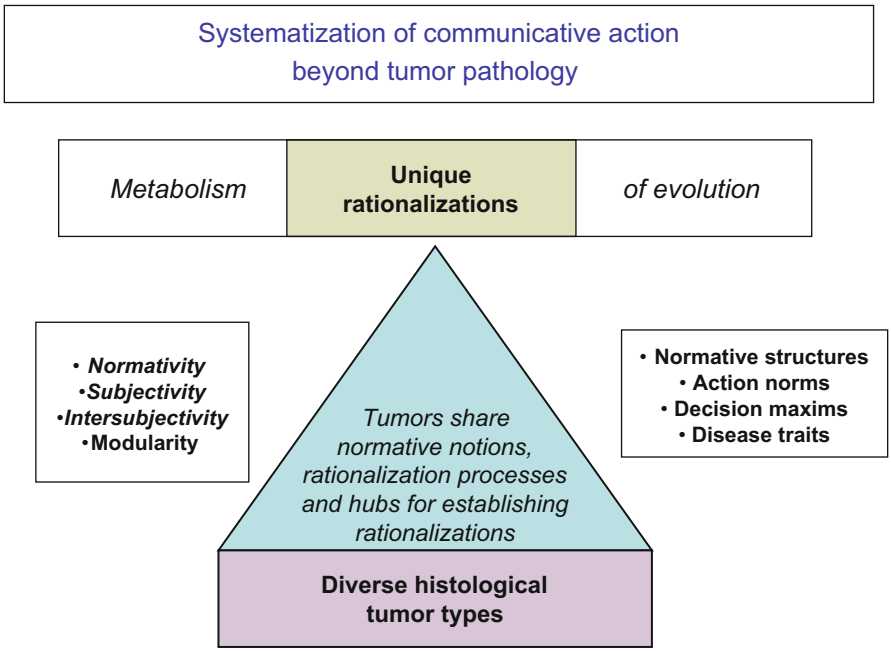

Fig. 12.4 Tumors share normative notions, rationalization processes and hubs for establishing rationalizations. Therefore, a novel categorization of histological tumor types is possible (evolution-adjusted tumor pathophysiology) according to shared tumor-associated normative notions and corresponding rationalization processes, evolutionary confined modular knowledge of systems participators and adaptive intersystemic exchange processes

Communication mediums and communication lines are easily accessible and comparable among rather different biologic systems. The reconstruction of their situative communicative validity and denotation—particularly in pathological circumstances (metastatic tumors)—necessitates further studies. These investigations should include not yet routinely operated methodologies, so that a distinct communication tool of interest can be assessed within its situational context.

Specific Conditions of Compliance Aimed at Redeeming Validity

Specific conditions of compliance aimed at redeeming validity are facilitating relations between communication technique (specified modular therapy approaches) and distinct tumor-associated situation-engraved systems stages. A holistic communication-based model now opposes reductionist systems views. The tumor's living world is, for example, uncovered by redeeming validity of communicative tumor processes through the implementation of modular knowledge in the cellular and external environment (for instance for therapeutic requirements): The tumor's entire communicative system is subjected to modular interventions pursuing the integration of complex biochemical systems processes (Fig. 12.4).

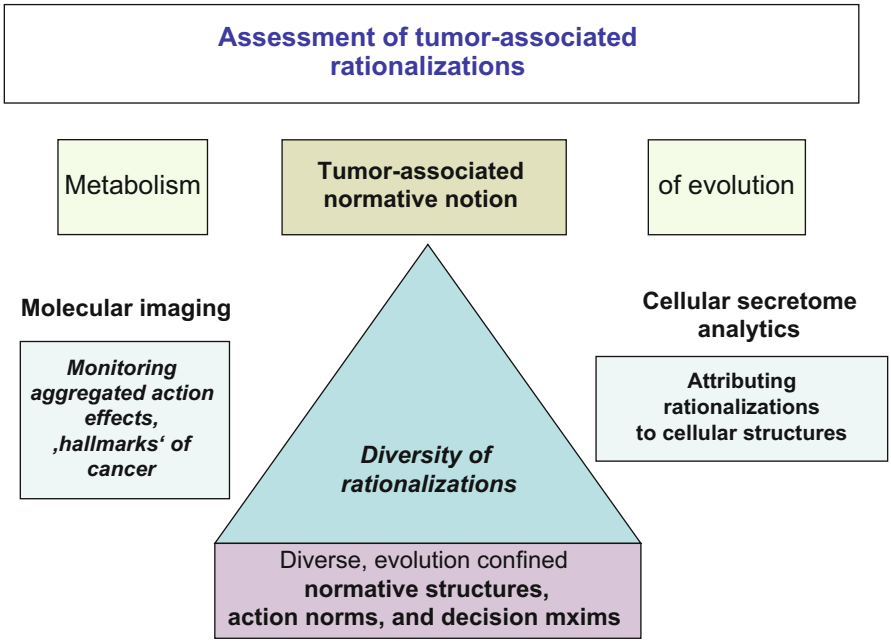

Fig. 12.5 Multifold technologies may be merged in a novel diagnostic setting to study the constitution of situative rationalization processes

Prepositional Communication Acts

Prepositional communication acts attribute validity and denotation to systems objects, constitute modules together with respective systems participators, and define the communicative expression of systems objects. The evolutionary communicative status is prerequisite for purpose-oriented (e.g., lineage fate, cell function) as well as communicative activities within the 'living world' [76].

Normative Biological Systems Structures

Normative biological systems structures include (1) cellular structures (morphological cellular and extracellular structures, including molecular-genetic or genetic aberrations, and modules), (2) compartmentalized action norms (diverse structures promoting angiogenesis, inflammation, immune response, robustness, cell death executing mechanisms, evasion of immune surveillance, glycolytic production of ATP also under aerobic conditions, the Warburg effect, compromised cell death programs, self-sufficiency in growth signals, tissue invasion, metastatic potential, limitless proliferation, stress phenotypes, such as metabolic, oxidative, mitotic, DNA damage stresses etc.) (Fig. 12.5), and (3) decision maxims (nodes, hubs) [33, 68, 77].

Of particular interest for the **preservation of normative systems structures** is the continuously proceeding process, through which internally- or externally-derived modular knowledge is implemented during the communicative exchange with the environment. The resulting situative communication profile enables—according to communicative rules—a steadily moving but distinct configuration of systems objects' validity and denotation, which is aimed at (1) maintaining robustness on the basis of definitely rationalized biological systems or (2) at rationalizing the tumor's living world to create non-linearly developing systems, i.e., tumor systems. In the course of evolution, the living world must be communicatively rationalized by the inclusion of situatively available or modified systems objects. Normative contexts limit the number of relations between the systems objects.

Rationalization of Normative Notions

In biological systems, modularity and rationalizations organize normative systems structures and interfaces for intersystemic exchange [66].

Rationalizations describe how normative notions of biological systems are temporally, structurally and functionally constituted (e.g., inflammation, angiogenesis etc.) and organized (intersystemic exchange processes) to achieve normative benchmarks. Purposes are enmeshed in rationalized 'life-forms' of communication-driven cell systems in a way that we cannot oppose or circumvent them (Chap. 23). A broad pattern of genetic changes and multi-faceted rationalizations converge to a limited pattern of normative tumor systems structures: Convergent evolution via rationalization of normative systems structures is a ubiquitous phenomenon [62].

In an evolutionary process, tumor cells may exploit the whole extent of the rationalization features of stroma cells to implement the functional diversity of systems behavior aimed at maintaining homeostasis and robustness in tumor systems. The implementation of a new form of integration (rationalization) of stroma cells allows the evolutionary advancement of the systems complexity with the remodeled rationalization of cellular functions: The diversified resources of tumor growth-promoting cytokines are distributed among rather different stroma-associated cell types (redundancy).

Tumor- and stage-specific therapeutic accessibility of normative processes to induce response in histologically rather different tumor types indicates differential integration of normative notions into the context-dependent 'living world' of tumor compartments and corresponding tumor-specific rationalization processes. For example, inflammation-related activities are communicatively promoted and differentially adapted during tumor evolution. Empirically, differences may be detected in the modalities of developing evolutionary systems and in the acquired functional impact of inflammation-related systems. Biomodulatory therapies, administered as fixed modules, may contribute to the discovery and understanding of novel regulatory systems in tumor biology [3] (Fig. 12.6).

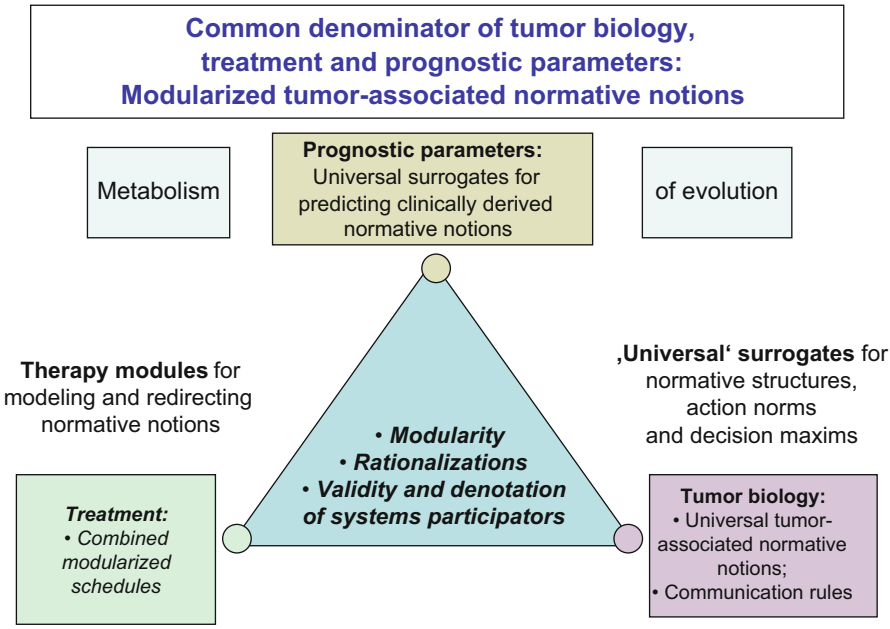

Fig. 12.6 Common denominator of tumor biology, treatment and prognostic parameters are closely interwoven tumor-associated normative notions. Tumor biology, treatment and prognostic parameters may be considered now from the perspective of consistent and inter-systemically comparable normative notions and make use of an integrative language

Intersystemic Exchange Processes

The complimentary reciprocal activity, which subsystems generate for one another, i.e., rationalizations, structures, action norms, and decision maxims, may be analyzed as currents of inter-systemic exchange. Therefore, from a therapeutic point of view, the systems biological model does not specify whether a normative notion has to be suppressed or stimulated to achieve tumor control: Inflammation control as well as stimulation of inflammation may control tumor growth, immuno- suppression, and immune stimulation. Contradictory decisions could be associated with the same capacity to achieve tumor control in a distinct tumor type [3] (Fig. 12.7).

Cellular Communication Acts

Cellular communication acts implementing redirection of modular events and rationalization processes do not generate experiences ('problem solutions'), but a **relief of activity**. Action systems may be rationalized with evolutionary constraints, in non-deterministical manner and in multifold directions [58].

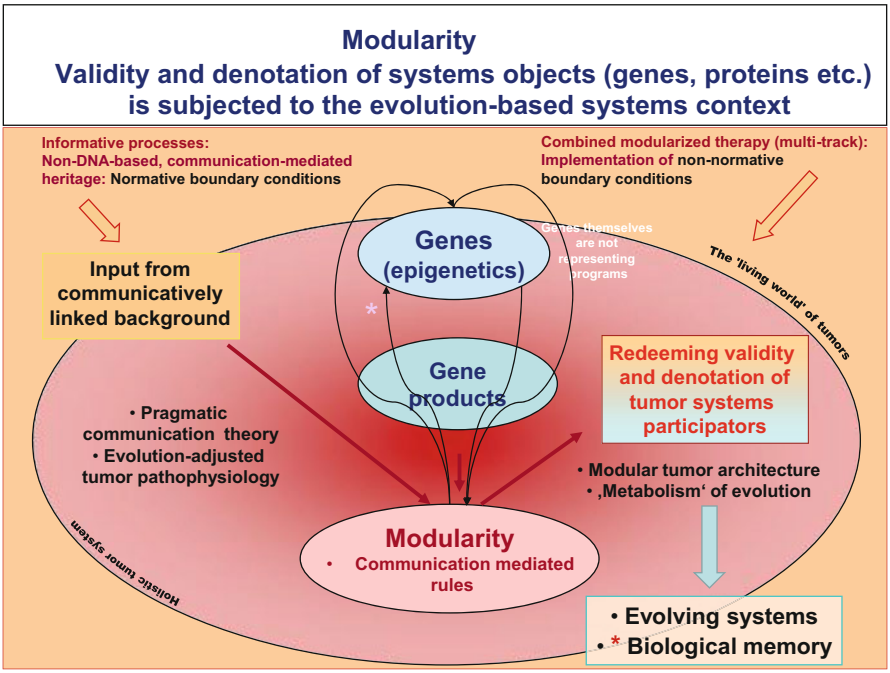

Fig. 12.7 Tumors allow experimental therapeutic access from inside in a comprehensive and reconstructive way (systems view) via modular (biomodulatory) therapy approaches and may be described as evolutionary developing systems. Modular therapies evolve the informative background, which redeems validity and denotation of tumor-associated objects. Therapeutically accessible pathologies may derive from the decoupling of functional cellular and systems 'world' and can be targeted by modular therapy approaches

Normative Tumor-Associated Benchmarks in Evolutionary Processes

Cell communities and cells constitute themselves, alternating in a close modular response to informative processes. Therefore, modular communication is usable as an internal systems-relevant and environmental communication mode: The evolutionary link between two different 'worlds', that of a systems participator and that of a system may be successfully constituted by a formal pragmatic communication theory.

Identity

In a reductionist sense, the object-associated identity serves primarily as a descriptive distinction towards the 'alter', the other systems object. Identity of a new 'organ', a separated system, or systems objects ('actors') is defined ex post from the perspective of an actively participating molecular and cellular systems world. The object-associated identity serves as a descriptive distinction towards the 'alter'.

The systems-associated identity of an actor, as the originator of a spontaneously accomplished communicatively driven action, may be only retrospectively assigned to already established identities (modular knowledge). The identity of an actor only occurs as a 'historical' feature. Identity is no inherent feature but is communicatively and situatively mediated, for example accomplished by the cellular fate within an evolving system [73]. The more evolutionary processes are involved, the more novel systems-linked identities of actors may be expected. Systems-specific redemption of validity and denotation is provided by the tumor's holistic communicative world, namely its 'living world' and endows identity, whereas noise, meaning that specific validity and denotation are not reified, does not specifically contribute to identity [66].

The claim for identity of rationality is confirmed in every ontogenesis (evolution history) [73]. In the systems-associated identity of an actor, as the originator of a spontaneously accomplished communicatively-derived action, the necessary structure of a subject and respective situative function are fused.

Robustness

Robustness (stability towards disturbances; complexity) and plasticity (switching to alternative rationalizations) may be described as rich branching (hubs!) and functional flexibility allowing dynamic switching of signals into alternative pathways in order to achieve nearly identical outcomes. Robustness is a fundamental feature of living systems. Evolution-trade-offs among the modalities robustness, fragility, resource demands and performance status provide possible benchmarks how biological systems evolve [78]: Multifaceted patterns of genomic alterations as well as diverse rationalization processes may initiate similar patterns of normative systems notions.

Tumor cell systems may recourse on differential rationalization processes (perlocutionary acts). Rationalization processes are symbolized by rather different communication lines and systems objects to maintain normative notions (robustness).

Basic mechanisms contributing to biological robustness are

- The steadily interwoven processes constituting the systems world and the functional world of systems objects
- The possibility to recourse on multi-faceted rationalization processes to fail-safe constitute normative notions
- Modular systems features by which a communication-derived decoupling from the former physical-chemical world may be established. The decoupling is based on the redirection of validity claims by communication-derived rules [73].

Local Penetration and Expansion (Colonization)

On the basis of the facticity of prepositional aspects, tumor cell colonization may lead to the complete destruction of non-regeneratory cell inventories. If 'traditional' organ-specific normative notions cannot be preserved, novel systems organizations

gain some kind of autonomy by neutralizing separation (identity) towards previous cellular functions or by the assignment of new functions [3].

Legitimation of Corrupt Rationalizations (Acceptance by an Established Organ)

Novel tumor-associated rationalization processes can be considered as strategies that allow systems objects and the respective modular arrangements to establish their 'corrupt' activities as justified, based on validity claims. Of interest are situative validity claims of a systems objects, which are grounded in the formal pragmatic communication theory and depicted with novel analytical approaches including mathematical specifications of 'modules' or functional 'fragments' [79; 80].

Participation in an Organ (Homeostasis)

Homeostasis—defined as the sum of processes available to maintain normative notions—can be explained on the basis of processes constituting robustness in involved organs and tumor lesions. Robustness is based on the multifaceted possibilities of systems objects to recourse on differential communication lines and rationalization processes to maintain normative notions. The impact of robustness in cellular systems, such as tumors, on the constitution of survival and reproduction is conspicuous. Robustness can be exemplified by therapy resistance in large series of metastatic tumors [81].

Redirection and modulation in systems rationalizations, induced by developing tumors, interferes with the affected organ and may destroy not-regenerative cell inventories. Thus, these changes not only alter previous ways of interactions among physiologic organ-associated cells, but also considerably affect the communicative infrastructure of rationalized communication within an affected organ. It is necessary to simultaneously decode paradox situations of cellular rationalization, deformation, and communication processes, i.e., to uncover inconsistencies within tumor cell compartments by means of a theory that includes the evolutionary development of a tumor as well as its biologic history in order to increase therapeutic options with systems-directed approaches.

Redistribution (Metastatic Process)

Each action system presents itself as an area of reciprocal interpenetration of subsystems. Each of these subsystems is specialized in reproducing basic functions facilitating tumor promotion. Successful tumor initiation is possible, if suitable normative boundary conditions, provided by the respective environment, prompt tumor cell proliferation, or if the tumor cell instigates a tumor-promoting stromal reaction. Non-normative boundary conditions might be responsible for insufficient communication with the tumor cell and consecutive 'smouldering' conditions or even tumor cell death.

Reproduction

One meaningful reproductive structure represents the genetic repertoire. The evolutionary reproductive function of tumor cells is underlined by molecular-pathologic data showing that molecular aberrations in the primaries determine tumor biologic behavior, for instance, early or late metastatic spread as well as metastatic sites [56].

The heritable inventory is evolvable via the tumor's living world [18]. This way, modular knowledge may be either incidentally or constitutionally acquired from the environment [18, 58]. Differential communicative interactions of environmental events with tumor cells results in molecular-genetic heterogeneity of tumors.

The second important reproductive structures are tumor-immanent rationalization processes (non-genetic equivalents to the genome): Despite of molecular-genetic heterogeneity in primary tumor sites or metastases, tumor-promoting rationalizations for normative notions are reproduced: Otherwise, we could not explain the successful modular access to metastatic tumor sites by implementation of non-normative boundary conditions. Biomodulatory therapies may induce continuous complete remission or long-term tumor control (Chap. 2). Reproduction of rationalizations has important implications for preventive strategies, for example in case of minimal residual disease, as well as for tumor control in the metastatic stage (Chap. 15, 23).

Combined modularized therapies show that modular events, assembled by the tumor's living world, are an additional evolution-constituting dimension, which is delimited by the respective aberrant genetic pattern, and the established tool of rationalization processes. Presumably, therapeutic redirection of the tumors' normativity is not based on genetic changes: Induction of biological memory might be an epigenetic process (Chap. 19).

Enhancement of Complexity, 'Corrupt' Rationalization

Tumor-related activities that seem to be operationally induced by the division of function, such as inflammation, neoangiogenesis, Warburg effect, immune response, extracellular matrix remodeling, cell proliferation rate, apoptosis, coagulation effects, etc. present itself from a systems perspective as an enhancement of complexity. Therapeutic efficacy of biomodulatory therapies has shown that tumor systems-directed therapies have the capability to use complex cellular communication acts as adjustable sizes to therapeutically modulate the tumor systems' stability, homeostasis, and robustness [46].

The formal-pragmatic communication theory allows the differentiation of the polarization between success-oriented and integration-oriented behaviors: Tumors equip biologically possible validities pegged to systems objects with the strength of facticity (corrupt rationalization) under the conditions of a perceivable incompatibility between facticity and validity. Between these conflicting priorities, the tumor disease unfolds and 'branches' according to rules that require further evaluation.

Stochastic principles or Darwinian selection models as a basis for clonal heterogeneity are commonly suggested as the driving regularity. At that stage, reciprocally acting communicative rules are added (Chap. 13).

Complexity may be explained as a function of modular knowledge and situatively available rationalizations to establish a **relief of function** within a systems context. If multifold prepositional communication acts are available for altering communicative expression of a communication line, modular knowledge may be implemented by redeeming validity and denotation of systems objects via therapeutically criticizable claims of validity.

The common starting point for understanding complexity is a phenomenon known as pleiotropy. Pleiotropy describes the observation that mutations simultaneously affect multiple normative notions. Experimental data reveal that evolution does not suffer at 'cost of complexity' because most mutations affect few traits and the size of the effects does not decrease with pleiotropy [82].

Relation Between Evolution Theory and Evolutionary History

Evolution theory cannot substitute evolutionary history, which is generated from a 'narrative' perspective, and which has to justify instructions for the solution of problems, and has to face aspects of criticism dependent on the chosen reference. Moreover, evolutionary history provides the basis for uncovering the rules of communicative expression linked to systems objects.

The necessary evolutionary historical constraints on retrospective explanations, i.e., selection, adaption etc., are relinquished by an evolution theory in favor of an in advance projected retrospective (prediction) derived from action-related, experimentally and therapeutically derived perspectives (e.g., biomodulatory therapies, efforts in transcriptional regulation, molecular imaging) [70]. The novel perspective may include particular claims on validity and denotation of systems objects within a novel evolutionary context, as well as evolutionary conserved communicative prepositions, which finally define validity and denotation within different evolutionary systems.

Evolution theories provide a basis for the therapeutic accessibility of evolutionary processes and an experimental frame to detect evolution-guiding communicative rules. They have no history and cannot be reproduced within narrative scenes due to their universal validity. The only restriction given: The contemporarily used communication tool is necessarily determining the answer of an investigated system, and the system itself is timely and locally positioned.

By introducing a pragmatic communication-theoretical approach, the intentionally uncovered structural levels are resolved in equivalent communicative structures linked to the respective systems objects. Now, the socially interwoven tumor and stroma cell community evolves as a holistic communication-driven structure, which provides internal access via modular therapy approaches, thereby disclosing its modularly designed architecture [39, 70, 83].

Communicative tumor (sub)-systems do not obey nominal conditions in an evolutionary process but adhere to rules to meet the validity of communication processes: Phenotypically distinguishable individual tumor disease may constitute within the predetermined range of—at least to some degree—autonomous tumor development. These self-evident presumptions compromise the phenotypical homogeneity of tumors. Induction of complete remission or long-time disease stabilization with combined modularized therapies, indeed indicate that rationalizations of normative notions within a tumor disease are frequently homogeneously organized (Chap. 2).

> How may be a theory about evolutionary processes ('metabolism' of evolution) linked with 'narrative' forms of evolutionary comprehension?

Theories about the development of distinct forms of behavior reductionistically undermine as hypothesis-driven theories (capable to explain experimental data) the traditional 'narrative' presentations about evolutionary processes.

However, starting point of evolution-theoretical considerations remains the reductionistically, in any arbitrary evolutionary system detected reference of a tumor systems object (cell, oncogene-addicted pathway, etc.) and the primarily hypothetically phrased tumor-inherent normative structure, to which a systems object can contribute.

Experimental or therapy derived data on communication-derived rules among tumor-associated rationalizations facilitate to resituate systems objects as systems subjects, which have been integrated in novel evolutionary based systems contexts. Evolutionary based communication-derived constrains may attribute tumor systems objects novel tumor type- and stage-dependent patterns of references, based on their modular knowledge.

Evolution theories or histories for describing tumor development should contribute to broaden the therapeutic instruments. The competing evolution historical and the evolution theoretical model systems show quite different model-creating determinants. Evolution theory may provide the basis to include non-oncogene addicted targets [38], and drugs with poor or no monoactivity into the therapeutic calculus and aims at targeting the tumor- and stage-dependent communicative expression, which is steadily involved in the 'metabolism' of evolution.

An Evolution Theory Provides the Scientific Tool to Answer Central Questions in Future

> May be an evolution theory consulted for the assessment of competing evolutionary histories about the same phenomenal domain?

By adding evolution theoretical considerations, tumor systems biology becomes an operatively accessible size. Evolution-adjusted tumor pathophysiology describes object-subject relations independent of the starting point, which is operated by evolution historical considerations. The main challenge for an evolution theory is

to converge the experimentally derived results, which have been generated ensuing from rather different experimental starting points (Chap. 22), and to describe communicative rules that are involved in tumor progression [66].

Competing evolutionary histories may be resolved by the introduction of communication derived validity claims of systems objects. Validity claims put communicatively linked systems objects in an evolutionary context. Validity claims of systems objects are based on communicatively derived pre-suppositions within a particular systems context, and they position preclinically-derived references of objects as situative systems subjects, which may be characterized by novel denotations in non-linearly evolving tumor systems.

The necessary structure of a systems subject and the respective situative function are fused in the systems-associated identity of an actor, as the originator of a spontaneously accomplished and communicatively-derived action. The novel 'selection' rules, based on modularity and rationalization processes may be uncovered by retrospectively establishing spaces for primarily non-heritable evolutionary developments, if modular events are implemented.

> May a universal history of evolution be described, which can be based on multiple particular descriptions of evolutionary solutions, or particular reconstructions of problem resolution?

A universal history may only arbitrarily approximate to the problem of the 'driving forces' of evolutionary processes, as selection processes always anticipate predetermined and often heterogeneous normative references. The 'metabolism' of evolution is experimentally (for example by gene transfection, knock-out models etc.), and in case of tumors also therapeutically accessible (biomodulatory therapies). General communication-derived rules assessing modular knowledge of tumor systems objects and the prepositional circumstances for a distinct communicative expression should explain multifaceted 'problem solutions'.

As rationalization processes are inherent in biological systems, inconsistencies, Achilles' heels, deformations or missing inter-systemic exchange processes are implicitly emerging features of such systems architectures: On this background, the claim for 'survival of the fittest' should be revised. 'Selection' in the Darwinian sense relies on reductionist based observations, which do not necessarily account for the 'metabolism' of evolution as the original texture. The Darwinian notion has originally established the fundamental biological feature, namely evolvability of communicatively linked cell systems. The assumption of modularity and rationalization processes is sufficient to explain that distinct tumor-associated genotypes may acquire stage- and context-dependent denotations, for example during the course of a tumor disease [40, 65].

> Is a formal pragmatic communication theory a methodological instrument to explain diverse ways of 'problem solutions' with unique scientifically accessible principles?

If we separate evolutionary structures (metabolism of evolution, modular knowledge of systems objects, communication rules, molecular genetics) as activities intending to transfer empirically derived objects into situative systems subjects, we do not need to establish continuity (unidirectionality), necessity or irreversibility of the course

(genomically induced aberrations), and selection to explain diverse ways of 'problem solutions'.

Competences of systems objects may be reconstructed only if they are therapeutically or experimentally accessible to the contemporary scientific objectivity.

An analysis of developmental logics may escape from fallacies, if the analysis does not inductively pick up the hierarchically arranged structural patterns ('ontologies'), but, if it systematically justifies that the respective 'higher', more complex niveau for 'learning' is based on the interaction of the holistically communicating systems world and the functional world comprising its systems' objects. Formally, there remains no space for 'superior' or 'higher' organization to the preceding one, but tumor-related activities achieve an enhanced level of complexity.

The inclusion of evolutionary based principles and communicative rules into the therapeutic calculus, i.e., modularity and rationalization processes, besides the whole genome analysis, allows to feature a stage- and tumor-type dependent personalized tumor pathophysiology and to set the stage to select among combined modularized tumor therapies, dependent on a tumor's genetic- and communication-derived systems status.

At this stage, the technical and theoretical instruments are available to explore, whether it is possible at all that the developmental logical processes (implementation of modular knowledge) and genome-based processes are not the same involving different levels of tumor systems alterations, as the genome theory is suggesting [2].

Environmental carcinogenesis may be explained with the presented evolution theory by continuous implementation of non-normative boundary conditions in biological systems, independently of the qualitative feature of the boundary conditions [84, 85].

Evolvability is commonly assumed as the ability to respond to a selective challenge by a genetically based phenotypic change [16]. The term evolvability is now extended to the non-genomic, but also digitalized working systems world of a cell: Systems objects are continuously exposed to modular events by externally (non-normative boundary conditions) or internally implemented (redeemed) modular knowledge (pathophysiological processes). Beyond the (molecular-) genetic heterogeneity of tumor cells at primary and metastatic sites, rationalization processes for tumor-promoting normative notions can be preserved (Chap. 2, [22]).

Accessibility of Evolutionary Processes (Communication Acts)

As nature is interwoven with communication and is represented and reproduced through communication acts, communication associated rules and constraints should be made scientifically accessible and reconstructible with appropriate methodologies:

- **Diagnosis of normative systems structures and there therapy-derived changes** (cellular secretome analytics, molecular imaging techniques, epigenetics of mononuclear cells in the peripheral blood, assessing rationalization processes of tumor-immanent normative notions) [50–52].

- **Comparative uncovering of tumor systems biology by** implementation of non-normative boundary conditions ('top-down', 'bottom-up' approaches, Chap. 22) and modular knowledge, with the aim to detect rationalization structures and intersystemic exchange processes [68].
- **Diagnosis of developmental problems in tumors:** Inconsistencies, deformations, aggregated action effects, Achilles' heels, robustness, i.e., multiplicity of available rationalizations to maintain normative notions [3].
- **Detection of communicative presuppositions**, which may facilitate evolutionary based, systems-restricted combinations of transcription factors on a genome-wide scale (modular knowledge), and which can specify regulatory elements ultimately responsible for both cell identity and situative cell type-specific response to diverse signaling inputs [73].

The newly uncovered systems perspective, which is frequently underestimated, moves its focus to the discrepancies that develop between the functional world of tumor-associated cell systems and the functional requirements imposed by rationalization processes and triggered by a tumor's systems 'world'.

Assessment tools of tumor systems biology are (corrupt) rationalization processes, inconsistencies, deformations (Achilles' heel), altered intersystemic communication, and the topology of aggregated action effects (enhancement of complexity), robustness (recourse on alternative rationalization processes), homeostasis, intersystemic exchange processes, reproduction (proliferation, apoptosis-resistance, dysplasia etc.), local penetration and expansion (colonization), and redistribution (metastatic process). The proof of discrepancies is suitable to identify communication-derived rules. Without these rules, evolutionary processes would not function [3].

Up to date, still insufficiently processed remains the evaluation of communication acts establishing identity (new 'organ', separated systems), legitimation of corrupt rationalizations (acceptance by an organ, factual acknowledgement of criticizable denotation claims), and participation of a neoplastic process in an organ (homeostasis). Interestingly, transplantable identity is not necessarily bound to acquired aberrations mediating neoplastic disease [86].

The therapeutically relevant acquisition of the 'language' of communicative cellular expression gives hints on the 'metabolism' of evolutionary tumor development. Supported by the therapeutic possibility to implement non-normative boundary conditions into the tumor' living world, the situative and specific redemption of validities of communicative processes may facilitate the promotion of a tumor's evolutionary development. The procedure is closely linked to the differential development of novel denotations of the systems objects: Via communication-relevant processes, systems objects are acquiring novel references within the tumor's living world without at first substantially altering the functionality of the entire communicative system.

Practical Relatedness of an Evolution Theory for Tumors

Communication Derived Tumor Pathophysiology

Assessment of Evolution–Mediated Rules: The 'Metabolism' of Evolution

Modular therapies exemplarily give indications of the 'metabolism' of evolutionary processes. Evolutionary processes are symbolized by redirected and modulated validity claims and denotations of systems objects, by object-subject-relations, by a realignment of normative structures, functions and decision maxims. The dissection of the holistic communicative tumor system in scientifically assessable systems terms advances the metabolism of evolution into the diagnostic and therapeutic focus [46].

Implementation of modular knowledge by primarily multi-track approaches ('top-down approaches'), such as combined modularized therapies, single-track approaches ('bottom-up' strategies), such as gene transfections, cell transplantations, cellular stress etc. may result in substantial evolutionary processes within the frame of the tumor's 'living world'. Non-normative boundary conditions, maintained by therapies, noxa or non-malignant processes (e.g., inflammation) have the capacity to induce molecular-genetic aberrations in tumor and stroma cells. Even transplantable non-malignant stroma characteristics may be induced [18, 86]. Vice versa, the (molecular-genetically altered) microenvironment facilitates clonal evolution of tumor cells [58].

All these therapeutically induced modular changes aimed at evolving biological systems are reproducible: This indicates that modularity and the **organization of rationalization processes is digitalized** and ascertainable in communication-derived rules. Digitalization does not exclude analogous working steps, for example represented by hubs.

The most important task for a communication-derived tumor pathophysiology is to look for common systems features within different tumor types to get action-theoretically guided classifications of distinct evolutionary systems processes. Furthermore, classification is essential because classification is the basic language of medicine and of systems organizations across different tumor types, which need to be clearly defined. The uncovering of common evolutionary features in different tumor types is only the beginning. Lymphomas could soon be classified according to their activation of inflammatory signaling pathways [87, 88]; common stroma gene expression sets may be detected in response to tumor invasion: and neoplasias may be classified according to their responsiveness towards combined modulation of transcriptional networking [46]. Another attempt may be the formulation of stroma scores or cellular secretome signatures [50, 89]. Tumor systems may be assessed according to rationalization aspects of normative notions—how are e.g., the hallmarks of cancer multi-dimensionally constituted, which cell types are contributing to a normative notion (Chap. 17)?

Systems that are based to a high degree on division of functions seem to be less susceptible to reductionistically designed therapeutic perturbations. Tumor cells in

such rather robust systems are characterized by multifold chromosomal aberrations [62]. Studying a tumor's robustness that means, assessment of perlocutionary processes supporting a normative notion will be of further therapeutic interest and could contribute to novel decision criterions for the selection of therapy. Failure of systemic standard therapies may be a measure for the resistance of these tumor systems towards external perturbances. Robustness is retained by the tool of the systems participators' background knowledge [90].

Evolutionary Reconstruction of Tumor-Associated Systems

Redeeming validity is tailored on the relation of modular communication to the objective features of the tumor compartment, the reconstructible evolutionary (modular) systems [68]. Modular events (biomodulatory therapies) serve as a prerequisite for the reconstruction of the tumor's living world, in which cells are symbolic communicative figures with—to some degree—exchangeable references connected by modular prepositional structures: Consecutively, communicatively derived systems may be described by rationalization processes, deformations, and intercellular exchange [3].

The application of available methodologies in novel indications, i.e., cellular secretome analytics, assessment of transcriptional regulation, molecular imaging etc., facilitates to decode paradox situations of cellular rationalization, deformation, inconsistencies, Achilles' heels of communication processes in the tumor cell compartments by means of a theory that includes the evolutionary development of a tumor as well as its biologic history: Aim is to increase therapeutic options with applied systems-directed therapies [3; Chap. 2]. In the mirror of evolutionary processes, the functional 'world' of cell systems may be recognized under systems-therapeutic conditions and vice versa [46, 91].

Situation-Related and Stage-Dependent Communicatively Explicable Evolutionary Constraints

Novel normative systems structures and rationalizations may evolve by continuous implementation of non-normative boundary conditions. Darwinian 'selection pressures' are depicted in an communication-driven evolutionary process by stage- and tumor type-dependent situative non-random communicative constraints (modularity, rationalization), by non-deterministic communication acts of systems objects, which are characterized by intersubjectivity, normativity, and subjectivity, and by stochastic communicative events (carcinogens, 'instigations', non-normative boundary conditions) [84, 92]. The availability of a **non-deterministic communicative window** again underlines the therapeutic accessibility of communication associated rules and constrains (Chap. 23).

In the initial development of pre-neoplastic lesions, cellular proliferation is controlled by communicative interactions with other cells, the extracellular matrix, and by soluble or insoluble growth factors [58]. Clonal expansion is permitted by gain of

function mutations in 'oncogenes', loss of function mutations in tumor 'suppressor genes', and disruption of normal senescence pathways. Evolution historical considerations provide explicit 'selection mechanisms' for single mutations, as indicated in the classical Fearon-Vogelstein model of colorectal carcinogenesis [93]. Vice versa, this model is indicative for a distinct feature of communicative rules, namely the availability of evolutionary conserved prepositions in a tumor systems context, which facilitate validity and denotation of tumor promoting communication lines. The evolution theory adds the non-deterministic communicative window, both for tumor development and for tumor control. Hierarchical organizations arise within the contextuality of validity claims of systems objects and may contribute in so far to the truncation of developments [94].

Definition of Evolutionary Conserved Communicative Structures

Hereditability of communicative presuppositions and consecutively of the systems objects' communicative expression is symbolized by evolutionary conserved communicative structures. Communicative expression is an inherent biologic feature of a communication line or a tumor-associated communication tool. The following prerequisites for the communicative exchange may release us from the necessity to evaluate communicative expression in a novel evolutionary context and may give hints for hierarchical orders: The availability of (1) universal suppositions for a distinct communicative expression of a communication line or medium, (2) the universal reciprocity of the communication act's immanent obligations, (3) universal clarifications of an intersubjective use of communication paths, (4) the possible universalization of action-associated norms (e.g., evolutionary conserved apoptotic pathways), and (5) the intersubjective commonality.

Whether communicative expression of a communication line is really **evolutionary conserved** may be assessed by evaluating the prepositions of systems objects' validity claims. The institutionalization of communication acts facilitates the attainment of evolutionary conserved, seemingly hierarchically organized systems. A communication concept, which enables the possibility for a generalization of prepositional circumstances constituting communicative expression, may be useful to evaluate legitimating-critical actions, e.g., cell fate determination. The development of multicellular organisms (tumors) is associated with complex rationalization processes for the relief of functions, and involves 'progenitor cells' endowed with evolutionary conserved complex communication concepts, which give rise to cell types with specialized functions (functional world) within a distinct systems context (systems world).

Implementation of communication-derived tumor pathophysiology in parallel to histopathology The classic methodology of pathology is comparatively classifying. The theoretical core is formed by assumptions about the structural differentiation of cells (histopathology) in functionally specialized systems of interaction. These assumptions are sufficient for supporting the observation that the

structural integrity of tumor compartments needs to be maintained to sustain appropriate tumor-stroma-cell communication for tumor progression. Thereby, functional considerations are not sufficiently separated from structural ones in such a way that the disposed concurrence between methodological strategies may unfold.

A further competitive research approach exclusively investigates the rationalization of functional systems in the course of evolutionary growth complexity during tumor development and tumor spread under the aspect of different purposes. The aspect of rationalization may be elucidated by the analytically defined functional spectrum (references) of fibroblasts or macrophages within a cellular system: Macrophages and other inflammatory factors do more than just foment angiogenesis in tumors, i.e., they actively aid cell movements that produce metastases, thereby calling tumor cells to the vessels. On the other hand, they may act as tumor-antigen presenting cells for tumor control. This out- lined functional 'world' of macrophages gives an impression of rather divergent options of rationalizations within a systems context.

A third approach pins down the tumor pathology at disordered intersystemic exchange processes, at the imbalance of mediators.

Each of these research approaches and viewpoints described brings about the separation of subject and object. In other words, none of the approaches considers it necessary to uncover the object. A tumor's systems biology is also a scientific subject, a co-subject of the scientist that interests not only as an approach for observation, description, and explanation of cellular behavior. Even more, it serves as a communication partner, for instance via biomodulatory therapies, and thus as an approach of hermeneutic comprehension. This approach represents a scientifically new aspect for understanding tumor biology, implicating a decisive broadening of therapy options that arise from the evolutionary consideration of tumor development [3]: **The systems objects' subjectivity is now scientific object** (Chap. 23).

Therapeutic implications of a communication derived tumor pathophysiology Criticizability of validity claims is the starting point for biomodulatory therapy approaches. Validity claims, which are generally associated with an action norm, may be solely redeemed with justifications based on the permissibility and the factual acknowledgement of criticizable denotation claims (subjectivity of each communication act): Therefore, single objects of a system share competences, i.e., **modularity, background knowledge, intersubjectivity, normativity, and subjectivity**.

The therapeutically relevant acquisition of the 'language' of communicative cellular expression gives hints on the 'metabolism' of evolutionary tumor development. Supported by the therapeutic possibility to implement non-normative boundary conditions into the tumor' living world, the situative and specific redemption of validities of communicative processes may facilitate the promotion of a tumor's evolutionary development. The procedure is closely linked to the differential development of novel denotations of systems objects: Via communication-relevant processes, systems objects are acquiring novel references within the tumor's living world without first substantially altering the functionality of the entire communicative system.

Targeting structures of intersubjectivity means to alter the communicative medium of systems subjects capable of acting and able to communicate on the basis

of their modular knowledge; to modulate the symbolic character of communication acts, and the communicative expression; to involve a tumor's living world and the modular knowledge of systems objects into the therapeutic calculus by implementing non-normative boundary conditions.

Targeting structures of normativity: The availability of normative systems structures in biological systems shows, that patterns of 'disparate' oncogenes and tumor suppressor genes may contribute to the constitution of different normative notions, to differential organizations of rationalizations, and modular arrangements of normative notions [62; Chap. 17)]. Targets are normative tumor structures (cell organelles, functional compartmentalisations, 'modules'), action norms (normative notions), and decision maxims (hubs and nodes). The assessment and appraisal of situational normative notions (for example, the hallmarks of cancer) is influenced by functional analytics (cellular secretome analytics, epigenetics, molecular imaging etc.) and the evaluation of perlocutionary processes, which may contribute to robustness.

Targeting structures of subjectivity ('to be an object in a biological system') means to alter the interpretation of a situation (i.e., nodes, hubs), the direction (orientation) of actions, the intention and motivation (instigation). Targeting structures of subjectivity may be realized, when the evolutionary systems status has been evaluated with appropriate technologies (Chap. 23).

Concurrent Evaluation of Evolutionary Processes in Different Normative Tumor Structures

Data derived from biomodulatory therapy approaches indicate that tumor-promoting rationalization processes are frequently preserved at the metastatic sites of an individual tumor disease, although cytogenetic heterogeneity of tumor cells is a common feature during clinical tumor progression [56; Chap. 23]. Evolutionary preservation of rationalization processes for maintaining distinct normative notions reveals that analyses of rationalizations constituting tumor-promoting normative notions play an important role to depict evolutionary processes, besides the traditional reductionist analyses (whole genome analytics; histopathology) (Chap. 15). The two perspectives open up methodologies enabling differential therapeutic access to metastatic tumor systems.

Obviously, the environment of metastatic organ sites defines restrictions and the scale, by which rationalizations are communicatively and contingently evolving, to sustain characteristic tumor-promoting normative notions (Chap. 16, 19). Evolving genomes in tumor cells are rather heterogeneous, but non-random—and considering this concrete restriction, we must assume that the arising chromosomal patterns are communicatively well organized to ensure the tumor cells' robustness and reproducibility. Non-randomness, evolutionary persistence and specific biomodulatory accessibility within an individual tumor disease is a frequently occurring feature of tumor-promoting rationalization processes, but—vice versa—redundant

background knowledge of cellular systems participators allows many ways to organize rationalization processes, just to constitute one single normative notion, like tumor-associated inflammation, angiogenesis, immune response etc. [3, 68; Chap. 2]. Compilation of these differential rationalization processes is the task of an evolution-adjusted tumor pathophysiology.

The hypothesis-triggered differential comprehension of evolutionary processes results in diversified, but equivalently applicable therapeutic approaches, i.e., the 'bottom-up' and the 'top-down' strategies (Chap. 22). These strategies have to be appropriately selected and adapted to the evolutionarily confined systems stage for achieving tumor control. In case of metastatic tumor diseases, it would seem the thing to use 'top-down' strategies to overcome cytogenetically based tumor heterogeneity (Chap. 2, 22).

Cellular Therapies In Situ

Cellular therapies in situ may be established by implementation of non-normative boundary conditions into the tumor's systems context and represent evolution-inducing therapies.

On the background of successfully administered biomodulatory therapy approaches, we propose that drivers of carcinogenesis (stress, noxa, chronic inflammation etc.) primarily induce adaptive changes via rationalizations and modularity. Redirection and modulation of the tissues' normativity is enabled by local or systemic modulation of tissue architectures and functions, which may be—as shown—consecutively digitalized as acquired (molecular-) genetic aberrations [18]. A full understanding of cancer biology and therapy through a cataloguing of the cancer genome is unlikely unless it is integrated into an evolutionary that means in a communicative context, explicated by an evolution theory. Tumor cell systems are getting evolved by the contemporarily restricted possibilities for redemption of external and internal modular knowledge by the respective systems objects. Aberrant tumor-associated genetic patterns are now pending to be reinterpreted on the background of modular and rationalization processes.

Hitherto existing perspectives favoring unity of patient care and contextualism are likely to consider qualitatively heterogeneous communicative actions, including modularly-designed tumor therapies, as too weak and presumably inefficacious. The reason for this view is that all hierarchies, that have developed by intentionally acquired knowledge (evolution historical considerations), are leveled to be discharged in a continuum of contingency programming, modularly-evolving systems features, and in continuous inter-systemic communicative exchange processes. On the other hand, the methodology of communicative therapeutic intervention (modular therapy) seems to be highly potent from a contextualist perspective. This view may be caused by the fact that incommensurable 'worlds', such as non-DNA-heritage and DNA heritage or different techniques for implementing modular knowledge and various modular tumor architectures, turn out to be pervious, despite their qualitatively

rather heterogeneous features [83]. Non-DNA-heritage and DNA heritage share a digitalized operative action pattern.

Adaptive Trial Design by Monitoring Changes in Normative Systems Structures (Systems Stage-Adapted Therapy)

Implementation of modular knowledge, rationalization processes and normative systems structures now enter the therapeutic calculus for establishing stepwise evolution-adjusted therapies and adaptive trial designs [83].

Biomodulation means to configure normative systems structures of tumors by the metronomic (e.g., continuous daily) implementation of non-normative boundary conditions, mostly via non-oncogene addicted targets, both in tumor and adjacent stroma cells. Implementation of modular knowledge facilitates to adaptively slow down the evolutionary growth promoting process of cancer, and not to unwisely accelerate evolution when applying classic cytotoxic drug therapy: 'Overtreatment' and long-term toxicity due to biological memory (epigenetic and genetic changes) may have an overall negative effect and can accelerate cancer evolution (secondary malignancy) following cytotoxic therapy.

Important questions yet still unanswered can be solved by an evolution theory:

- How can we use systems homeostasis to constrain cancer by modulating simultaneously multiple homeostasis systems in individuals?
- Is it possible to apply the evolutionary provided or therapeutically induced communication principle to slow down cancer progression?
- Can cancer be directed into a slow growth phase that will not trigger much heterogeneity? Evolution and therapeutically induced evolutionary processes drastically change the cooperative and competitive relationship between cancers and host. Cancer may be therapeutically directed to enter into a highly homogeneous phase, then constrained by therapeutically induced systems homeostasis mechanisms (Chap. 2, 19).

Drug Repurposing

Compared to conventional pulsed chemotherapy, biomodulatory therapy strategies are thought to be less susceptible to the development of drug resistance and to cause less toxicity [46]. Taking into account that the combinatorial use and repurposing of biomodulating agents might potentiate the antineoplastic effects without causing life threatening toxicities, targeting communicative expression of tumor systems objects (multi-dimensional rationalization processes) is judged to be a promising approach in tumor palliation. Drug repurposing research still remains a challenge for systems biological considerations [95].

Discussion

Dobzhansky wrote, "nothing in biology makes sense except in the light of evolution", and this, has not been sufficiently recognized yet by medicine [96]. Modular therapies exemplarily give indications of the 'metabolism' of evolutionary processes in tissues.

Three main factors emerged as our starting point for evolution theoretical considerations, an unmet medical need (systemically pretreated patients with metastatic tumors), a hypothesis-driven vision (the formal pragmatic communication theory) and technological advances to pursue that vision (biomodulatory therapy approaches, cellular gene transfection etc., clinical proteomics, epigenetics and molecular imaging techniques) (Fig. 12.8).

The present evolution theory on tumor development arises from a formal pragmatic communication theory and has been originated, starting from the three looming mainstays of acquiring new insights into novel therapy approaches assuming modular features (biomodulatory therapy) in diseased tissues, (1) the change from the classic conclusion logic (indicating a pathway responsible for cell death) to that of normative statements (how to control systems-associated processes with therapy modules to achieve response); (2) the change from object-associated to situation-associated systems interpretations (biomodulatory therapies in metastatic tumors); and (3) the change from an intentional (reductionist) to an evolution-based systems explanation (systems behavior and response) [68].

At this time, for situation-associated systems interpretations, we may use terms derived from theoretical considerations (evolution theory) on a tumor's modular systems architecture and on intercellular rationalization processes. The two methodological pillars (reductionist versus holistic) for creating tumor models complement each other in the same way as the benchmarks of communicative systems correspond to the components of which functional sequences are composed**.**

The proposed evolution theory on tumors aims at specification of novel therapy approaches of metastatic tumors (biomodulation, cellular therapy in situ) and at uncovering of modular systems structures (novel tumor pathophysiology). This approach is realized by a pragmatic communication-theoretical method for understanding communicatively linked systems objects, biochemical processes, and cell functions by communication-technical terms, namely the validity and denotation of systems objects.

The formal-pragmatic communication theory exceeds information theoretical approaches as well as the game theory, because normativity, modular features, subjectivity and intersubjectivity of systems objects are acknowledged beyond the simple exchange of information via communication lines or the reductionist assignment of functions.

Particularly, communication theory specifies the communication related prepositional circumstances, which are prerequisite to attribute particular systems objects

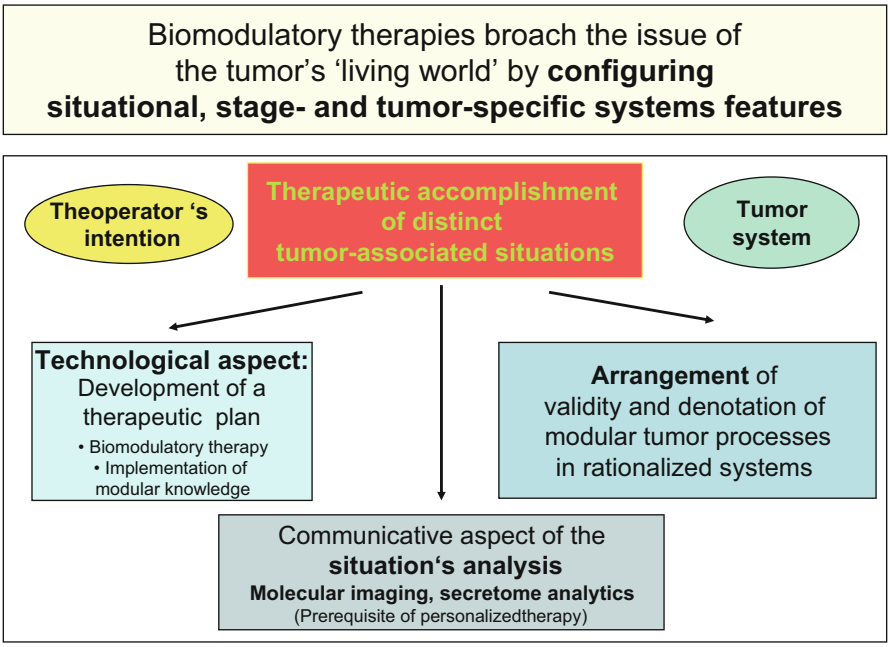

Fig. 12.8 Biomodulatory therapies broach the issue of the tumor's 'living world' as a holistic and therefore self-contained communication process by configuring situational, stage- and tumor-specific systems features. The tumor's **evolutionary-derived stages** are separated episodes of the tumor's 'living world' with respect to distinct issues or intentions, namely the aspired growth control of respective metastatic tumors

distinct communicative expression, and which define, whether a communicative expression is evolutionary conserved or whether the respective systems object is used in a novel communicative context (modular knowledge).

The theory is based on statements about retrospectively recognizable and scientifically accessible evolutionary processes, which contribute to a relief of function within a communicative modus, frequently, by enhancing complexity. In future probably other structures than the actually established cognitive-instrumental and practical structures are accessible for a reconstruction of evolutionary processes (Fig. 12.8). Our knowledge about communication processes is limited in so far as it is dependent on currently available communicative instruments and available evolution historical data.

What Does an Evolution Theory Accomplish?

A main task of an evolution theory will be to uncover more examples of rationalization processes constituting common normative notions of tumors, based on a very broad, but non- random pattern of acquired genetic aberrations. Multifaceted rationalization processes are utilized by respective tumor systems for constituting distinct

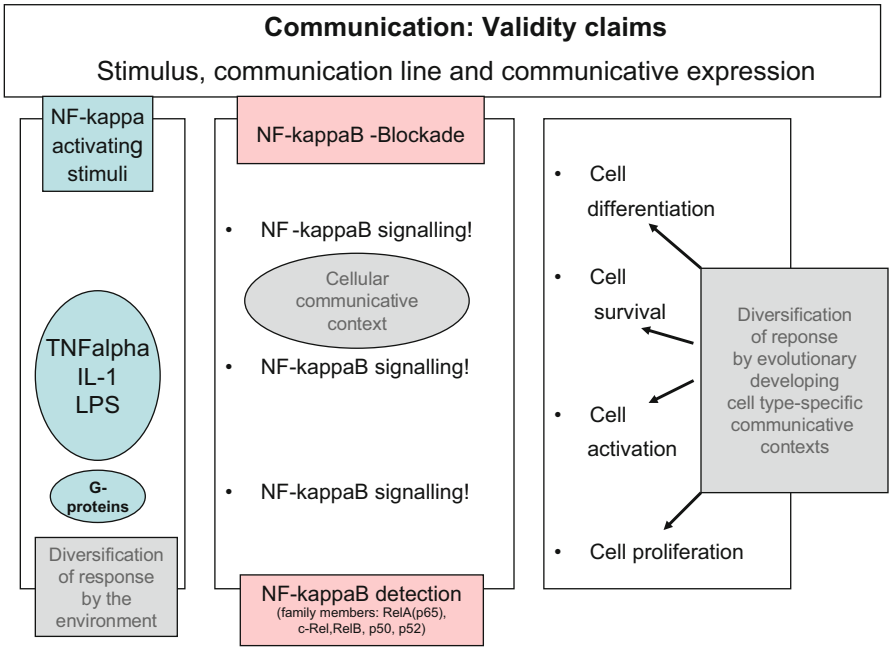

Fig. 12.9 The communicative expression of the activated NF-kappaB signalling pathway is modulated by extrinsic, environmental parameters and by intrinsic, evolutionary developing communicative contexts

tumor stages and tumor types and for supporting evolutionary confined normative systems structures, differential interfaces and correspondingly adaptive intersystemic exchange processes. The introduction of biomodulatory therapy regimens for treating metastatic tumors allows versatile involvement of clinical treatment strategies in communication- technically accessible novel tumor pathologies (evolution-adjusted tumor pathophysiology).

The implementation of therapies interfering with evolutionary tumor processes serves as

- A detector for therapeutic targets, which are derived from modular tumor architectures and rationalization processes. Biomodulatory therapies ('top-down' approaches) are "targeted" therapies using ubiquitously available targets, present on tumor and stroma cells, and aim at targeting holistic communicative structures (rationalization processes). The implementation of non-normative boundary conditions facilitates to redeem validity and denotation of specific systems objects within communicative tumor processes (Fig. 12.9).
- Therapy-relevant action-theoretical approaches may uncover the interwoven modular tumor architecture. This way, we can describe modular textures on a molecular

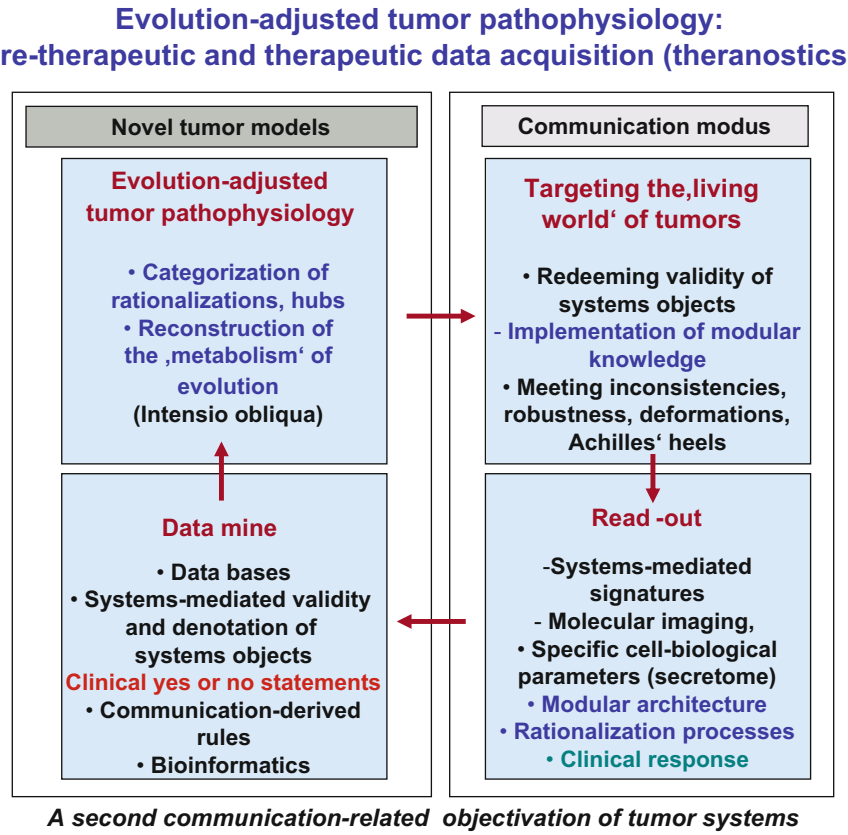

Fig. 12.10 Theme-dependent and closely interrelated areas of knowledge are the basis for reductionist approaches to uncover systems biology. According to reductionist systems interventions, scientists are observers of subject-object relations. However, if references of studied systems objects resolve during evolutionary tumor development, and systems objects are anticipating novel systems-related rationalization processes (e.g., differential integration of inflammation), then methodological considerations guided by 'intentio obliqua' are appropriate to reconstruct evolutionary systems stages (modular approach)

basis, (1) on the background of altered cell functions in the course of rationalization processes, and (2) with novel therapy-guiding 'universal' biomarkers (cellular secretome analytics) (Chap. 15).

In each new tumor case a few small regulatory changes may be detectable, sufficing to redeploy rationalization processes, which are robust, adaptable, and which create novel interfaces. By applying novel indirect methodologies ('intensio obliqua') for uncovering the architecture of rationalization processes, hubs within rationalization processes, or for identifying deformations and Achilles' heels in tumor systems, vulnerable nodal points of rationalization processes could be targeted in future with 'bottom-up' strategies (Fig. 12.10).

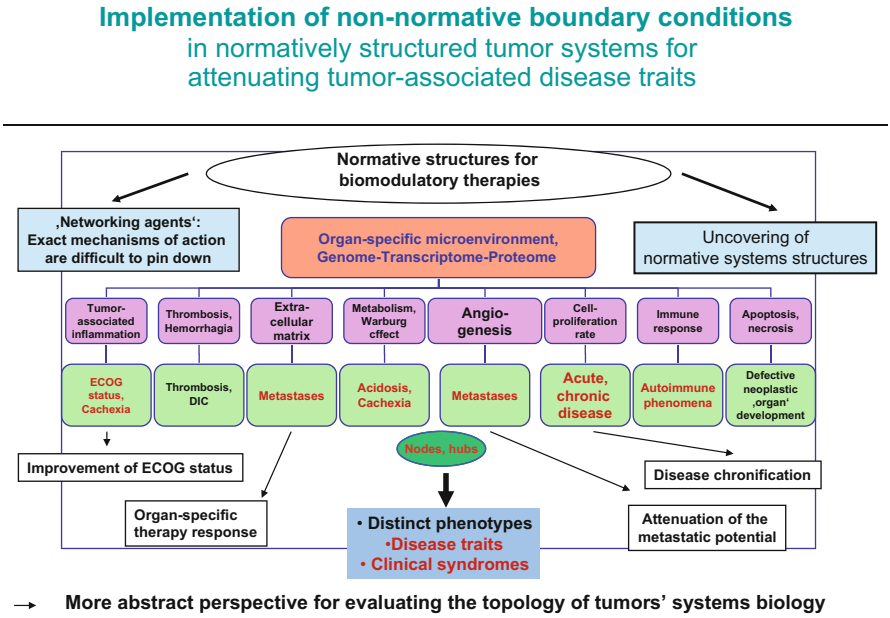

Fig. 12.11 Modular therapy approaches facilitate the detection of new networking interactions and the reconstruction of normative notions. Thereby, the context of discovery (modulation of tumor associated disease traits, biomarkers) has to be consistently separated from the context of justification (rational for a biomodulatory therapy approach). The currently established genomic/non-genomic biomodulatory therapies may lead to novel and more abstract perspectives for viewing the topology of tumor systems biology, inconsistencies, deformations, and Achilles' heels

Up to now, the success of cancer screening programmes solely depends on the assumption that small, primary tumors are curable if detected early enough. This field, however, could be further personalized by considering communication derived tumor pathophysiology, particularly rationalization processes (Chap. 15).

> An evolution theory allows a possible virtualization of the engagement to get experiences via implementation of non-normative boundary conditions (Pragmatic virtualization of communication acts)

Tumor models are based on normative systems structures; the 'metabolism' of evolution is linked to criticizable claims of validity and the redemption of validity and denotation on the background of a holistically acting communicative systems context; evolutionary conserved communicative structures may be newly defined by universal suppositions for a distinct communicative expression of a communication line or medium; the theoretical and technical instruments to evaluate the evolutionary and therapeutic impact of heritable/non-heritable evolutionary developments on the genetic and rationalization level, respectively, are now available (Fig. 12.8, 12.9, 12.10, 12.11, 12.12, 12.13, 12.14, 12.15).

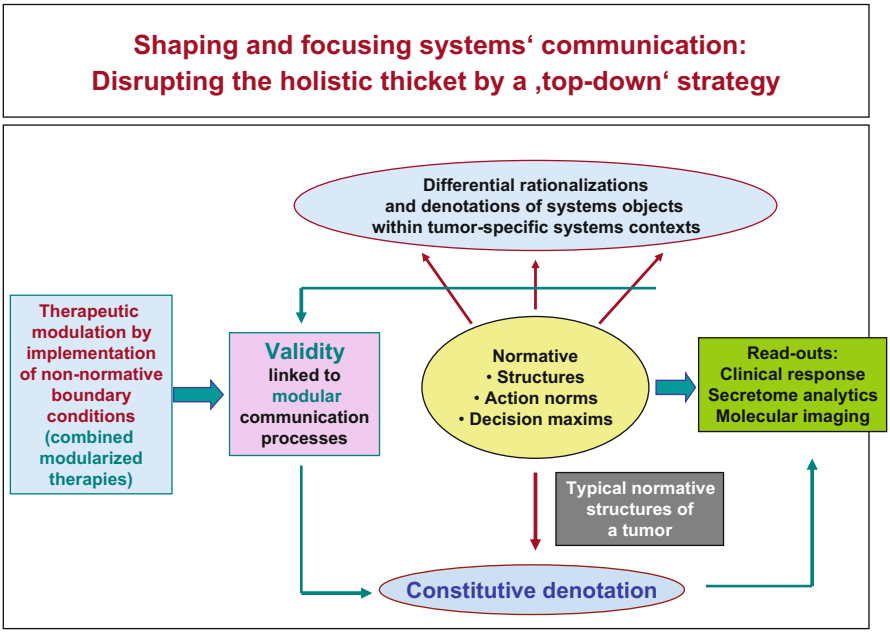

Fig. 12.12 Shaping and focusing systems' communication: disrupting the holistic thicket

The endogenous monitoring of time-related processes (**time-consciousness**) must be imminent in biological systems. Particularly, the operative interplay of functional and systems world could be a main cause for generating time-consciousness in biological systems (Chap. 11).

The newly established pragmatic communication-theoretical approach shows that causality in any particular form does not need to be a feature of every successful scientific explanation: Primarily the 'know that', the communicative rule accomplishing modular knowledge, which is mediated by the activity of biomodulatory therapy approaches is sufficient, whereas the 'know how' has to be further evaluated, again in a reductionist sense (Fig. 12.12). Introduction of communication derived rules may pragmatically resolve the problem of mutual causation of two phenomena [97].

A currently published 'evolution theory' proposes as evolutionary benchmarks some deeply grounded biological perspectives, i.e., 'natural selection acting on multicellular organisms to mold barriers and restraints, natural selection acting on infectious organisms to abrogate these protective mechanisms, and oncogenic selection which is responsible for the evolution of normal cells into cancerous cells' [98]: This way, biological systems disintegrate in the particularism of suggested relevant cuttings of the 'living world' in the sense of a neopragmatism.

An evolution theory allows a possible virtualization of the engagement to get (therapeutic) decisions via implementation of non-normative boundary conditions

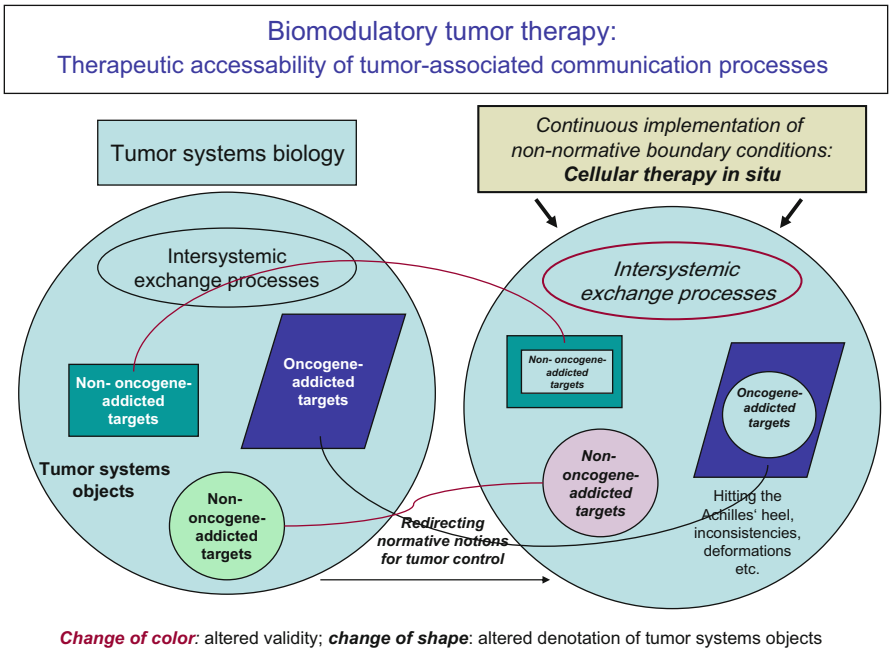

Fig. 12.13 In an evolutionary process, tumor and stroma cells may exploit the whole extent of evolutionary restricted rationalization features and tools of systems objects' modularity to implement the functional diversity of systems behavior aimed at promoting tumor growth, maintaining homeostasis and robustness towards perturbances. By therapeutic implementation of non-normative boundary conditions, rationalization processes and modular tools may be accessed for modulating normative notions of tumor systems (attenuation of tumor growth) via redemption of systems-constrained validity claims and consecutively systems objects' denotations

Biomodulatory therapy recommendations may be based on evolution theoretical considerations. The claim for objectivity on systems-biological processes studied via biomodulatory therapy approaches is based on a possible virtualization of the engagement to get experiences or decisions (Fig. 12.13). The virtualization is enabled by a discursive evaluation of hypothetical requirements for the validity of systems objects in a systems-biological model and hereby allows the generation of provable knowledge. These new methodological approaches for studying systems biology by a therapy-guided method may be an important supplementation of the established analytical/empirical studies on functional genomics in systems biology [50, 51, 68]. Therapies can be adapted (adaptive trial design) to situation-related and stage-dependent communicatively explicable evolutionary constraints by implementation of externally and internally derived modular knowledge.

Diversifying Palliative Care for Patients with Metastatic Cancer: Toxicity of therapy approaches and pharmaco-genomic aspects may be decisive in co-morbid or medically non-fit patients for decision-making. Communication-derived tumor

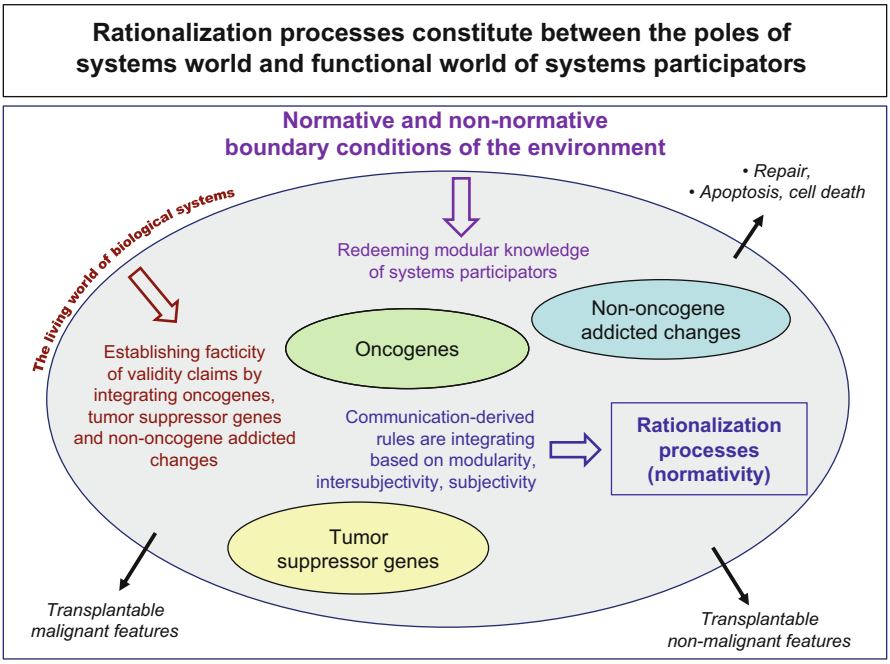

Fig. 12.14 Rationalization processes constitute between the poles of systems world and functional world of systems participators. Modulating determinants are the tumor's living world, normative as well as non-normative boundary conditions and the systems participators' modular knowledge. Rationalization processes are an attainment of tumor and stroma cells

pathophysiology will be a prerequisite for targeting multifaceted rationalizations of tumor-promoting normative notions (Fig. 12.14) (Chap. 19).

Personalizing Tumor Therapy by Novel Adaptive Trial Designs: By the possibility to virtualize the engagement to get situative experiences about tumor systems (communication-derived tumor pathophysiology) and decisions to tailor biomodulatory therapies, the possibility of an evolutionarily adapted modeling of cancer (cellular therapy in situ by adaptive therapies and novel adaptive trial designs) will continue to increase our understanding of tumor pathophysiology and may contribute to an evolution-oriented design of systems biological strategies. Adaptive trial designs aim at diagnosing and clinically managing tumor diseases on a novel personalized level (theranostics). Basic science is getting directly involved in the reconstructive process, even though an approach has been established directed from bedside to bench aiming at implementing clinical practical care (adaptive trial designs) as scientific object in patient care (Fig. 12.15).

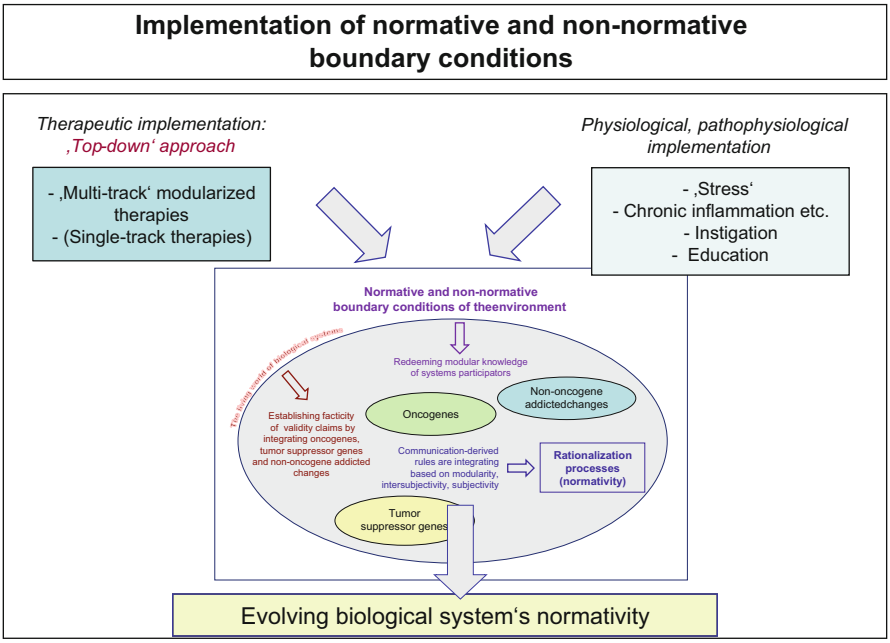

Fig. 12.15 Implementation of normative or non-normative boundary conditions to redirect and modulate biological systems' normativity: 'Top-down' strategies concertedly target rationalizations of tumor-promoting normative notions. 'Bottom-up' approaches aim at targeting single tumor growth promoting communication lines. Both approaches have in common that their efficacy is based on the redirection and modulation of rationalization processes (Chap. 22)

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Chapter 13
Modularity, Subjectivity, Intersubjectivity and Normativity: Clinically Applicable Operative Benchmarks

Albrecht Reichle

Abstract In the old tradition of disease management, an abbreviated relation was consistently established between experimental and clinical observations compiled in a reductionist manner and the daily practice of diagnosis and therapy. By now, the gap between medical theory and therapeutic practice has been further operationalized by a formal-pragmatic communication theory that tends to only care for the transparency of basic concepts, i.e., the universality of scientific description and situative systems-immanent validity claims. Because of its 'multilingualism', a formal-pragmatic communication theory may uncover unexpected coherencies, for example, between experimentally and clinically detectable normative notions, i.e., normative tumor structures, action norms (rapid displacing growth, the 'hallmarks' of cancer, etc.), decision maxims (hubs), and their corresponding established rationalization processes that are concretely responsible for the fail-safe constitution (robustness) of tumor-associated normative notions. The first diagnostic step is to reconstruct the prerequisites for the situative identity and function of the systems objects (cells, pathways, etc.) in a tumor system. The results of these efforts—which constitute evolution-adjusted tumor pathophysiology—may advect in completely new therapeutic approaches. Such approaches may consist of biomodulatory multi-targeted therapies that are directed at hubs of tumor-associated rationalization processes constituting distinct normative notions. The communicative nature of configuring novel tumor pathophysiology may launch and shape discussion levels also from a clinical point of view. Clinically oriented tumor pathophysiology is pragmatically deployed by the inclusion of communication-derived benchmarks, i.e., modularity, subjectivity, intersubjectivity and normativity. Necessarily, the starting points for categorizing rationalization processes are pragmatically selected for their final presentation and therapeutic implementation. Intersystemic comparison of rationalization processes plays a crucial role. The pluralistic procedure indicates the pragmatic function of a communication theory for personalizing tumor therapy.

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A Formal-Pragmatic Communication Theory as a Pluralistic Conception

A formal-pragmatic communication theory has a pluralistic conception. Its basic idea does not compose an own 'language', let alone a unique system: The theory demands scrutinizing the situative validity and denotation of systems objects in evolutionarily developing systems and aims at attributing evolutionarily confined communicative expression [1]. At this stage, novel instruments and resources may be provided for the reconstructive acquisition of evolutionarily founded scientific knowledge in evolution-adjusted tumor pathophysiology. The usefulness of these instruments has been demonstrated by means of reconstructing therapeutic results on biomodulatory and multi-targeted therapies [2]. The supposed universality of scientific descriptions and denotations may be derived from the position of a particular observer or from the evolutionarily modified validity claims of systems objects that arise from the view of a systems participator. This universality may be accounted for by rules, to which communication lines and communicative expressions adhere, but also by irrefutable presuppositions that are necessary to define the communicative expression of systems objects [3].

The 'Multilingualism' of a Formal-Pragmatic Communication Theory

A formal-pragmatic communication theory only cares for the transparency of basic concepts, i.e., the universality of scientific descriptions and situative systems-immanent validity claims. Because of its 'multilingualism', such a theory may uncover unexpected coherencies, for example, between experimentally and clinically detectable normative notions, i.e., normative tumor structures, action norms (rapid displacing growth, accumulating tumor cells due to apoptosis resistance, dysplasia, the 'hallmarks' of cancer, etc.), decision maxims (hubs, nodes) and their corresponding established rationalization processes. Such processes are concretely responsible for the fail-safe constitution (robustness) of tumor-associated normative notions. In this way, basic assumptions of a formal-pragmatic communication theory branch into biological structures and functions at multifaceted discussion levels of communicative behavior. All these possible starting points for communication-derived pathosphysiological considerations must be newly created and pragmatically systemized.

Reconstruction of Cellular Identity and Function

The first step in reconstructing the prerequisites for cellular integrity, identity, and function within a tumor system results in the notion of the tumor's 'living world' (Table 13.1) [1]. The functionality of the tumor's 'living world' is characterized of

Table 13.1 Evolution-adjusted tumor pathophysiology: Prerequisite for an evolution theory

- Systemizing tumor-associated normative notions and corresponding rationalizations under pragmatic aspects
- Systemizing presuppositions for distinct communicative expressions of systems objects (communication lines, cells etc.)
- Recording the 'background knowledge' of a systems object (the tool of multifaceted communicative expressions dependent on various evolutionary linked presuppositions)
- Categorizing patterns of chromosomal aberrations and corresponding rationalization processes
- Figuring out critical hubs in conflicting cases, which provide normative regulations of strategic interactions on the basis of acquired chromosomal aberrations (tumor development) or may lead to interruption of essential communication processes (lack of communication may lead to cell death)

how, for instance, effects and side effects of therapeutic interventions may be intercepted within a social cellular system. By interception, cellular integrity (robustness, intrinsic resistance) can be maintained if the cellular system is threatened by the tension between facticity (therapeutic intervention) and corresponding validity claims within evolutionarily constrained tumor-associated system contexts.

The communicative nature of configuring evolution-adjusted tumor pathophysiology may launch and shape the discussion levels also from a clinical point of view: Which rationalization processes may, for example, constitute rapid tumor growth (Fig. 13.1)? Can these processes be categorized (Table 13.2)? Do 'universal' hubs exist among rationalization processes that may serve as 'universal' targets? What are the critical hubs in conflicting cases that provide normative regulations of strategic interactions on the basis of acquired chromosomal aberrations (tumor development) or that may interrupt essential communication processes? What tumor-associated normative notions (e.g., disease traits) should be targeted to improve overall survival in metastatic cancer as well as to specify palliative care? To answer such questions, clinical tumor pathophysiology is pragmatically deployed by the inclusion of communication-derived rules that are covered by a tumor's living world, which represents a holistic communicative system. Necessarily, the starting points for categorizing rationalization processes are figured out comparatively and are discursively selected for their final presentation and therapeutic implementation [4]. The pluralistic procedure indicates the pragmatic function of a communication theory for establishing evolution-adjusted tumor pathophysiology.

Evolution-Adjusted Tumor Pathophysiology

Evolution-adjusted tumor pathophysiology is now ready to be established for diagnosing the situative pathophysiological status, synonymously with the evolutionarily

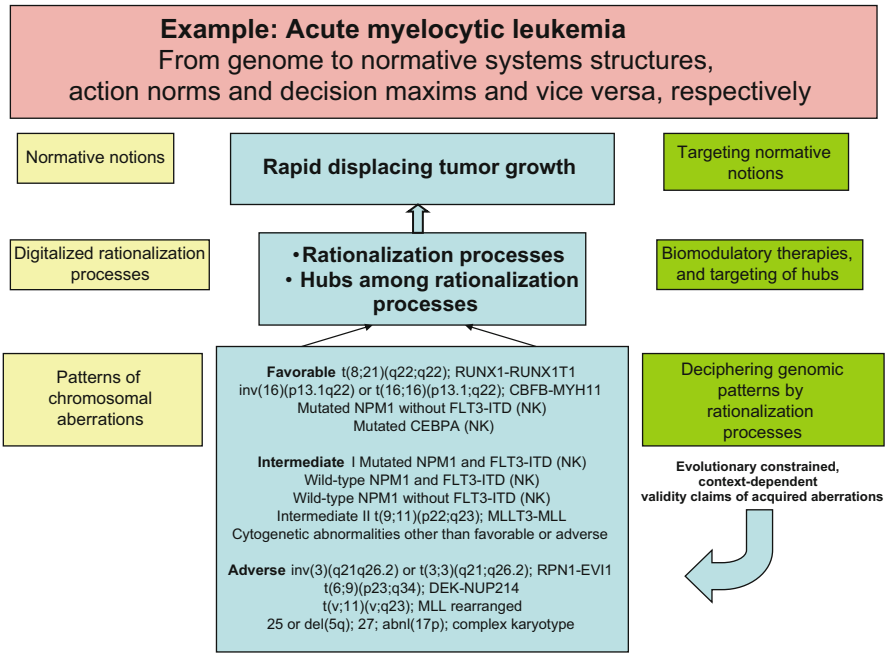

Fig. 13.1 The normative notion, 'acute displacement of normal hematopoiesis in bone marrow' is a common feature in 'acute' leukemia. This leukemia-associated notion may be realized by a myriad of non-random chromosomal aberrations. Tumor-immanent rationalizations processes for 'rapid displacement' must be readily comprehensible because of a manageable number of possible tumor-associated structures. Thus, common hubs are postulated that may be triggered by multi-faceted pathways on the background of diverse chromosomal aberrations. Combined modularized (biomodulatory) therapies aim at modulating and redirecting tumor-associated normative notions and help decipher genomic patterns via the identification of rationalization processes

Table 13.2 Benchmarks of a communication theory as essential part of an evolution theory

- Describing systems' robustness
- Working out the polarization between success- and integration-oriented behavior
- Evaluation of modular systems features
- Categorizing rationalization processes of normative notions beyond tumor histology
- Characterizing nodes among rationalization processes

constrained systems-mediated identity and function of systems objects (cellular compartments, 'driver' aberrations, etc.) in a tumor. Pathophysiology in the novel sense does not aim at presenting 'glass patients': A formal prerequisite for the coherency of a physician's conceivability of a disease is the comprehension of the basic identity of the pathophysiology of a tumor disease [5]. By contrast, evolution-adapted tumor pathophysiology complements classic pathophysiology as well as pathology with completely new aspects: the communication-derived, situatively multifaceted identities and functions of systems objects, rationalization processes, and modularity, including the 'background knowledge' of systems objects. Evolution-adapted tumor pathophysiology aims at diversifying diagnostic and therapeutic instruments [6]. The personalization of tumor therapy is now being enhanced by applied systems biology.

Traces of exclusive scientific normativism peter out in the dilemma. The substance of the current scientific knowledge about tumors dissolves in its exclusive object-related configuration and is ready to be newly arranged as a systems-mediated communicative subject. Thus, current scientific knowledge may be not substantiated, neither in the common teleology of an evolution history nor in the constitution of systems objects themselves nor from the random fundus of successful explanations for evolutionary leaps.

However, the formal-pragmatic communication theory allows—in concurrence to practiced normativism—the attribution of communicative competence to objects of a biological system to give them the status of scientifically accessible systems subjects. This attribution contrasts with the current assumption that systems objects are characterized by inherent normative and practical competence, which is seemingly independent of evolutionarily changing communicative presuppositions (universality of scientific descriptions and validity claims).

Consequently, the physical evidence of a systems object must be supplemented by the systematic evaluation of its context-dependent, evolutionarily constrained validity and denotation for establishing evolution-adapted tumor pathophysiology. Comprehending evolutionary constrained systems behaviors is of even higher interest, the broader the ascertained variability of acquired genetic aberrations constituting a distinct tumor-associated normative notion, i.e., normative structures, action norms, and decision maxims within a distinct histological tumor type or among different histologically specified tumors. Novel tumor pathophysiology may initiate the deciphering and categorizing of patterns of chromosomal aberrations and corresponding rationalization processes for therapeutic purposes (Fig. 13.1). The evaluation of the situative identity and function of systems objects is now gaining therapeutic interest and affords the routine introduction of novel technologies, such as imaging techniques, secretome analytics, etc. Evolution-adjusted tumor pathophysiology supplies the situate description of the evolution-based identity and function of systems objects and works hand in hand with classic pathophysiology, histopathology, and molecular pathology.

The Gap Between Theory and Practice

In the old tradition of disease managing, an abbreviated relation was established between experimental and clinical observations compiled in a reductionist manner and the daily practice of diagnosis and therapy. By now, the gap between medical theory and therapeutic practice has been further objectivized and operationalized by a formal-pragmatic communication theory. Novel pathophysiological considerations could impede trial designs that repetitively confirm the gap between the universality of scientific descriptions and situative systems-immanent validity claims.

The old reductionist tradition got in the focus of normative issues and corresponding therapeutic procedures: An acquired chromosomal aberration, a 'driver' mutation, etc. is considered independently of its context, although this context has the capacity to decisively regulate the validity and denotation of the respective tumor systems object, namely its communicative expression. To put it in exaggerated terms, the further development of reductionist comprehension leads to the therapeutic implication that physicians should simply reach into their 'magic box' to select small molecules for targeting 'driver' aberrations analyzed by whole genome analytics. This procedure may be possible in tumors with consistently reproducible sequences of genetic and pathway alterations in tumorigenesis. However, the constitution of normative notions of a phenotypically (clinically, pathologically) characterized tumor type by multifaceted patterns of genetic aberrations increases the importance of reconstructive activities and the systemizing capacity of evolution-adjusted tumor pathophysiology.

Orientation Towards Validity Claims of Tumor Systems Objects

The formal-pragmatic communication theory for biological systems allows an orientation towards validity claims of tumor systems objects, which may be a basis for categorizing pathophysiological tumor processes. One priority is the facticity of the identity and function of a systems object derived from basic science and disease models that are derivated from arbitrary biological systems. The second—and conflicting—priority is the corresponding evolutionarily confined validity and denotation of the systems object in another (clinical) biological system, for instance, a patient with a tumor disease. These conflicting priorities may be resolved by consequent pre-therapeutic reconstructive activities, such as clinical pathophysiology. The results of these efforts that constitute evolution-adjusted tumor pathophysiology may advect in completely new therapeutic approaches. Such approaches may consist of biomodulatory multi-targeted or targeted therapies that are directed at hubs of tumor-associated rationalization processes constituting distinct normative notions, to some degree probably even irrespective of the histological type of tumor.

Table 13.3 Accessibility of evolutionary processes and practical relatedness

Accessibility of evolutionary processes:
• *Evaluation of the therapeutic scope for the implementation of non-normative boundary conditions (primarily multi-track therapy as 'top-down' strategy)*
• *Providing technologies for monitoring evolutionary constrained validity claims via reconstructive efforts (molecular imaging techniques, cellular secretome analytics, epigenetics, monitoring of tumor dissemination etc.)*
Practical relatedness of an evolution theory for tumor therapy:
• *Personalizing tumor therapy by implying validity claims of pathophysiologically relevant systems objects*

Rules Imposing Constraints on Systems Objects

Communication-derived pathophysiology involves rules that impose constrains on systems objects. For instance, a systems object is compelled to adapt its behavior in correspondence to the attitude of a strategically acting systems object or to non-normative, therapeutically implemented boundary conditions (biomodulatory therapies). Communication-derived rules may also serve to unfold integrative forces by imposing obligations on systems objects, which is only possible—according to the given prerequisites—on the basis of intersubjectively acknowledged normative validity claims (Table 13.3). Concomitance of factual compulsion—on the basis of acquired chromosomal aberrations—and a legitimate validity claim may cause the disposition of stroma cells to follow and support the aberrant cell compartment.

Redirection of Normative Notions: Success-Oriented and Integration-Oriented Behavior

This novel tumor pathophysiology allows the differentiation of the polarization between success-oriented and integration-oriented behaviors, as tumors equip a biologically possible validity pegged to a systems object with the strength of facticity (corrupt rationalization) under the conditions of a perceivable incompatibility between facticity and validity. Between these conflicting priorities, the tumor disease unfolds and 'branches' according to rules that require further evaluation. Stochastic principles or Darwinian selection models as a basis for clonal heterogeneity are commonly suggested as the driving regularity (Table 13.4) [7]. At that stage, reciprocally acting communicative rules are added.

The facticity of 'anyhow' altered systems objects—due to acquired tumor-associated molecular-genetic aberrations—meets the other acting systems objects within their living world, which comprises their background knowledge for integration-oriented behavior. Then, the living world is not any more appreciated

Table 13.4 Defining the term tumor disease communication-technically

Evolution-adjusted tumor pathophysiology allows the differentiation of the polarization between success-oriented and integration-oriented behaviors, as tumors equip a biologically possible validity pegged to a systems object with the strength of facticity (corrupt rationalization) under the conditions of a perceivable incompatibility between facticity and validity.
Between these conflicting priorities, the tumor disease unfolds and 'branches' according to rules that require further specification by reconstructive activities.
Basic communicative benchmarks in biological (tumor) systems are: Normativity, modularity, modular knowledge, subjectivity, intersubjectivity, interfaces of rationalization processes; epitomized 'flexible' and 'fixed' reasons are constitutive for a tumor's living world; normative or non-normative boundary conditions of a system

from the formal-pragmatic perspective of a participator but reified from the perspective of an observer. In case of conflict, the communicative actors face multifaceted alternatives, such as the termination of communication that is probably associated with communication-deprived cell death, activation of cell death pathways, recruitment of repair mechanisms, development of resistance; in more abstract wording, the horizon of the metabolism of evolution provides a solution within the evolutionarily constrained living world of a tumor [8].

A solution to the conflicting goals is offered by a normative arrangement via novel rationalization processes, to which the participators may agree within evolutionarily constrained limitations. The paradox nature of such agreements is shown on the background of the premises that facticity and validity are dissociating in two mutually exclusive dimensions for the respective acting systems objects [9].

For success-oriented actors within an evolutionary process, all components of a situation convert into facts, which are appreciated by the addressees. These facts start with the physical and biological preferences of the actors, whereas integration-oriented actors depend on a general comprehension of a situation in the frame of processes maintaining robustness. Relevant facts are interpreted within 'routine' intersubjectively acknowledged validity claims.

If such success-orientation and integration-orientation is to represent a mandatory arrangement for sufficing two contradictory requirements for all participators—which would be a realistic alternative for the respective tumor systems objects as actors—then scientifically accessible rules must be available facilitating the actors to simultaneously fulfill these requirements. Such rules impose constraints, which alter the interpretation of the available data profile in such a way that—on the background of a strategic acting systems object—a respective addressee is forced to take over even 'corrupt' behavior, as it is the case in tumors. On the other hand, the rules have to unfold a socially integrative function by imposing obligations on the addressees. This balancing communication act is only possible by the intersubjective acknowledgement of normative validity claims, which are realized in concrete rationalizations. The respective norms have two qualities, i.e., they exert compulsion and—on the background of their legitimacy—disposition after the compulsion.

Therefore, the norms are able to regulate the perceived incompatibility of facticity and validity [9].

An important task of evolution-adjusted tumor pathophysiology is to assess respective norms and corresponding rationalizations. Biomodulatory therapies may contribute to uncover communication-derived rules by exhausting the evolutionarily constrained tools of rationalizations available for attenuating tumor growth [10].

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Chapter 14
Turning Upside Down the Mode of Science to Emphasize and Harness the Impact of Environmental Communication

Albrecht Reichle

Abstract Bridging theory and practice remains the very own endeavor of clinicians; such bridging is aimed at expelling science from the 'niches' and at establishing the upside down of the scientific mode. The suggested steady on-going transition of the phenotypes of biological systems objects has become an object of institutionalized scientific interest to ultimately conceive transient evolutionarily confined systems stages or even heterogeneity among metastatic tumor sites. Providing methodologies for reconstructing the situative communicative expression of systems participators is the novel field of evolution-adjusted tumor pathophysiology. The evolution theory is based on the assumption that biological processes are interwoven with communication and represented and reproduced through communication acts to facilitate communicative expression: A tumor system not only consists of diverse cell types and pathways—termed 'tumor systems objects'—but also comprises all components of action insofar that these components are oriented in terms of diverse cell types. The components of action are organized in communication acts. Communication within a biological system is closely linked to descriptively accessible 'learning' processes, contingency programming, adoption of the players, and the systems objects within a tumor system. An evolution theory should operationalize the 'metabolism', facilitating the spinoff of novel systems functions. Furthermore, such a theory is aimed at covering some practical, i.e., diagnostically and therapeutically relevant issues to convince the scientific community that the evolutionary concept lacks proper appreciation, both for diagnostic and therapeutic issues. For many diseases, such as metastatic tumors that have undergone countless years of evolution, a stepwise and evolution-adjusted therapy rather than drastic therapeutic interventions based on theme-dependent knowledge may be an alternative for achieving medical improvements. Thus, paradox situations of cellular rationalization, deformation, and communication processes need to be decoded or, in other words, it is necessary to uncover inconsistencies within tumor cell compartments or distinct topologies of aggregated action effects.

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Introduction: The Reductionist Mode of Science

Scientists are interested in separating new insights from already existing knowledge. Nevertheless, researchers want new knowledge to be delivered well-tried in the setting of familiar circumstances.

The daily content of scientific output should be exceptionally new, but the presentation of scientific data should always remain the same. Thus, scientists are wary of the mixed bag of biological phenomena they encounter daily, about the potpourri of inter-individual, even intra-individual differences in tumor diseases in real life.

Researchers rely on scientific journals, because articles are reviewed with certain background knowledge on particular experimental circumstances, which again rule supreme in the studied niches. This way, habituation and order is generated in contrast to innovation. Scientists know with certainty that the objects under investigation do their particular job in familiar systems. Any reported change of the phenotype of a systems object gives rise to suspicion: What is the difference to former experiments? Are there any mistakes in the experimental procedure, or has the denotation of the systems object changed? Did the 'communicative' circumstances perhaps change the validity and denotation of a systems participator?

Boundary Conditions Make up the Difference

In contrast, physicians are challenged by these 'suggested' individual biological 'variants' every day. Therefore, the call for standard operating procedures arises to bring into line some therapy-relevant normative notions [1].

Basic science commonly provides clinicians with knowledge about reductionistically derived therapeutic targets: In the reductionist view, the presence of distinct targets in arbitrary systems stages is sufficient for these targets to be assigned to situatively invariant communicative expressions, independently of their evolutionarily confined context (Fig. 14.1). The evolutionary context, i.e., steadily moving and evolving boundary conditions in which systems participators are embedded, is based on variable acquired genetic or molecular-genetic aberrations [2]. To rudimentarily elaborate evolutionarily confined contexts, the term 'disparate' oncogene has been generated, indicating that the validity and denotation of oncogenes have changed within an evolutionarily confined systems context.

Clinical standard operating procedures principally refer to reductionist considerations on targets and their communicative expression but represent the essential back-up for both, physicians and patients. Invoking 'experience' and separation of multifold evolution histories are the commonly used alternative strategies for bridging discrepancies between theory and practice. To some extent, those normatively acting back-ups provide innovative lineaments and may expose and highlight a single 'case' exemplarily.

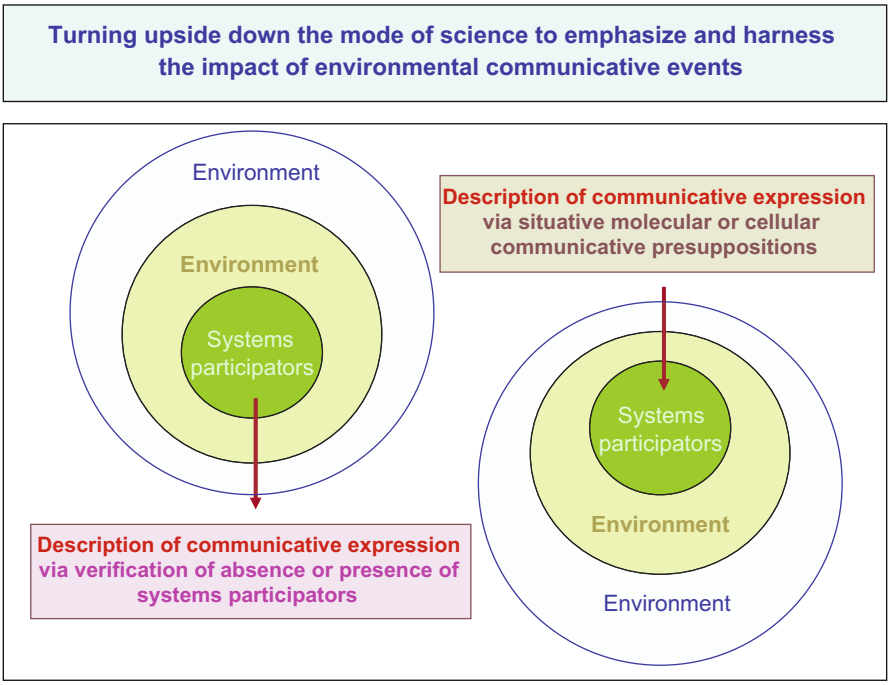

Fig. 14.1 Turning upside down the mode of science to emphasize and harness the impact of environmental communicative events

Bridging theory and practice remains the very own endeavor of clinicians [3]. Therefore, physicians take on the role of providers of consistent starting points. Evolution-theoretical considerations make novel instruments available for integrating reductionistically derived knowledge into the interpretation of evolutionarily confined situative systems stages. This unavoidable reconstructive step pragmatically implements a communication theory, now also for biological processes.

Expelling Science from 'Niches': From 'Bottom-up' to 'Upside down'

Only communication-derived tumor models can turn the mode of science upside down: The described formal-pragmatic communication theory facilitates the introduction of an evolution theory, contrasts traditional evolution histories, and takes over the view of a systems participator. Thereby, this theory conveys that evolutionarily changing communicative presuppositions in tumor systems (1) account for the therapy-relevant situative validity and denotation of systems participators,

(2) are available for systematic reconstruction, and (3) serve as novel targets for tumor therapy.

Sometimes, seemingly 'stale news' have to be gingerly renewed and novel contents need to be integrated into the tool of available reductionist knowledge.

Turning the mode of science upside down appears to be a scientific revolution: The suggested steadily on-going transition of the phenotypes of biological systems objects becomes the object of institutionalized scientific interest to ultimately conceive transient evolutionarily confined systems stages or even heterogeneity among metastatic tumor sites. Providing methodologies for reconstructing the situative communicative expression of systems participators is the novel field of evolution-adjusted tumor pathophysiology.

From 'Everything Flows' to the 'Metabolism' of Evolution

Everything flows (Πάντα ῥεῖ) is a famous aphorism used to characterize Heraclit's thoughts (a pre-Socratic Greek philosopher, c. 535–c. 475 BC) that was phrased by Simplicius, a neo-platonist. The quote from Heraclit 'all entities move, and nothing remains still' appears in Plato's *Cratylus*. But what are the benchmarks of the 'metabolism' of evolution in biological systems, driving the 'flow' of phenotypes, validity, and denotation of systems participators between the two poles of success-oriented and integration-oriented behaviors?

Taking Account of the Steady Vagueness of the Validity and Denotation of Systems Participators

Evolution-adjusted tumor pathophysiology makes a point of pursuing the situative validity and denotation of seemingly familiar tumor-associated systems objects. Particularly, the modular knowledge of systems participators matters to physicians when designing novel and more specific and personalized therapeutic strategies. The context-dependent validity and denotation of systems participators alerts that systems objects cannot be assigned to a distinct phenotype independent of the knowledge about evolutionarily confined communicative circumstances [4]. These communicative circumstances evolving in a multifaceted manner in tumor systems are often unpredictable.

Which particular facet of the modular knowledge of a systems participator is realized in an evolutionarily confined stage? Is it known at all? At that stage, we have to deal with the vagueness of situatively arising validity claims of systems objects and their modular but digitalized 'flows' ('metabolism' of evolution) within evolving biological systems.

Adapting Communication-Derived Rules

If communication is reciprocal, it is also subjective, thus representing a continuous obstacle for basic sciences, particularly as long as communicative presuppositions are evolutionarily confined and changing in a therapeutically relevant manner, as observed in most tumor types [5]. However, subjectivity may be objectified based on the digitalized, non-arbitrary mode of modular communicative interactions. Digitalization, which does not principally exclude analogous processes, such as threshold setters, provides the starting point for keeping track of communicative rules. Finally, communicative rules find their counterpart in the digitalized genetic system. Again a digitalized continuum has been re-established by introducing communication-theoretical aspects. Simultaneously, a window could be opened for reinterpreting tumor-specific genomic profiles.

We should find the strength to resolve the communicative obstacles by systematically addressing the situatively arising, evolutionarily confined 'flowing' phenotypes of systems objects and the concrete situative validity and denotation within evolving systems.

Distinct evolutionarily developing communicative presuppositions decisively alter the validity and denotation of systems objects in a modular way, thereby subjecting systems participators to the inevitable requirements of tumor systems at a distinct stage. Commonly, we uncritically assign our reductionistically derived perceptions of the communicative expressions of systems participators to newly arising biological systems, as we rely on familiar communicative expressions derived from arbitrary 'historical' experimental settings. Suggested communicative expressions may be situatively valid in case of an evolving biological system (i.e., tumor system), as long as the communicative context of respective systems objects only supports the communicative expressions originally observed in experimental settings. In these cases, the respective communicative expression of systems participators would be called evolutionarily conserved, but the communicative expression of systems participators is frequently not conserved in tumors!

Tumor Disease: A Communication-Technical Perspective

Multifold and not necessarily predictable, but non-randomly acquired chromosomal aberrations in tumors cooperate in a life-maintaining manner: Thereby, tumors equip a biologically possible validity pegged to systems objects with the strength of facticity ('corrupt' rationalizations) under the conditions of perceivable incompatibility between facticity and validity. Between these conflicting priorities, tumor disease is unfolding and 'branching'. Success-oriented 'corrupt' behaviors may exploit the entire modular knowledge of systems participators in the range of the evolutionarily confined 'living world' of tissues. Such a communication-based definition of the term tumor disease refers to the polarization between success-oriented and integration-oriented behaviors in biological systems.

Normative Notions are Characteristic Systems Features and thus Specifically Rationalized

Communication between systems participators of a tumor disease is subjected to claims from the systems participators and simultaneously to claims from the tumor system. Decisive relevance for differential therapy strategies is achieved by intentions to communicate, the selection of normative notions for therapeutic targeting, the multifaceted rationalizations of normative notions at the tumor site (inflammation, angiogenesis, immune response, etc.) [6]. How, for example, is tumor-associated inflammation organized in an evolutionary context? May inflammation control or promotion be a starting point for attenuating tumor growth or, at least, in a concerted and combined modularized therapeutic approach?

The multifaceted rationalizations constituting distinct tumor-associated normative notions are traditionally faded down in the scene of science. However, the physical origin of normative notions is subjective, i.e., dependent on the requirements of biological systems as well as on the identity and function of their systems participators: The systems context challenges the validity claims and denotations of tumor-relevant systems participators within the range of their 'modular knowledge', thereby generating specific communication-associated pathologies. These new kinds of pathologies should be systematically uncovered for answering the questions, which cell compartment predominantly promotes a distinct normative notion, which cell types are the teammates, what kind of communication lines are initiated, where are the communication-technical bottlenecks to therapeutically meet the Achilles heel?

Broadening and Diversifying Therapeutic Approaches

When considering intersystemically comparable normative notions among tumors and different therapeutic intentions, it becomes obvious that discursively acquired agreements among scientists and pragmatists must gain unrestricted importance within the scientific world (formal-pragmatic communication theory). Mutual consents serve as pragmatic instructions for starting therapy-relevant communication strategies in tumor systems, for instance, by implementing non-normative boundary conditions into a tumor's systems world ('living world') [1]. The aim of combined modularized therapies is to specifically redirect the always interdependent and tumor-immanent normative notions, which concertedly support tumor expansion (success-oriented behavior).

The communicative management of tumor diseases becomes the centre of attention for therapeutic considerations—besides the traditional strategies aimed at targeting particular tumor-associated systems objects regardless of their situative, evolutionarily confined validity and denotation. By redirecting communicative presuppositions, it is feasible to therapeutically attribute altered validity and denotation to originally tumor-relevant and growth-promoting systems objects for attenuating tumor growth, as shown in a series of phase II trials [4, 7].

Expanding Technologies in a Novel Setting

How can we advance knowledge about communicative presuppositions in a scientific way to harness evolutionarily confined communicative circumstances for therapeutic approaches?

The routine pre-therapeutic and follow-up evaluation of the communicative expression of tumor participators may be pragmatically used to establish novel therapies designed for implementing non-normative boundary conditions into a tumor systems' world as well as a novel kind of adaptive trial design.

Combined modularized therapies (biomodulatory therapies) seem to be easily manageable and tractable (adaptive trial designs) by routinely introducing cellular secretome analytics (defining the function and identity of tumor-associated cell compartments), molecular imaging techniques (focusing on monitoring the 'hallmarks' of cancer), or by comparative uncovering the biology of tumor systems by modularly targeting tumor systems [8–11]. The focus on uncovering the boundary conditions of systems participators to comprehend their situative communicative expression in comparable tumor systems turns the mode of science up-side down and requires the establishment and expansion of the mentioned technologies in a novel setting.

Systematization and Integration of Tumor-Associated Normative Notions in a Scientific Context

Normative notions of tumor systems are commonly pooled as the so-called 'hallmarks' of cancer [12]. This synopsis immediately suggests a limited pool of normative notions that promote tumor growth. But these notions have been continuously extended and presented within a closed circle, indicating that rationalizations of normative notions are closely interconnected via intersystemic exchange processes. However, the classic 'hallmarks' of cancer are incomplete for defining the multifaceted qualities of normative notions at a tumor site, thus requiring extension: Tumor-associated normative notions comprise rather different qualities, i.e., cellular structures, action norms, as well as decision maxims (hubs within rationalization processes). The tool of tumor-associated normative notions can be pragmatically selected and depends on discursively attained (subjective) understandings as well as on the historical reductionistically derived knowledge available. Completely 'new' hallmarks may be singled out. For example, common hubs within different rationalization processes constituting a distinct normative notion would provide pivotal targets. Such targets (hubs) do not necessarily have to be derived from oncogenes or tumor suppressor genes!

Focusing on the investigation of tumor-associated normative notions necessitates the selection of adequate, intersystemically comparable normative notions. Corresponding rationalization processes should be experimentally accessible and detectable within diverse groups of histological tumor types. Evolution-adjusted tumor pathophysiology introduces a completely new tumor classification based on the

diversity of rationalizations for single normative notions and commonly operated hubs within rationalizations.

The pre-selection of tumor-relevant and especially therapeutically important normative notions for comparative purposes is the starting point of scientifically outlining the diversity of rationalizations that constitute distinct tumor-associated normative notions meant to detect the multifaceted tools available for realizing distinct normative demands in tumor systems (redundancy). Systematization of rationalizations available for selected normative notions leads to a novel systematization of tumor pathophysiology regardless of tumor pathology.

Reconstruction of Normative Notions: Diagnostic Approach of the Novel Tumor Pathophysiology

The convenience of reconstructing tumor-associated normative notions entails methodologically aligning 'corrupt' rationalizations promoting tumor growth, with non-normative boundary conditions available to be therapeutically operated for attenuating tumor growth. Rationalizations can be specified for the different assignment of therapeutic targets. A novel scientific tool presents itself that is not only utterly underestimated but also ethically of pivotal relevance.

The reconstruction of rationalizations that constitute distinct normative notions involved in promoting tumor growth is a novel clinically oriented approach. As mentioned beforehand, rather different technologies are available for establishing evolution-adjusted tumor pathophysiology and for re-categorizing tumor systems, particularly for therapeutic purposes. Here, reconstruction is available from the view of a systems participator, which makes us cope with the vagueness of the validity and denotation of a systems participator in an evolutionarily confined systems context. Classic pathology takes up the position of a systems observer, thereby informing us about the presence or absence of systems participators and communication lines. Classic pathophysiology implies 'historical' relationships derived from arbitrary biological systems to be applicable in novel evolutionarily confined systems stages. All views are absolutely complementary.

Routine evolution-adjusted reconstruction provides completely new pathophysiological insights, which results in a novel pathosphysiologic classification, making accessible communication-relevant therapeutic targets that may be seized by therapy strategies designed for redeeming modular knowledge of systems participators with the aim of attenuating tumor growth.

Evolution-adjusted tumor pathophysiology brings diagnostics and therapy closer to the demands of a personalized tumor therapy by reconstructing multifaceted situative boundary conditions of systems participators arising in evolving tumor systems. The evolutionarily confined communicative context ultimately defines the communicative expression of systems participators in a therapy-relevant manner. Considering communicative boundary conditions within an evolution theory

detaches attempts of rigid stochastic explanations of cell fate decisions and establishes reciprocal communicative response between 'stem cells' and their environment as permanent feature [13, 14].

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Part VI
From Genome- or Organ-centric to Rationalization-centric Systematization of Tumors

Chapter 15
Purposive-Rational Tumor Therapy: Exploiting the Tumor's 'Living World' for Diversifying, Specifying and Personalizing Tumor Therapy

Albrecht Reichle and Gerhard C Hildebrandt

Abstract Evolution-adjusted tumor pathophysiology introduces the view of systems participators to assess evolutionarily constrained validities and denotations of systems participators, and contrasts with the manacle of the classic disciplines, pathology and pathophysiology, which provide the view of observers. The differential perspective of communicative interaction applied by an evolution-adjusted tumor pathophysiology (1) involves the comprehension of tumor's systems features at diagnosis by accentuating the communicative aspects of a situation's analysis, (2) allows situating identity and function of systems participators as systems subjects during therapies modulating communication, (3) facilitates to describe the tumor's 'living world' comprising (all) endogenously or therapeutically redeemable validity claims and denotations of systems objects, (4) contributes to select and specify purposive aspects at diagnosis and during therapy to pragmatically configure and modulate available evolutionary based rationalization processes of normative notions (theranostics), and (5) affects the technologies to interfere with communication based pathologies in a tumor (adaptive trial designs). Evolution-adjusted tumor pathophysiology provides contently and methodologically novel approaches to succeed in personalizing tumor therapy, and should be introduced as clinically orientated discipline, equivalent with traditional disciplines, thereby increasing their value and accomplishing ethical demands. A tumor type-specific, systems stage-specific, metastatic site-specific or disease trait-orientated therapy seems to be within grasp.

Introduction

> Science does not ask, what did us provoke to aim at something; rather, it negates that we had aimed at, and reckons that something other has happened,—briefly, that the belief in 'will' and 'purpose' seems to be an illusion . . . . Its task is absolutely unresolved. (Friedrich Nietzsche. Will to power. 667. Kröners 78; 1930)

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The presence of tumor-promoting normative notions cannot be neglected. In contrary, they are the impulse for organizing normative structures, action norms and decision maxims of tumor and adjacent stroma cells. Tumor-associated normative notions may be comprehended under the aspect that they aim at achieving concrete purposes of a tumor system. Knowledge about purposes of tumor systems objects' communicative expression (i.e., of cells, pathways etc.) strongly depends on the current reductionist knowledge derived from arbitrary (pre-clinical) biological systems and from empirical data, which have been collected from highly diverse evolutionary confined biological systems, including those in human tumors [1, 2]. Based on these empirical data, additional identities of systems objects may be uncovered in developing biological systems due to the presence of novel evolutionary constrained contexts [3].

Evolution-adjusted tumor pathophysiology may engage in therapeutic decision making and employs data derived from reconstructive studies on communication-mediated, stage-dependent identities of tumor-associated systems objects [4, 5]. The assessed communication-derived data tools allow for isolating rationalization processes of tumor-immanent normative notions (the explicit organisation of normative notions, e.g., the 'hallmarks' of cancer) and facilitate to attribute situatively divergent validities and denotations of systems objects dependent on the present evolutionary confined biologic system.

Reconstructive activities (i.e., biomodulatory therapies, molecular imaging techniques, cellular secretome analytics etc.) assess with novel, and yet evolving diagnostic tools (1) communication-based presuppositions, which are positioned by the respective tumor system and to which systems participators are situatively subjected, and (2) the modular knowledge of systems objects, that means the given frame whereby respective identities and functions of systems objects may be preserved and changed (differentiation, de-differentiation, transdifferentiation) (Fig. 15.1, 15.2) [4–7, 8]. Aim of reconstructive studies is to describe the evolutionary confined validity and the denotation of the respective systems object as prerequisite for any 'personalized' tumor therapy.

Reconstructive approaches comprise all levels of scientific comprehension and should be applied in practice, both diagnostically and therapeutically, if evolutionary processes are expected to play a central role, as to be suggested in developing tumors. The present paper aims at unfolding the practical impact for routinely establishing reconstructive methodologies (clinical pathophysiology), i.e., to specify cancer screening, diagnosis, staging, treatment planning, response assessment and clinical trial design in the frame of an evolution-adjusted tumor pathophysiology.

The task of reconstructive approaches is to transform theme-dependent knowledge acquired by reductionist considerations in initially arbitrary systems contexts into evolution-adjusted knowledge on tumor pathophysiology through the introduction of reconstructive methodologies in daily routine diagnostic care [9].

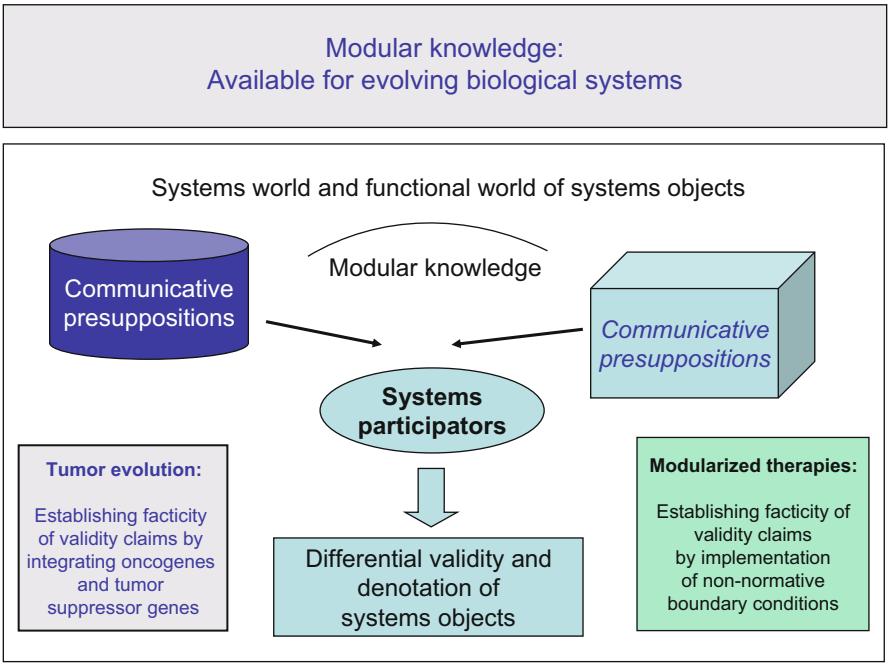

Fig. 15.1 Modular knowledge of participating systems objects is an attribute on which the 'metabolism' of evolution draws on during tumor evolution ('corrupt' rationalizations) or during implementation of non-normative boundary conditions with combined modularized therapy elements for attenuating tumor growth

Screening: Monitoring the Evolving Stroma-Tumor-Cell Interactions

Population-wide risk evaluation and 'individualized' risk estimation is currently guided by biomarker profiles or signatures from quite heterogeneous observational levels, which have been originally assessed in arbitrary evolutionary systems, then theme-dependently transferred in human pre-malignant or malignant lesions and empirically proven to be predictive in large (patient) populations [10]. These markers, however, are used irrespectively of their evolutionary constrained situative validity and denotation [11].

The perspective of a participator affords to evaluate the situative validity and denotation of tumor or pre-malignant systems objects of interest. The evolutionary confined situative identity of tumor-associated systems is principally difficult to validate for therapeutic purposes, secondary to unknown and poorly functionally interpretable, sometimes complex molecular-genetic signatures in both, tumor and adjacent stroma cells [12–14].

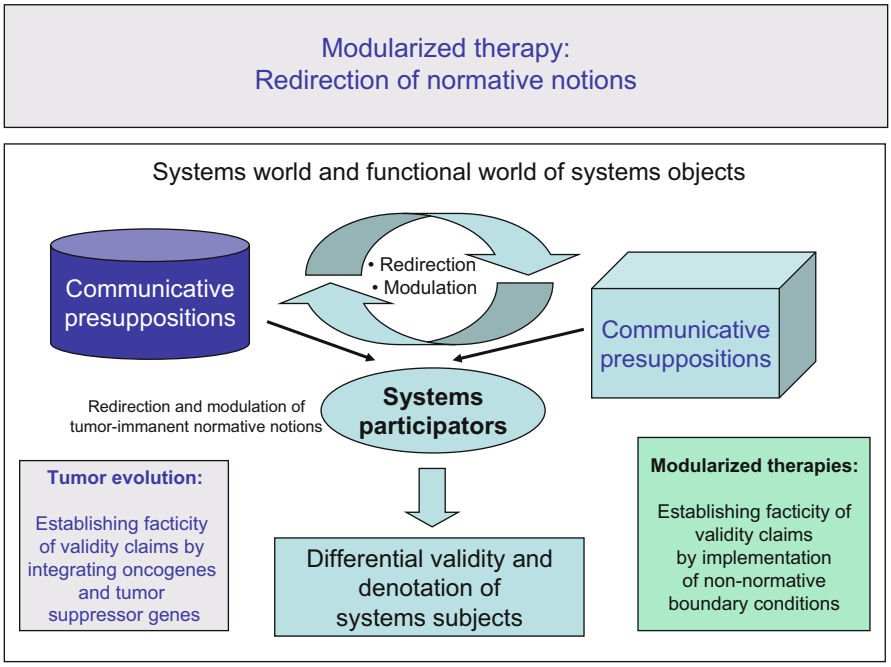

Fig. 15.2 By implementing non-normative boundary conditions the communicative presuppositions may be redirected and modulated to alter validity and denotation of tumor-relevant systems objects

Biomodulatory preventive approaches Data derived from evolution-adjusted tumor pathophysiology provide information how tumor cells develop rationalizations for distinct tumor-promoting normative notions, and how these rationalizations are therapeutically accessible with biomodulatory therapies. With this knowledge in background, biomodulatory preventive approaches could be established in future, if tissues have been exposed to known noxa posing a risk to develop a tumor disease. Concerted modularized targeting of tumor-promoting rationalizations and accessing interactions of the environmental 'niche' with tumor cells could attenuate initiating steps in the presence of metastatic tumor dissemination and minimal residual disease, or may prevent progression of the lesions. Long-term redirection of communicative interactions between tumor and stroma cells might be an appropriate therapeutic approach and an realistic alternative to the attempts to eradicate genetically heterogeneous disseminated tumor disease with classic 'bottom-up' strategies.

Comprehending how rationalizations are multi-dimensionally organized In case of established tumor diseases, commonly used empirical data might suffice for prediction of risk and for therapy stratification, if latency or very slow progression of stepwise evolving genetic aberrations is expected in the tissue of interest [15, 16]. However, if quite heterogeneous tools of aberrations constitute unique phenotypes,

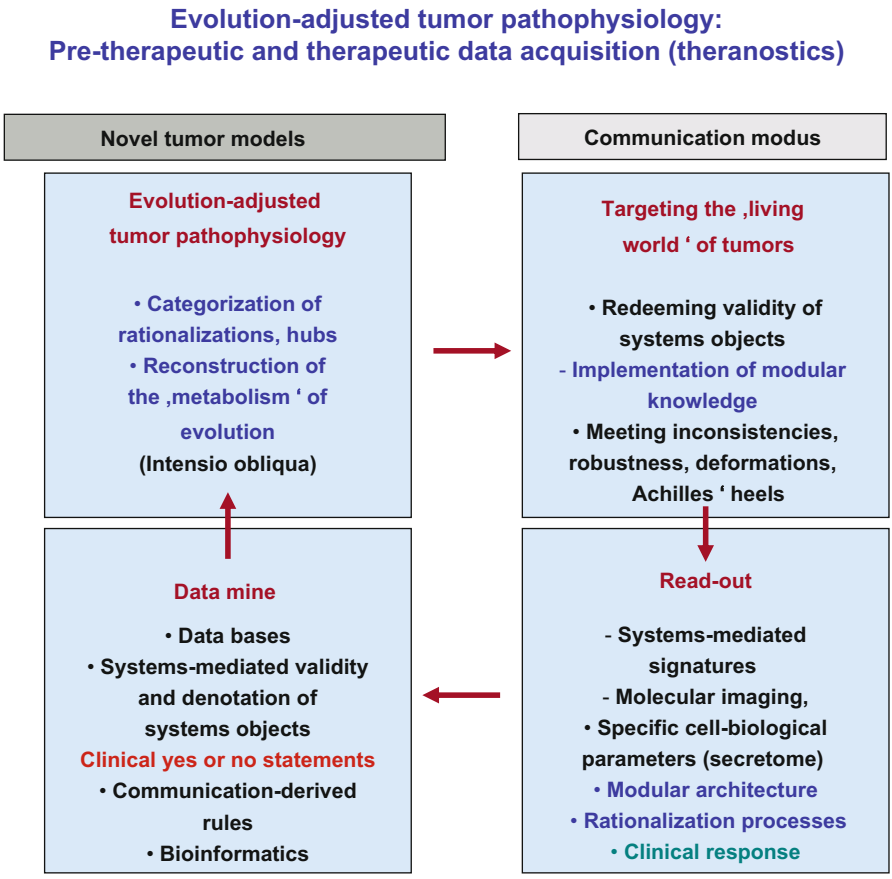

Fig. 15.3 Reconstructive analyses of tumor-specific evolutionary processes may be achieved by iterative cycles of differentially structured combined modularized therapy elements and evaluation of modular systems and tumor response: Modular therapies generate systems-related read-outs, consecutively leading to decision-relevant yes or no statements. Qualitative and quantitative systems analyses may be supplemented and broken down to an analytical level by complementary molecular-biological data mining. Thereby, systems-relevant functions may be assigned to specific structures within stage-specific rationalization processes resulting in systems classification

for example rapid tumor growth in 'acute' leukemias [17], or if lesions are suspected to rapidly acquire additional aberrations (e.g., transformations in lymphomas), then reconstructive approaches may be appropriate to comprehend how rationalizations are multi-dimensionally organized (Fig. 15.3).

The application of reconstructive methods is suitable for longitudinal studies on changes in pre-malignant lesions or in malignant tissues. Prerequisite for efficacious

monitoring would be a broad pathophysiologic knowledge e.g., about the cellular secretome of cell compartments in a lesion and respective changes during progression. It must be assumed that mostly single biomarkers will not be predictive, but rather biochemical or imaging-derived signatures, which define individual tumor systems stages by depicting single tumor-associated normative notions (e.g., 'hallmarks' of cancer) and respective rationalization processes ([7], Chap. 18).

Diagnosis: Reconstruction of Tumor-Associated Rationalization Processes

Current therapy decisions for metastatic tumors are predominantly organ-of-origin based. Correspondingly, textbooks are contentual organized. Contrast-enhanced imaging techniques to evaluate tumor size, localization, spread and anatomical complications represent central diagnostic instruments for decision making. Histology, immuno-histology and at an increasing rate molecular (-genetic) markers serve to further specify therapy decisions and commonly offer great promise to improve the outcome of tumor therapy [18].

The current strategy for personalizing tumor therapy aims at collecting a growing amount of data on quite heterogeneous cellular, molecular-biologic and cytogenetic levels. Biotechnology provides multifaceted tools for the measurement of myriads of 'omics' data across multiple observation levels (DNA, RNA, protein, metabolomics etc.) [19]. Commonly chosen 'starting points' for biomarker research are samples from tumor biopsies. The resulting collection of data presents snapshots in time, which have to be interconnected with bioinformatics to outline systems biological relations between distinct detected systems objects. The supposed communicative expression of systems objects is interpreted on the basis of historic data collections, which have been derived from other biological systems, from in vitro studies, animal models and clinical trials. Aim of these concerted diagnostic approaches remains to improve personalized medical therapy of (metastatic) tumors by reductionist systems considerations. For categorizing this data-collecting approach so called 'ontologies' must be implemented to organize available data hierarchically [20].

Reconstructive methods In contrast, the introduced formal-pragmatic communication theory allows physically detected systems objects to anticipate situative identities, i.e., communicative expressions, which are mediated by communicatively relevant presuppositions lying in the respective evolving tumor systems context [21]. If context-dependent changing presuppositions may alter the communicative expression of systems objects in the frame of the 'metabolism' of evolution, reconstructive methods fill in an important data gap (Fig. 15.1, 15.2, 15.3): They accomplish the requirement to introduce diagnostically and therapeutically relevant data on the situative identities of systems objects, including tumor-related rationalization processes.

Systems objects gain altered validity or novel denotation in a situative evolutionary context of an individual tumor disease [7, 22, 23] Evolutionary confined identities of systems objects are relevant to interpret systems objects' communicative expression, and therefore, are decisive for comprehending systems biology in the proper meaning. Reconstructive methods open up a new diagnostic and therapeutic window: These partially novel methods decisively contribute to guide therapy selection and longitudinal response monitoring (theranostics).

Depicting rationalization processes and establishing functional genomics The assessment of rationalization processes is worthwhile to get answers to the question, how known systems objects are integrated in rationalization processes on the basis of novel validity claims and denotations. The way, how rationalization processes are physically realized concerning normative structures, functions and decision maxims (hubs), may help to decipher communicatively interactive gene patterns (rationalization-based gene signatures) and to establish functional genomics. Hereby, reconstructive activities contribute to the interpretation of whole genome analyses in tumors or allow to functionally separating important gene signatures. Currently, interpretation of gene signatures is at its beginnings [24].

Main components of rationalization processes are cellular compartments in the tumor as important functionaries [25]. Due to the fact that multiple cell compartments are able to contribute to maintaining a distinct normative notion, and as they are often replaceable by alternative 'players' (robustness), we cannot attribute functions or normative notions stereotypically to the same structures, especially in unknown evolving tumor systems, as commonly anticipated e.g., for vascular endothelial growth factor (VEGF), NFkappaB etc. [23, 26, 27].

Rationalization processes integrate tumor microenvironment and tumor cell system Therapeutic requirements are best met by reconstructive activities, as these—by evaluating the situative communicative expression—contribute to clarify, which cell type or biological systems unit carries and promotes distinct functions within a unique tumor system. Rationalization processes integrate tumor microenvironment and tumor cell system communication-technically to open the window for biomodulatory therapies [25].

Categorizing rationalization processes Reconstructions of rationalization processes in tumors add information about potential additional targets for tumor therapy, besides the yet known reductionist derived ones. In so far, categorizing rationalization processes broadens the basis for personalized tumor therapy and facilitates the implementation of biomodulatory therapy approaches by establishing modularized non-normative boundary conditions, i.e., predominantly communication guiding conditions, for attenuating tumor growth.

The comparative uncovering of how distinct normative notions are implemented and molecularly organized—frequently ensuing from quite different acquired (molecular)-genetic starting points (e.g., in acute leukemias)—establishes transparency about validity claims of tumor systems objects in a situative context and facilitates to uncover hubs, which are equally operated by different rationalization

processes aiming to constitute similar normative notions in probably histologically quite different tumor types [7, 28]. The search for common hubs within rationalizations for distinct normative functions occupies a central place in searching for novel targets [2].

Basic limitations of tumor models The novel category of investigations on tumor systems biology, the reconstructive efforts, try to attribute evolutionary confined functions to systems objects, and aim to evaluate the constitution of multidimensionally organized rationalizations and interconnecting hubs—a global picture may be created from site of tumor systems participators with their context dependent changes of validity and denotation. Here, the difference between pathology and evolution-adjusted pathophysiology becomes most obvious [29]: Pathology has its limitations in its basic starting point, the observer site; evolution-adjusted tumor pathophysiology only realizes the participator site.

Assessing identity and function of systems objects Within the frame of an evolution-adjusted tumor pathophysiology, the investigative focus may be pragmatically chosen for clinical and experimental purposes: The identity and function of systems objects can be monitored globally, e.g., in serum by cellular secretome analytics, epigenetic monitoring of mononuclear peripheral blood cells, or locally in the tumor tissue, e.g., by monitoring transcriptional regulation (epigenetic modifications), and may be deepened by studying single systems objects, their evolutionary linked validity and denotation (late-stage biomarkers) as well as their interplay with other subsystems (intersystemic exchange). Molecular imaging techniques or cellular secretome analytics are able to depict normative notions, structures, action norms and decision maxims in follow-up [4–6].

Staging: Assessment of Rationalizations Across Different Tumor Histologies and of Tumor Heterogeneity

Classic staging procedure Currently, staging procedures predominantly monitor the anatomic extent of tumor diseases. Molecular markers supplement anatomic results to estimate the risk of non-response and poor survival, and growingly, molecular (-genetic) parameters or scores serve as positive predictors and/or simultaneously as therapeutic targets [30, 31]. The contemporary kind of conceptualization does not take account of the frequently assessable tumor heterogeneity, which may be substantiated either clinically (mixed or transitory response), or histopathologically and molecular-genetically, both in tumor and adjacent stroma cells [32, 33].

Systematizing tumors according to unique rationalization processes In contrast, evolution-adjusted tumor pathophysiology delineates that the physical constituents of rationalization processes or particular hubs within rationalization processes may be shared across histologically different tumor types [34]. Rudimentary knowledge about differential rationalizations of pro-angiogenic processes among different

histological tumor types seems likely to be the reason for the weak activity of currently administered angiogenesis inhibitors and the associated lack of prognostic parameters [27].

Tumor cells may exploit the whole extent of rationalization features of both stroma and tumor cells to implement the functional diversity of systems behavior aimed at maintaining homeostasis and robustness in tumor systems and to establish distinct, communicatively 'allowed' rationalizations [35]. Differential biomodulatory accessibility of tumor-associated normative notions, i.e., normative structures, action norms, and decision maxims, for mediating clinical tumor response is indicative for corresponding multifaceted integration of distinct tumor-associated normative notions, e.g., inflammatory processes, proangiogenic impulses etc., into a tumor's systems context.

Assessing tumor-immanent rationalizations Staging in an evolution-adjusted manner comprises the comprehension, how rationalization processes of normative notions are constituted [36] and what kind of common communication technical hubs are available as therapeutic targets. Hubs are suggested crossing points, to which rationalizations draw on, if genetic starting points are heterogeneous, such as in the case of 'acute' myelocytic leukemias [17].

Additionally, staging must provide signatures to efficaciously predict attenuation of tumor growth or objective response via modulation and redirection of tumor-associated normative notions. Prerequisite for a specific therapeutic modulation of rationalizations is to know, how e.g., pro-angiogenic processes are situatively organized. Do they occur in the context of inflammation or immune reactivity? Which cell types contribute to maintain normative notions? Knowledge on robustness and on the organization of intersystemic processes in a distinct evolutionary constrained context becomes important. Cellular secretome analytics, molecular imaging techniques for normative notions, e.g., the 'hallmarks' of cancer, are diagnostically indicated for categorizing rationalization processes in follow-up as well as to detect commonly operated hubs.

Additional communication-technical tumor characteristics may be evaluated during staging procedures. Exemplarily, the following modalities have been used for assessing tumor systems behavior in recent phase II trials by modularly targeting tumor-associated inflammation [7]:

1. **Inconsistencies** may be therapeutically met, if a therapeutic approach leads to rapid tumor response by hitting the main weakness of a tumor system (Achilles' heel). Paradox processes, such as weaknesses, may develop on the basis of a systematic congestion caused by rationalizing the functional 'world' of tumor-associated stroma and tumor cells. This rationalization results in an overload or restriction of communicative infrastructures or in a decoupling of systems and the functional world of cell systems.
2. **A series of phase II studies demonstrated differential modularized accessibility** of tumor-associated normative notions, i.e., normative structures, action norms, and decision maxims, for mediating clinical tumor response.

3. **Disturbances in intersystemic exchange processes** are suggested, if biomarkers (e.g., C-reactive protein) or signatures depicting the redirection of normative notions (here tumor-associated inflammation) show a low sensitivity to predict clinical benefit.

Assessment of tumor heterogeneity remains mostly not considered as there are no established therapy approaches meeting this important issue. Heterogeneity in tumor cells and microenvironment counteracts the efforts for personalizing tumor therapy [32, 33].

Modularized therapy approaches could provide a rational for problem solutions. Tumor and stroma cell heterogeneity can be considered as the result of evolutionary processes, the latter adhering to rules, which are given by the 'metabolism' of evolution [21]. The perspective from inside, which is acknowledging the tumor's communicative system and its evolutionary constrained responsiveness to communicative challenges, e.g., development of resistance, repair, or implementation of novel aberrations due to environmental stress factors, should allow choosing non-normative boundary conditions for therapeutic purposes to prevent further development of heterogeneity.

Treatment Planning: Purposive-Rational Tumor Therapy as Cellular Therapy In Situ

Hypothesis-driven pathophysiological models are the basis for considerations about the functional behavior of tumors, particularly for uncovering communication-based pathologies and novel therapeutic targets [34]. The operative tools, provided by the traditional theme-dependent pathophysiology, are now opposed by the evolution-adjusted, synonymously; the perspective from outside, i.e., the observer position is now contrasted by that from inside, i.e., the position of a systems participator. Tension between both perspectives arises: Identity of tumor systems objects and evolutionary systems contexts are irrevocably linked, but cannot be situatively pinned down in steadily evolving systems (vagueness of systems objects' situative validity and denotation); however, they may be retrospectively described and predicted on the basis of communication-derived rules, which constitute the metabolism of evolution and are depicted by situative signatures indicating identity and function of systems participators [8].

Vagueness of systems objects' situative validity and denotation The traditional treatment planning ignores the potential vagueness of the evolutionary confined communicative expression of tumor systems objects. Tumor-associated systems objects are only allegedly familiar on the background of novel evolving (molecular-) genetic signatures. Context generating, evolutionary confined, communication-relevant presuppositions facilitate novel validities and denotations of tumor systems objects. Systems participator plus communicative presuppositions define the communicative expression of a therapeutically accessible module. Consequently the smallest therapeutic units are modularized.

Currently, therapies are classically organ-of-origin-, stage- and molecular marker-based The concept allows to specify therapy and—as an important result—to significantly improve survival. This has been proven e.g., in breast cancer with amplified epidermal growth factor receptor 2 (HER2) gene (trastuzumab), in KRAS-mutated metastatic colorectal cancer (cetuximab or panitumumab) or in chronic myelogenous leukemia (tyrosine kinase inhibitors), in ALK positive T-cell lymphomas (crizotinib) and B-cell lymphomas (anti-CD20 rituximab) etc. [31, 37–39]. Synthetic biology comprises a further development of highly specific targeted therapy approaches, e.g., the bispecific antibody CD3-CD19 [40, 41], currently in clinical evaluation. All these rationally designed, molecularly targeted therapeutics are successfully based upon a reductionist pathophysiological concept and on biomarkers/targets of a particular neoplasia. In all these examples of molecularly targeted therapy, cancer-specific biomarkers and drugable molecules or signalling pathways were the bases for subsequent preclinical and clinical development and for successful licensing of the respective agents.

Conventional cytotoxic agents share some features with targeted therapeutics, as they show tumor histology-related activity profiles [42, 43]. Nevertheless, they are generally considered as non-targeted therapies, as their clinical introduction is predominantly based on empirical data: Nitrogen mustard induced long-term cytopoenias in surviving soldiers, so the observation during the First World War.

Classic targeted therapies In contrast to a cytotoxic agent, whose toxicity is not limited to cancer cells, but may also affect normal proliferating cells, molecularly targeted agents are suggested to more specifically trigger apoptosis, by inhibiting oncogenic signals, inducing cell cycle arrest or differentiation of tumor cells. However, achievement of an isolated cancer cell restricted effect of those drugs seems illusional. Molecular marker-based drugs usually show activity profiles in a narrow range: Activity is constrained by the availability of the target and the evolutionary systems signature, in which the target is embedded—as shown for the target VEGFR (Chap. 7). Principally, the on-target effect involves both tumor and adjacent stroma cells. Therefore, the distribution of the cellular compartments and their functional impact to maintain a normative notion is decisively responsible for the clinical outcome. Off-target effects may be highly specified and more diversified than those of cytotoxic drugs. Either on-target or off-target effects depend on the distribution of the target in a distinct tumor tissue. Therefore, both target effects are tumor histology-specific, but may be also tumor site-specific, as shown for the administration of tyrosine kinase inhibitors in different tumor stages and diseases (Chap. 7).

Biological agents may be molecularly-targeted, e.g., anti-CD20, rituximab, but they can be also non-targeted, as in the case of interferon-α, dexamethasone, pioglitazone [28]: Their targets are ubiquitously available in quite different cell compartments. Other novel classes of drugs, such as histone deacetylase inhibitors or immunmodulatory agents (IMIDs), metronomic low-dose chemotherapy belong to non-targeted, biomodulating agents as well.

Possible aspects of the tumors' normative notions can be modified by classic targeted therapies, 'bottom-up' approaches, as exemplified by targeting vascular endothelial growth factor receptor inhibitors (Chap. 7).

Table 15.1 Novel communication-derived targets

• ***Targeting normativity*** by purposive rational therapy approaches: Modulating and redirecting rationalizations of normative notions, i.e. tumor-associated normative structures, action norms and decision maxims (hubs)
• ***Targeting intersubjectivity:*** Modulating validities of communication lines
• ***Targeting subjectivity:*** Altering the systems interpretation by modulating hubs, the orientation of actions, intentions or motivations by instigating signals

Targeting rationalization processes Now, the reciprocal, 'top-down' step can be taken: Tumor therapies may directly target rationalization processes of tumor-associated normative notions, e.g., the 'hallmarks' of cancer [44]. Signatures derived from serum or plasma depict differential tumor cell compartments' evolutionary based situative identity and function and can be used for designing and guiding biomodulatory therapies [4].

Targeting normativity by purposive rational therapy When considering simultaneously on- and off-target effects of targeted therapies, the ubiquitous availability of targets ('non-targeted' therapies in a current pharmacological sense), or the tumor histology- and site-specific activity profiles (e.g., VEGFR targeting agents), then we approach the idea of biomodulation, the implementation of non-normative boundary conditions, which launch a communicative tumor-associated system to change normative notions (targeting normativity by purposive rational therapy), with the aim to modulate validities of communication lines (targeting intersubjectivity) or to alter the systems interpretation by modulating hubs, the orientation of actions, intention or motivations by instigating signals (targeting subjectivity) (Chap. 22) (Table 15.1).

'Corrupt' rationalizations The tumor has the potential to evolve and to gain progressive autonomy by implementation of 'corrupt' rationalizations—that means by giving facticity to possible validities, among those comprising the modular knowledge of systems objects. The same way non-normative boundary conditions aim at stripping down tumor-immanent normative systems features, and at disconnecting tumor-immanent facticity linked to distinct validities of systems objects, to attenuate tumor growth or to achieve healing [28].

Distinct normative benchmarks may be targeted by combined modularized therapies, while concentrating on certain main functions of the tumor, i.e., robustness, local penetration and expansion (colonization), participation in an organ (homeostasis), redistribution (metastases), and reproduction, whilst taking into account tumor-specific rationalization processes, which may be operated across different histologies.

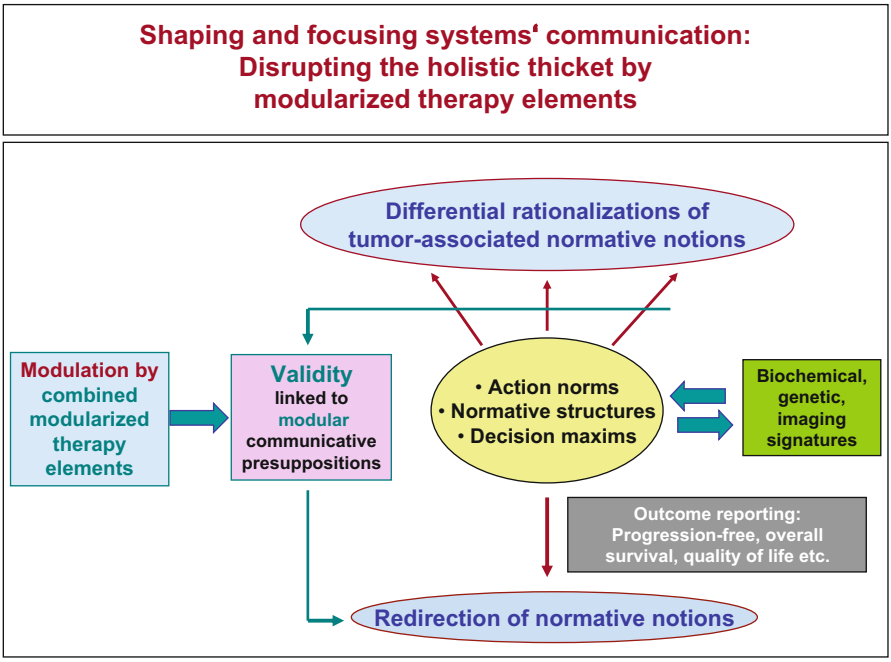

Fig. 15.4 Shaping and focusing systems' communication for disrupting the holistic communicative thicket with modularized therapy elements: Tumor-associated normative notions are realized by differential rationalizations. Validity of systems objects or, in a larger scale, of rationalizations may be redirected or modulated by combined modularized therapy elements and consecutively adapted according to biochemical, molecular-genetic or molecular imaging signatures

'Non-oncogenes' and 'oncogenes' Rationalization processes include both, 'non-oncogenes' and 'oncogenes'. From a communication technical view it is more interesting to differentiate between systems-mediated situative functions of oncogenes, which are described in the literature as 'disparate' oncogenes [45], and to assess the way, how oncogenes are implemented in communication-relevant modules, than to formally separate non-oncogene and oncogene addicted targets [46].

Cellular therapies in situ Biomodulatory therapy schedules successfully include modularized therapy approaches targeting the communicative expression of oncogenes [28]. They aim at operating in situ the communicative system of tumor and stroma cells with modularized therapy elements, thereby modulating and redirecting the tumor's normative notions (Fig. 15.4). Cellular therapies in situ are representing evolution-based and -inducing therapies, established by implementation of non-normative boundary conditions.

Implementation of non-normative boundary conditions On the background of successfully administered biomodulatory therapy approaches, we propose that—vice versa—drivers of carcinogenesis lie also in inducible (e.g., cellular stress), adaptive

changes, which are enabled by local or systemic modulation of tissue architectures and functions, and which consecutively may become digitalized in form of acquired (molecular-) genetic changes [47]

Non-DNA-heritage and the DNA heritage are incommensurable based on reductionist knowledge, but in the evolution theoretical view they share the fact that they are digitalized, both rationalizations and corresponding gene signatures. This way, non-DNA-heritage and the DNA heritage, as well as different techniques for implementing modular knowledge and various modular tumor architectures, turn out to be pervious and homogeneous for communication-driven systems biologic considerations on the background of an evolution theory (formal pragmatic communication theory), despite the qualitatively rather heterogeneous features of the 'heritages' and the presence of analogously working processes: Gene signatures have their digitalized counterparts in multi-level normative notions and their corresponding multifaceted rationalization processes [48].

Modularly targeting rationalizations Modularized therapies stand out from the other available therapy approaches, i.e., targeted therapy, conventional chemotherapy, biological therapy and cellular therapy. By modularly targeting rationalizations of normative notions, e.g., tumor-associated inflammation, angiogenesis, in histologically different tumor types, tumor systems' rationalizations and intersystemic exchange processes may be comparatively uncovered as the tumor systems' communicative feedback is immediately available for data mining in follow-up (theranostics). This way, biomodulatory therapies contribute to detect communicative systems structures [7].

The three mainstays of acquiring new insights into novel therapy approaches implementing modularity by non-normative boundary conditions are

1. the change from the classic conclusion logic (indicating a pathway responsible for cell death) to that of **normative statements** (how to control systems-associated processes with therapy modules to achieve response)
2. the change from object-associated to **situation-associated systems interpretations** (biomodulatory therapies in metastatic tumors)
3. the change from an intentional (reductionist) to an evolution-based **systems explanation** (systems behavior and response, rationalizations of normative notions). For situation-associated systems interpretations and systems explanations, we may now use terms derived from theoretical considerations on a tumor's modular systems behavior and intercellular rationalization processes (evolution theory) [7].

Efficacy of biomodulatory therapies depends on the communicative capacity to concertedly focus on the redirection of normative notions with respective therapy modules. Thereby, the modular interaction of biomodulatory drugs may implement non-normative boundary conditions and finally facilitates to focus on the redirection of tumor-specific rationalizations based on tumor-associated normative notions [28]. Rationalization processes of an individual tumor disease must be uniquely accessible for combined modularized therapies to failsafe ensure systemic tumor control (Chap. 2).

Constrains of biomodulatory therapies are suggested to lie in the constitution of the metabolism of evolution, the structure and extent of the communicative architecture (rationalizations), the tumor's living world. Preceding radiotherapy seems to explicitly alter rationalization processes in such a way that biomodulatory therapy—while controlling other non-irradiated lesions—fails to control relapsed tumor in the radiation field (Chap. 2).

Communication-derived therapeutic targets Vice versa, the therapeutic challenges lie in detecting communicative weak spots, i.e., Achilles' heels, inconsistencies, deformations, accompanying rationalizations and intersystemic exchange processes, hubs of rationalizations, the background knowledge of systems objects, namely the multifaceted identities, which can be adopted under various evolutionary-linked communicative presuppositions.

Diversification of Response Assessment: Monitoring the Redirection of Tumor-Associated Rationalizations (Theranostics)

Response assessment in current clinical practice is based on clinical evaluation, on examination findings, frequent serial assessments by imaging techniques (e.g., RECIST criteria), and other clinical and molecular markers.

Purposive rational therapy concepts The introduction of molecularly targeted therapies in gastrointestinal stroma tumors brought up the question, how to monitor biological changes in tumor systems during therapy, which could be indicative for growth attenuation prior to tumor shrinkage [49]. Evolution-adjusted tumor pathophysiology provides multifaceted novel response parameters or signatures for therapeutic decision making ([28], Chap. 18). The evaluation of altered situative identities of tumor cell compartments or the redirection and modulation of rationalization processes during biomodulatory therapies is now implied in therapeutic considerations by front-line patient selection or outcome adaptive trials. Multifold assessment techniques of changes in tumor-associated normative structures, action norms and decision maxims (hubs) can be pragmatically selected for guiding therapy and for predicting tumor response. This way, purposive rational therapy concepts may be established: The modular comprehension of both, tumor systems and therapy elements diversifies the instruments for attenuating tumor growth, even for adapting therapies to the concerns of individual patients' tumor disease and tumor-associated disease traits.

Modulation and redirection of the tumors' normativity While starting from the concerted modulation and redirection of the tumors' normativity (e.g., the 'hallmarks' of cancer), biomodulating techniques gain in therapeutic importance for diversifying tumor growth control by their modularized structure. Biomodulatory therapies are realized by implementation of non-normative boundary conditions via adaptively

selected and combined therapy modules. During therapy, the main focus will be to selectively monitor—as pragmatically indicated—therapeutically induced changes in normative notions, inclusively the traditional hallmarks of cancer, to generate evolution-adapted biomarkers and signatures, which are indicative for efficacious growth control (Chap. 22).

Assessment techniques for systems response are multifaceted, if redirection and modulation of selectively targeted normative notions is being monitored. Cellular secretome analytics in serum, epigenetics in peripheral mononuclear cells provides information about the status of single tumor-associated cell compartments and changes in identity and function during biomodulatory therapy approaches [4, 5]. Molecular imaging techniques for studying therapy-relevant normative notions of metastatic tumors may indicate therapeutically efficacious modulation [6]. Additionally, empirical data provide information about the link of biological surrogate parameters (signatures) depicting normative notions and clinical outcome parameters, tumor growth attenuation or cell death.

Theranostics Biomodulatory tumor therapies with their multifaceted accesses to tumor-associated modularized rationalization processes contribute to uncover systems biologic structures in tumors and comprise important diagnostic aspects. Theranostics, a portmanteau of therapeutics and diagnostics, is realized by implementing non-normative boundary conditions into the tumor systems context to tailor biomodulatory treatment approaches based on biomarker signatures (Fig. 15.5). Signatures are reflecting identity and function of even complex systems objects on the background of varying evolutionary confined presuppositions.

Response assessment of biomodulatory therapies is primarily confined to the monitoring of tumor-associated rationalizations and their redirection during biomodulatory therapy based on evolution-adjusted signatures. In parallel, correlations are possible between signatures depicting identity and function of tumor cell compartments and clinical outcome parameters.

Communication derived pathologies The concrete selection of distinct tumor-associated normative notions as target for combined modular therapies primarily neglects therapy-relevant intersystemic communication processes among tumor-associated, evolutionary constrained rationalizations, although all action units within a system are in an organized close trade-off. Empirical data on the correlation of molecular or imaging signatures, which indicate the redirection and modulation of tumor-associated normative notions, with clinical outcome parameters contribute to detect inconsistencies, the main weakness of a tumor system (Achilles' heel), paradox processes, such as weaknesses, disturbances in intersystemic exchange processes, systematic congestion caused by rationalizing the functional 'world' of tumor-associated stroma and tumor cells. Those pathologies in rationalization processes result in an overload or restriction of communicative infrastructures or in a decoupling of systems and the functional world of cell systems [21]. The field of communication derived pathologies may be only uncovered and categorized by an evolution-adjusted tumor pathophysiology.

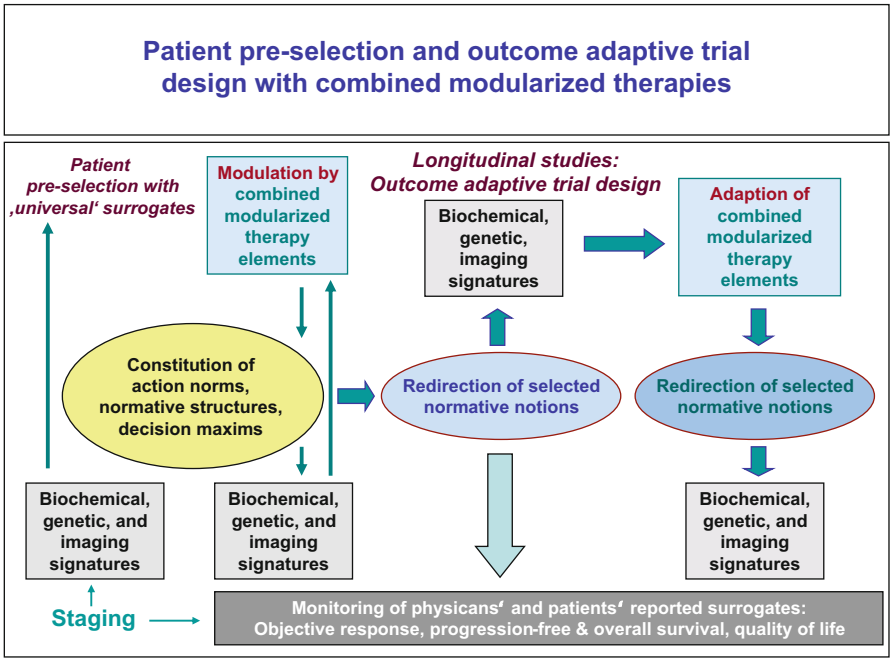

Fig. 15.5 Biomodulatory therapies provide information about communicative structures of the respectively treated tumor systems. The modular constitution of the therapy elements facilitates to generate concrete reference points to appropriately adapt biomodulatory procedures during the course of treatment. The reconstructive procedure promotes a pre-study patient selection, but may also facilitate a rapid adoption of therapy modules by implying data in therapeutic considerations, which have been generated during longitudinal pathophysiological observations with already administered biomodulatory therapy approaches (theranostics). Consecutive therapeutic approaches may be rehanged—as appropriate—at alternative rationalization processes (e.g., pro-inflammatory processes, pro-angiogenetic, immunomodulatory processes, tumor metabolism, and hubs)

Clinical Trials: Incorporating Systems-Relevant Information in Clinical Trial Designs for Metastatic Tumors

Novel generation of adaptive clinical trial designs Adaption of modularized therapy approaches according to longitudinal information about the physical and communicative (modular) feature of tumor systems and the patient's individual tumor-associated disease traits is an important therapeutic aim, which may be seized by novel adaptive clinical trial designs in medical oncology (Fig. 15.5).

Currently, stop and go designs of adaptive trials are orientated at dose levels (dropping arms, adding additional doses, optimization of dose-outcome), at the responder, non-responder population (extension of the study in the responder population, the proportion of patients, which is randomized in the particular study arms), and at patient accrual rates. Adaptive trial designs allow answering multiple questions at once and therefore, they are more informative [50].

Cellular therapy in situ Based on evolution theoretical considerations, the tool of covariables available for adaptive trial designs significantly increases. Reconstructive activities on the communicative expression of therapeutically relevant systems participators and analyses of tumor-associated rationalization processes refer to methodologies provided by an evolution-adjusted tumor pathophysiology. Dissecting tumor systems for pragmatic purposes in modular structures and rationalization processes may provide concrete clues how to modify pathophysiologically defined modules or rationalization processes with combined modularized therapy approaches, i.e., cellular therapy in situ.

Longitudinal modelling of tumor systems on the basis of evolution-adjusted signatures assessing identity and function of cellular compartments in the tumor by cellular secretome analytics or appropriate molecular imaging techniques would facilitate to predict primary end points. Using a Bayesian perspective longitudinal information about identity and function of cell compartments would provide the basis to generate a novel generation of efficacious outcome adaptive trial designs.

Pre-study patient selection and therapy adaption The reconstructive procedure promotes a pre-study patient selection, and facilitates a rapid outcome triggered adaption of therapy modules by implying follow-up data, which have been generated with already administered modularized therapy approaches (theranostics) during longitudinal observations: Longitudinal changes in signatures indicating the redirection of tumor-associated normative notions enter therapeutic decision making (Fig. 15.5). Genomic signatures may be reinterpreted on the background of characteristic rationalizations of tumor-associated normative notions, evolutionary confined communicative presuppositions determining communicative expression may be therapeutically considered, as well as communicative weak spots. This way, reconstructive activities provide novel tools of possible covariables for stratifying modularized therapies in metastatic cancer or for facilitating outcome adaptive trial designs. Both, at diagnosis available and during therapy generated evolution-adapted pathophysiological knowledge may ideally modify the trial's course.

Modularized therapies allow purposive rational therapy designs, which are aiming at targeting the tumor's characteristic normative notions via concerted modifications of the tumor's modularly linked systems objects, striving to attenuate tumor growth and to induce cell death, either via classic death pathways, or alternatively, via the breakdown of essential communicative processes, as suggested from the pragmatic communication theory.

Evolution-adjusted biomodulatory therapies Implementation of modular knowledge, redirection of rationalization processes and accompanying normative systems structures, now come into the therapeutic calculus for establishing stepwise evolution-adjusted biomodulatory therapies to slow down growth promoting processes in cancer (Fig. 15.5). Therapies aimed at redirecting important tumor-associated normative notions, including tumor-associated disease traits (e.g., tumor-associated inflammation, angiogenesis etc.) for growth control, are predestined for a novel generation of adaptive trial designs.

Facilitating Drug Repurposing

Another important aspect seems to be drug repurposing for biomodulatory therapy approaches [51, 52]. Recent phase II trials on biomodulatory therapies in a broad variety of metastatic cancers have shown that drug repurposing within modularized therapy schedules may play a crucial role, as communicative drug interactions, even at low single doses are the therapeutic key element, and whereas maximizing doses is less important.

In recent phase II trials the combined activity of transcriptional modulators has been used for tumor control. Interestingly, metronomic low-dose chemotherapy with a drug, which when clinically used alone at normal dosages can be inefficacious (e.g., capecitabine in castration-resistant prostate cancer), it may demonstrate comparatively high activity when combined with transcriptional modulators in modularized combined schedules [28, 53–56].

Discussion

Evolution-adjusted tumor pathophysiology provides the diagnostic, therapeutic, and biometric instruments for data mining to meet important prerequisites for getting closer to the demands of personalized tumor therapy (Table 15.2). The emerging discipline permeates all medical levels, modelling of tumors, diagnostics, tumor monitoring, therapy and study design, thereby providing a whole claviature of novel therapeutic targets (modules, rationalizations) and longitudinal reference points for adaptive therapeutic interventions. In contrast to the traditional targets, the novel ones are explicitly categorized according to their communicative expression, i.e., validities and denotations in a systems context (Table 15.3-15.5).

Two tumor-immanent obstacles prevent a stereotypical assignment of situative, evolutionary confined validities and denotations to tumor-associated systems participators: (1) Tumors are steadily subjected to evolutionary processes by the ‘metabolism’ of evolution and (2) multifaceted molecular-genetic signatures including repetitively occurring (molecular-) genetic aberrations in both, tumor and stroma cells, may constitute unique normative notions, for example ‘acute’ replacement of normal haematopoiesis in acute leukemias [17].

The resulting vagueness about the situative validity and denotation of tumor-associated systems participators, including oncogenes, necessitates decidedly the evaluation of tumor systems objects’ situative communicative expression (reconstructive methodologies) to conceive the detailed constitution of rationalization processes either for diagnostic or therapeutic purposes. The evolutionary confined multifaceted identities of systems objects are subsumed in their diversity as ‘modular knowledge’. Modular knowledge represents a characteristic benchmark for a systems object. Context-dependent changing validities and denotations of systems objects have been exemplarily described in the term ‘disparate’ oncogenes [45].

Table 15.2 Methodological diversification of approaches for personalizing tumor therapy

	THEME-DEPENDENT ('Bottom-up') REDUCTIONIST APPROACHES	***EVOLUTION-ADJUSTED ('Top-down') HOLISTIC APPROACHES***
Screening	• *Population-wide risk evaluation* • *'Individualized' risk estimation according to* ***biomarker profiles*** *(population-based)*	• ***Assessment of identity and function of cellular compartments*** *in premalignant lesions (secretome analytics)* • ***Assessment of evolutionarily confined validity claims*** *of systems objects in premalignant lesions*
Diagnosis	• ***Organ-of-origin-based*** • ***Histology-based, molecular marker-based*** • ***Genome-centric exploration*** • ***Translation of validity claims*** *of systems participators irrespectively of the communicative context and communicative expression*	• ***Assessment of rationalizations*** *of the tumor's normative notions (evolution-adjusted tumor pathophysiology)* • ***Assessment of normative structures, action norms, decision maxims*** *(probing cellular activation states)* • ***Correlation of rationalization processes with aberrant genetic patterns*** *(both digitalized systems)*
Staging	• ***Anatomic extent of tumor disease*** • ***Molecular/genetic risk*** • ***Assessment of genetic tumor heterogeneity*** *at primary tumor site and metastases*	• ***Cellular secretome analytics, imaging of normative notions*** *(rationalization-based gene signatures)* • ***Normativity: Common rationalization processes among metastatic sites*** *(identifying key hubs in diseased tissues)*
Treatment planning	• ***Typically organ-of-origin-based*** • ***Stage-based, genome-based*** • ***Molecular marker-, pathway-, oncogene-based*** • ***'Eradication'*** *of cell compartments via single or multiple targets, which are frequently expressed in a distinct tumor tissue. 'Knocking down' tumor-promoting pathways*	• ***,Top-down', multi-track modularized therapy*** *for targeting rationalizations, remodeling and redirecting identity and function of cell compartments in the tumor* • ***Combined modularized shaping of*** *robustness, local penetration and colonization, participation in an organ, and reproduction; overcoming genetic tumor heterogeneity* • ***Targeting hubs*** *of ,corrupt' rationalization processes*
Response assessment	• ***Based on clinical evaluation, examination findings*** • ***Frequent serial assessments*** *by imaging techniques (RECIST criteria, tumor shrinkage), and other clinical and molecular markers*	• ***Monitoring of the redirection of rationalization processes*** *(secretome analytics, molecular imaging)* • ***Theranostics*** *(remodeling of signaling pathways)* • ***Comparative uncovering of tumor systems biology*** *by modularly targeting tumor-associated normative notions*
Clinical trials	• ***Oriented at maximum tolerated doses*** *or at the determined range of tolerable and active doses* • *Eligibility restricted to tumor marker patterns, histology (****single-track****), and prior treatment, with serial assessments; 'classic' adaptive trial design*	• ***Definition of the minimal active biomodulatory doses*** • ***Drug repurposing:*** *Combination of drugs with poor or no monoactivity, besides those with monoactivity (****multi-track****)* • ***Adaptive trial designs*** *for remodelling and redirecting the tumor's normative notions to attenuate tumor growth*

Table 15.3 Proposed categorization of tumor-associated pathologies

- **Normativity**
  - *Action norms:*
    Common rationalization processes across tumor histologies
  - *Decision maxims:*
    of rationalizations
  - *Normative structures*
    Histology; cell organelles (excessive number of mitochondria in oncocytomas) e.g., etc.
- **Alignment of genetic signatures to corresponding rationalizations**

  Reinterpretation of patterns of acquired gene aberrations
- **Communication-derived pathologies**

  Achilles 'heels
- **Modularity: Preposuppositons for validity and denotation of systems objects**
  - *Subjectivity*
    Results as communicative outcome between requirements of the system and the functionality of systems participators
  - *Intersubjectivity*
    The use of commuication lines with differential communicative expression

Table 15.4 Action norms: common rationalization processes across tumor histologies

Normative functions (examples):

- Velocity of tumor growth (sequence of molecular-genetic aberrations), dysplasias
- Homeostasis between host's organs and tumor, e.g. osteoplastic, osteolytic metastases etc.
- Tumor redistribution, reproduction
- ‚Hallmarks' of cancer: Inflammation, angiogenesis, immune response, metabolism etc.
- Organ tropism (colonization), local penetration and expansion
- Robustness: Alternative pathways to maintain normative notions, repair, intrinsic resistance
- Tumor-associated disease traits, i.e. kachexia, fatigue, metastatic spread,activation of disseminated tumor cells etc.

Table 15.5 Proposed categorization of communication-derived tumor pathologies

Communication-derived pathologies

Achilles ' heels:

- *Inconsistencies*
- *Weaknesses*
- *Systematic congestions*
- *Overload or restriction of communicative infrastructures*
- *Decoupling of systems and the functional world of cell systems*
- *Disturbances in intersystemic exchange processes*

Reconstruction of the communicative expression of tumor-associated systems objects (e.g., cells, pathways, genes), and of rationalization processes including the adapting intersystemic exchange processes, facilitates to consider evolutionary-linked communicative processes and their pathologies for therapeutic purposes. Tumor-associated normative notions, and their corresponding rationalization processes, have their therapeutic equivalent in combined modularized therapies, which facilitate for multi-level implementing non-normative boundary conditions and aim

at evolving tumor systems and redirecting communicative presuppositions of the systems objects' tumor-promoting communicative expression [7].

Reconstructive methodological approaches draw on population-based research The change of the starting point to pathophysiologically review tumor systems, namely according to pragmatically selected and inter-systemically comparable tumor-associated normative notions (structures, action norms and decision maxims) and their respective rationalizations, does not at all singularize the process of data mining and decision making in a confusing way: Rationalization processes are digitalized, as impressively supported by the specific activity of biomodulatory therapies, particularly, by the modular therapeutic accessibility of tumor-associated inflammatory processes [7].

The digitalized feature of rationalization processes is further underlined by the fact, that a nucleus transplanted into a species-divergent nucleus-free cell does not adequately establish communicative contact, and the cell dies [57]. Communication does not admit arbitrary interpretations of communicative challenges, and therefore, rationalizations may be categorized in a discursive and pragmatic manner, steadily adapted to novel issues.

Rationalization processes and genome signatures share their digitalized feature. Reconstructive activities on normative notions and their concrete rationalizations provide access to reinterpret and catalogue genome signatures: A full understanding of cancer biology and therapy by functionally cataloguing the cancer genome in terms of genome signatures is unlikely unless it is integrated into an evolutionary that means communicative context, explicated by an evolution theory [58].

From genome-centric to rationalization-centric systematization of tumors The counterpart of a tumor's rationalization processes represents functional genomics across different cell types of the tumor compartment. Categorizing rationalizations will be the basis for novel population-based considerations and across different histologically-defined tumor entities [7]. Only the investigated object is changing: Evolution-adjusted tumor pathophysiology focuses systematically on the evaluation of the identity of systems subjects, as systems participators are subjected to context-dependent, evolutionary-linked validity claims, thereby acquiring additional modular knowledge.

Personalization of diagnostics and therapy is not the exact opposite to population-based approaches, as frequently suggested [59]. By implementing reconstructive methodologies only the populations for systematic analyses are contentual changing [48].

Plurality of the reconstructive methodology Attributing modularized purposes to systems objects and—on a larger scale—to rationalization processes are scientifically well-founded issues, which facilitate the plurality of the reconstructive methodology and the openness for pragmatically confined categorizations of rationalization processes in an evolution-adjusted tumor pathophysiology.

Basis for reconstructive activities are reductionist data from biochemistry, pathology, classic pathophysiology and biomodulatory therapy. Evolution-adjusted,

reconstructive pathophysiology provides the therapeutically decisive information about the situative communicative expression of systems objects, e.g., their modular knowledge, their identity in an evolution-based context, and their integration in rationalizations. Vice versa reconstructive data pass the ball back to the classic disciplines to study rationalization processes and rationalization-linked hubs, or for designing classic targeted therapies directed to novel molecular targets, e.g., to rationalization-derived hubs.

Technologies for reconstruction are particularly selected for identifying situative validities and denotations of systems objects. The methodologies comprise partially novel and yet developing technologies, i.e., the targeting of communicative processes in modular biological systems (biomodulatory therapies), and comparative reconstructions of systems biology by modularly targeting normative notions [7]. These techniques are supplemented by cellular secretome analytics to get hints about how the cell compartments are contributing to maintain and propagate normative notions, by molecular imaging techniques for tumor-associated normative notions, inclusively the 'hallmarks' of cancer, and by studies on sequential transcriptional regulation etc.[4–6, 60].

Selecting and stratifying therapies Reconstruction-derived data on systems objects' validity and denotation are clinically applicable in a multifaceted fashion. The functional and structural evaluation of rationalization processes serves for selecting and stratifying therapies in (metastatic) cancer, and may be used as valuable instrument for longitudinal studies in tumor pathophysiology during clinical tumor observation or modularly designed therapies. Simultaneously, reconstructive approaches give novel input to specifically modify multi-targeted modularized therapies and clinical trial designs (theranostics). Evolution-adjusted tumor pathophysiology accomplishes the prerequisites to stepwise develop novel biomodulatory therapy technologies.

Biomodulation as novel universal therapeutic technique allows a communication-based highly specific therapeutic intervention, as shown by response data on combined modularized therapy approaches in multiple phase II trials [28]. Biomodulatory therapies provide modular access to rationalization processes by focusing on the implementation of non-normative boundary conditions into the tumor's systems world, and by using synergistic or at least additive communicative interactions of drug combinations. Communicative interactions within a tumor system are founded in a formal-pragmatic communication theory [21].

Even the combination of drugs with poor or no mono-activity, or of drugs with activating activity, e.g., activators of transcriptional processes, may clinically develop sufficient activity, as indicated by long-term tumor response, and complete remission [28]: Therefore, drug repurposing is a central theme in future, especially on the background that most drugs do not achieve their main aim to become licensed—due to missing mono-activity or missing activity combined with standard therapies (Fig. 15.6). The field of drug repurposing within biomodulatory therapy tools represents a great challenge and chance for developing and marketing drug combinations with moderate toxicity profiles and tumor-specific biomodulatory activities [52].

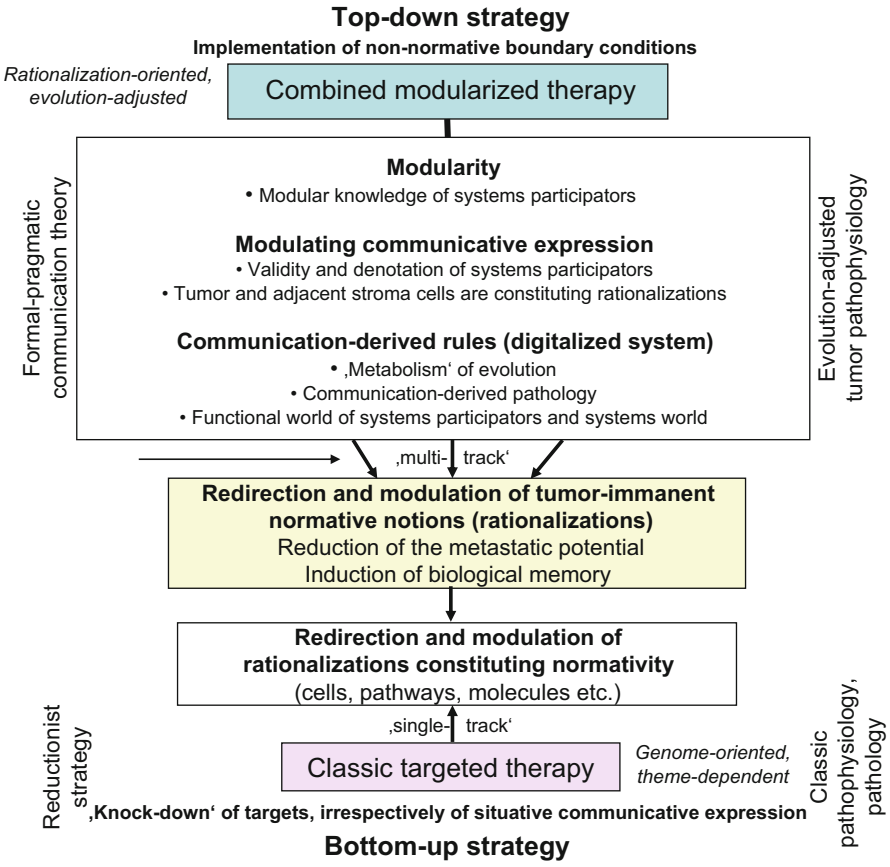

Fig. 15.6 Applied systems biology for the control of metastatic cancer: Shaping and focusing systems' communication by disrupting the holistic thicket with a 'top-down' strategy. Tumors are not any more considered as 'objects', which have to be destroyed with targeted therapy approaches, but as subjects within a communicative context, which allow to implement multifold novel therapies

Purposive-rational therapy designs consider both, the diversity of rationalizations constituting a unique tumor-associated normative notion and the differential impact of normative notions in evolutionary confined systems stages (e.g., rapid proliferation, apoptosis resistance, dysplasias). The broadened diagnostic basis allows to stratify tumor patients for therapy and to specifically select biomodulatory therapies for attenuating tumor growth via targeting of tumor-associated normative notions. Aim of redirecting and modulating therapy elements is to evolve tumor systems therapeutically; thereby tumor-associated modules are operated by combined modularized therapy approaches, i.e., cellular therapy in situ. Basically, normative notions, including tumor-associated disease traits, are the trigger to achieve tumor control or healing. Biomodulatory therapies point the way from theme-dependent to evolution-adjusted therapy approaches: A tumor type-specific,

systems stage-specific, metastatic site-specific or disease trait-orientated therapy (e.g., inflammation-associated disease traits) seems to be within grasp (Fig. 15.4).

Long-term tumor control and palliative care The techniques for redirecting normative notions are multifold, but selectively orientated at modulating and redirecting tumor-associated normative notions in a concerted communicative approach (biomodulation) [28, 56, 61–64]. Quite diverse normative structures, action norms and decision maxims may be selected for achieving tumor control via differential purposive-rational therapy designs. The main focus will be uncovering those biomodulatory drug combinations or normative notions with the most compelling link to clinical efficacy norms, i.e., disease stabilization, objective response, progression-free survival, and overall survival. In future, targeting normative systems features and tumor-associated disease traits could be an important contribution for long-term tumor control and palliative care.

Technical disposability of scientifically verifiable tumor-associated communicative processes Reconstructive activities within an evolution-adjusted tumor pathophysiology, adaptive trial designs, drug repurposing, purposive-rational therapy designs and cellular therapy in situ contribute to the technical disposability of scientifically verifiable tumor-associated communicative processes. Evolution-adjusted tumor pathophysiology closes a data gap to facilitate therapy selection and therapy adaption for patients, in contrast to the currently performed patient selection for therapy.

The differential perspective of communicative interaction applied by an evolution-adjusted tumor pathophysiology involves (1) the comprehension of the tumor's systems features at diagnosis by accentuating the communicative aspects of a situation's analysis, inclusively communication-derived pathologies, (2) allows situating identity and function of systems participators as systems subjects also during communication modulating therapies (theranostics), (3) facilitates to describe the tumor's 'living world' comprising all endogenously or therapeutically redeemable validity claims and denotations of systems objects (modular knowledge), (4) contributes to select and specify purposive aspects at diagnosis and during therapy to pragmatically configure and modulate available evolutionary based rationalization processes of normative notions by implementation of non-normative boundary conditions, and (5) finally affects the technologies to interfere with communication based pathologies in a tumor (cellular therapy in situ), promotes their further development and adaption to situative circumstances (adaptive trial designs) (Table 15.3).

The genome- and organ-centric perspective of tumor biology is complemented by a rationalization-centric systematization Therefore, evolution-adjusted tumor pathophysiology provides contently and methodologically completely novel approaches to succeed in personalizing tumor therapy. The novel comprehension of tumor systems biology supplements traditional efforts for personalizing tumor therapy, i.e., 'bottom-up' strategies and pharmacogenomics data on drug metabolism [65]. The novel discipline introduces the view of systems participators and contrasts with the manacle of the classic disciplines, pathology and pathophysiology, which

provide the view of observers, also when systems biological approaches are applied. Evolution-adjusted tumor pathophysiology should be introduced as clinically orientated discipline for routine tumor evaluation, equivalent with traditional disciplines, thereby increasing their value and accomplishing ethical demands (Fig. 15.6).

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Chapter 16
Including Rationalizations of Tumor-Associated Normative Notions in Pathophysiologic Considerations: Communication-Theoretical Implications

Albrecht Reichle

Abstract Currently, the individual experimental setting irrevocably attributes distinct communicative expressions to tumor systems objects, irrespectively of the situative evolutionary context. This reductionist view legitimizes the application of any of the large number of classic targeted therapies available, irrespectively of how tumor-immanent normative notions are physically rationalized. In our perception, normative notions, which are ubiquitous and not circumventable, include all observation levels, either the clinical or the experimental setting, i.e., 'high and low-grade' lymphoma, 'dysplasia', and the 'hallmarks' of cancer, etc. Evolution-adjusted tumor pathophysiology categorizes normative notions of tumors in a pragmatic and discursive manner; this way, aspects derived from basic and clinical science to comparatively uncover systems biological processes are equally acknowledged. From the formal-pragmatic communication theory we may delineate that normative structures, action norms, and decision maxims are concretely rationalized and that the (therapeutic) implementation of non-normative boundary conditions leads to remodeling and redirecting tumor-associated rationalization processes and their corresponding normative notions. Thus, the communicative expression of communication lines and communicative presuppositions facilitating distinct identities of systems objects are digitalized within the range of situative evolutionary constrains—a fact that does not exclude analogously or stochastically working subsystems. At that stage, genomic patterns become reconstructable on the basis of scientifically verifiable rationalization processes. Differential origins of rationalization processes, for instance, sequential long-term vs. short-term ('de novo') genetic aberrations, could impact systems robustness and intrinsic resistance. Rationalization processes are important therapeutic targets, particularly in metastatic tumor diseases, for overcoming the obstacle of 'bottom-up' strategies with genetically based tumor heterogeneity.

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A. Reichle (ed.), *Evolution-adjusted Tumor Pathophysiology,*
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Introduction

No communication line can be interpreted without assessing its communicative expression, i.e., without including normative notions. Normative notions that are ubiquitous and not circumventable in our perception include all observation levels, either in the clinical or in the experimental preclinical setting (Chap. 10).

Evolution-adjusted tumor pathophysiology systemizes normative notions of tumors pragmatically, irrespective of the observation levels. This way, aspects derived from basic science or from clinical observations to comparatively uncover systems biological processes of tumors are equally acknowledged [1, 2].

Systematization of Different Types of Neoplasia According to Normative Notions

Different types of neoplasia are routinely systemized according to normative notions from the very beginning, i.e., 'high and low-grade' lymphoma, 'acute' leukemia, 'chronic' leukemia, 'dysplasia', and the 'hallmarks' of cancer, etc. [3]. But neither are a tumor's normative notions systematically categorized—it would seem appropriate to distinguish normative structures from action norms and decision maxims—nor are normative notions really appreciated and broken down into their physical constitutions within a novel evolutionary context to comprehend the concrete situative rationalization of normative notions. In most cases, the constitution of rationalizations is not sufficiently depicted by the enumeration of reductionistically derived pathways and structures. The reductionistically derived repertoire of knowledge is commonly used to describe individual tumor diseases.

The frequently weak activity of classic targeted therapies in metastatic tumor diseases may be ascribed to the discrepancy between the mere availability of a target and its evolutionarily based validity and denotation. However, targets may participate in various rationalization processes, dependent on multifaceted patterns of acquired genetic aberrations. A huge number of genetic aberrations, for example, may realize rapidly displacing growth, such as in 'acute' leukemia. This fact is not sufficiently acknowledged, neither diagnostically nor therapeutically (Chap. 13).

Comparative considerations about the structural and functional constitution of rationalization processes, i.e., the physical constitution of distinct normative notions in an evolutionary context or the evolutionarily constrained identities of systems objects, are the starting points for novel pathophysiological considerations.

Reductionist considerations are supplemented within a formal-pragmatic communication theory by situative communicative expression and by respective physical presuppositions facilitating the evolutionarily confined identity of systems objects. Formal assignments, such as 'oncogene' and 'non-oncogene' to tumor systems objects, are not axiomatic functional features of systems objects.

Currently, the experimental setting irrevocably attributes distinct communicative expressions to tumor systems objects, irrespective of the situative evolutionary context. This characteristic procedure represents a common reductionist view, which legitimizes the application of any of the high number of classic targeted therapies. The attribution of evolutionarily confined situative identities, such as 'disparate' oncogenes or non-oncogenes, is the beginning of reinterpreting the communicative expression of systems objects and their biological importance [4, 5]. Consequently, the therapeutic observation ceases to fall behind the experimental observation, if communication-technical considerations are included in the explicatory calculus.

The usefulness of terms, such as oncogenes and non-oncogenes, is not being questioned. Oncogenes take over important tumor-promoting functions in model systems. However, their communicative expression may be modified in a therapy-relevant manner, as exemplified by the Philadelphia chromosome, if tumor evolution takes place, such as in acute lymphatic leukemia or in chronic myelogenous leukemia cells, or if additional acquired aberrations arise in distinct evolutionary contexts [6, 7]. Gene interaction studies in yeast have provided experimental evidence for these clinical observations [8].

Selection of Normative Notions

Normative notions for systemizing purposes of tumor systems should be discursively selected with pragmatic aims. Vice versa, we cannot phrase the pattern of normative notions of a distinct tumor type, as defined notions per se are a matter of the chosen starting point. Principally, we have to distinguish the view of an observer from that of a participator. Correspondingly, reductionist and holistic understandings are exerted to reproduce a situational stage of tumor disease: Differential perspectives of interaction are entangled with various levels of knowledge and consecutively with rather different therapeutic strategies [9].

The development of intrinsic resistance to drugs or drug combinations represents a strong clue for systems-relevant normative notions that are maintained to preserve cell identity and function. The detection of intrinsic mechanisms of resistance and their physical constitution should be interpreted in the first place as an important evolution-related pathophysiologic process, which features pivotally intrinsic tumor-associated normative notions and robustness [10–12]. Resistance mechanisms may reconstruct evolutionarily important tumor-associated rationalizations.

Rationalizations are Digitalized Similar to the Genomic System

The digitalized genome is commonly opposed to an assumed analogously and stochastically working system in the surrounding cell. Noble stated that 'the resulting patterns of gene expression are not only widely variable from one tissue to another, they themselves are not digital' [13].

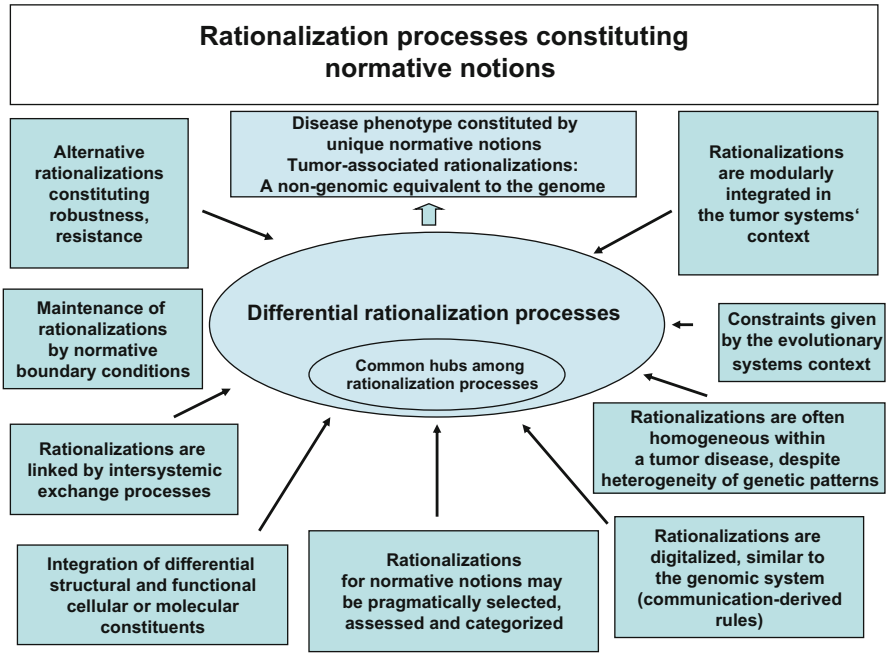

Fig. 16.1 From communication-theoretical considerations (formal-pragmatic communication theory) we may delineate that normative notions, i.e., structures, action norms, and decision maxims, are concretely rationalized. Additionally, the (therapeutic) implementation of non-normative boundary conditions into the normatively structured systems world of a tumor may lead to remodeling and redirecting rationalization processes and the corresponding normative notions according to testable communication-derived rules

From communication-theoretical considerations (formal-pragmatic communication theory) we may delineate that normative notions, i.e., structures, action norms, and decision maxims, are concretely rationalized. Additionally, the (therapeutic) implementation of non-normative boundary conditions into a tumor's normatively structured systems world leads to remodeling and redirecting rationalization processes and their corresponding normative notions according to testable communication-derived rules ([1, 2], Fig. 16.1). Non-normative boundary conditions may even have the capacity to implement reproducible heritable chromosomal changes in prostate cancer [14].

Therefore, the communicative expression of communication lines is digitalized within the range of situative evolutionary constraints. Consecutively, all communicative presuppositions facilitating distinct identities of systems objects have to be considered communicating in a digitalized way—a fact that does not exclude analogously or stochastically working subsystems. Communication-associated rules are constructed for enabling systems objects to acquire identities of systems subjects in a novel evolutionary systems context.

This novel view offers the opportunity to systematize normative notions and their corresponding multifaceted rationalization processes on the background of genomic patterns and acquired molecular-genetic aberrations. At that stage, genomic patterns become interpretable on the basis of scientifically verifiable rationalization processes that are experimentally or therapeutically reconstructable as well as accessible either to modulation and redirection by biomodulatory therapies or by epigenetic and genetic interventions.

The Formal Origin of Pathologic Rationalizations

The formal origin of pathologic rationalizations may be rather different—independent of the supported normative notion: Sequential rationalizations developing in the frame of reproducible sequences of genetic and pathway alterations during long-term tumorigenesis, such as colorectal cancer, may follow a rather different constitutive process than rationalizations developing in malignant diseases with principally heterogeneously 'de novo' acquired genetic origins, as, for example, 'acute' leukemia. Systems robustness and intrinsic resistance can be particularly influenced by the different origins of rationalization processes, either by the sequential development of genetic aberrations over many years, or 'de novo'—i.e., without clinically detectable 'pre-malignant' lesions—within a rather short time frame. These observations may be supported by chemotherapeutic experiences, which show that complete remission or continuous complete remission is more likely to be achieved in tumor diseases with 'de novo'-arising chromosomal aberrations (e.g., acute leukemia) than in metastatic tumors with a carcinogenesis over several years (e.g., colorectal cancer) [4, 5, 15].

Differential Constitution of Rationalizations Supporting Similar Normative Notions

Rationalizations supporting similar normative notions are frequently organized by completely different patterns of chromosomal or molecular-genetic aberrations. Convergent evolutionary processes should be categorized in novel evolution-adjusted tumor pathophysiology.

In more and more types of neoplasia, molecular-genetic aberrations are also found in adjacent stroma cells. Rationalizations constitute communicative expression, which again cannot be pinned down to tumor cells only, but have to include the reciprocal communicative function of stroma cells. Stroma cell functions may be multifaceted and vary from bystander functions to tumor-initiating functions and may even induce neoplasias in heterologous cell systems [16–20]. The term 'tumor stem cell' is relativized on the background of the multifaceted communication-mediated functions of tumor-associated stroma cells.

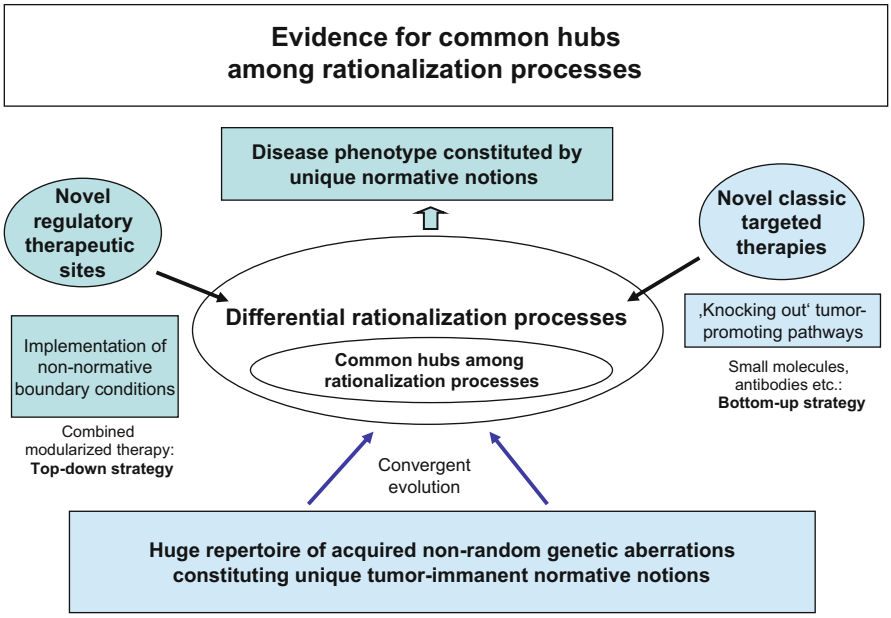

Fig. 16.2 Multifaceted combinatory tools of non-random chromosomal and molecular-genetic aberrations can constitute unique tumor phenotypes by propagating and maintaining diverse evolution-based rationalizations for distinct tumor-immanent normative notions. Therapeutic accessibility of comparable tumor-immanent normative notions among histologically heterogeneous tumor types with identical combined modularized therapies suggests the therapeutic availability of common hubs among these rationalization processes

Rationalizations Make Use of Hubs

A further important point is that rationalization processes draw on a limited number of biological structures and functions within a cell, such as tumor cells, that are specifically evolutionarily confined. Thus, differential rationalization processes constituting similar normative notions on the basis of rather heterogeneous genetic starting points within a histological tumor type or among different histologies may be linked by characteristic hubs or nodes, which are concertedly used to install action norms and which finally allow the configuring of tumor-comprehensive normative notions while recruiting limited functional and structural biologic resources according to communication-derived rules (Fig. 16.2).

These postulated rationalization-inherent hubs and nodes, which may be predominantly non-oncogene-addicted, would be excellent targets for biomodulatory tumor therapy approaches or again for classic targeted therapies aimed at modulating and redirecting decision maxims. The bcr-abl oncogene product (chimeric tyrosine kinase coded by the Philadelphia chromosome) seems to be localized in such a hub of rationalization processes that contribute to mediating apoptosis resistance and survival in chronic myelogenous leukemia (CML) [21].

Tumors driven by receptor tyrosine kinases seem to be more efficaciously treated by hitting signaling nodes that interconnect core pathways, i.e., rationalization processes sustained by receptor tyrosine kinases [22].

Non-oncogene-addicted targets, such as downstream oncogene effectors, could be detected to act as hubs by network modeling. The blockade of such a hub, for instance, the bet1 integrin subunit, may disrupt network gene expression and attenuates tumor growth in vivo [23].

Communicative Rules and Cell Death

To conceive every situative communicative expression of systems objects seems daunting: But the certainty that the identity of systems objects is redeemed in rationalizations in a digitalized manner and that these rationalizations may be categorized, confines the experimental expense for investigating evolutionarily constrained identities and functions of systems objects. Such studies may be supplemented by systemizing communicative prepositions that mediate the concrete situative identity of a systems object.

Systems objects are subjected to distinct structural presuppositions facilitating their communicative expression, frequently within multifaceted evolutionary contexts. These boundary conditions determine the systems objects' function within the frame of their modular knowledge. Evolutionarily confined communicative contexts access certain features of modular knowledge and provide the opportunity to therapeutically redeem distinct features necessary for tumor control. Systems objects can only be considered evolutionarily conserved if molecular or cellular constellations that have been observed in the original experimental setting are redeemed by a novel evolutionarily constrained communicative context. Thereby, evolutionary contexts may stretch to rather different observation levels and can thus be described on a subcellular, a cellular, or on a tissue level; however, such contexts can be also relate to the whole organism.

If communicative rules, the availability to mobilize alternative rationalizations (robustness, intrinsic resistance), or the possibility to redirect rationalizations collapse in response to the implementation of non-normative boundary conditions or any other therapies, the whole biological system becomes unable to maintain identity and integrity [24]. Consecutively, cell death will result. Thus, cell death may also be a consequence of fatal communicative interactions, if alternative rationalizations (normative notions), compensative pathways, or intrinsic resistance processes are lacking. Fatal communicative interactions by hitting the communicative Achilles' heel are inducible by implementing non-normative boundary conditions (biomodulatory therapies), as indicated by the very rapid tumor response observed [25]. Cell death would then be reduced to a communication-technical problem. The concerted activity of multi-targeted biomodulatory therapy approaches, including drugs with poor or no mono-activity, may indeed induce continuous complete remission, for example, in renal clear cell carcinoma and multisystems Langerhans cell histiocytosis [25].

Studying Rationalization Processes

Are rationalization processes an intellectual game or accessible to scientific scrutiny?

For many transcription factors and cell systems, context-dependent multifaceted situative identities and functions are known to ensue from different evolutionarily confined systems. Frequently, functions of distinct cell types are opposing or qualitatively rather different, for example, macrophages or the function of a central transcription factor during tumor progression, NFkappaB [26], or—as exemplified in the present book—the activity of VEGFR and corresponding tyrosine kinase inhibitors (Chap. 7).

The identity and function of cell systems may be directly investigated by cellular secretome analytics, by imaging techniques focused on finding, for example, the 'hallmarks' of cancer and therapeutic changes [27–29], or by the comparative uncovering of a tumor's systems biology by means of modularly targeting normative structures, action norms ('hallmarks' of cancer), or decision maxims [1]. The identity and function of the regulatory information of the genome may be investigated by sequence specific transcription [30]. Tissue-specific transcriptional control mechanisms are essential for the development of cell phenotypes [31]. Therefore, (combined) transcriptional modulation represents an important biomodulatory approach in cancer therapy [25].

Generally, proteins expand their identity as elements in networks of protein-protein-interactions, in which a distinct protein develops a contextual or cell-specific function within functional modules. The identity and function of multiply interconnected proteins is described, among others, by the attribute, enzyme, catalyst, signaling molecule, or by the building of distinct aggregates in cells [32].

By assessing the identity and function of tumor systems objects, the functional and structural sequences of rationalization processes may be reconstructed in detail. In parallel, novel interactions may be uncovered in the traditional reductionist way.

Rationalization Processes as Target to Overcome Genetic Heterogeneity of Tumor Cells

Long-term disease stabilization, long-term disease control at minimal disease and induction of continuous complete remission in various histological tumor types with combined modularized therapies is indicative that combined modularized therapies meet homogeneously constituted rationalizations for tumor-immanent normative notions within a distinct tumor disease despite the suggested molecular-genetic and genetic heterogeneity in the primary tumor site and in the metastases ([1, 25], Chap. 2). The observation is supported by results from the group of Fridman, showing that the constitution of immune response at the primary tumor site and in corresponding metastases of the lung is similar in colon cancer and renal cell carcinoma [33].

Thus, studying rationalization processes for tumor-immanent normative notions is pivotal to overcome genetically based tumor heterogeneity with combined modularized therapy approaches: Rationalizations represent a non-genomic equivalent to the genome.

Starting from the therapy results derived from combined modularized therapy strategies, one may speculate that the frequent failure of combined modularized therapies in controlling previously irradiated tumor lesion (chapter 2) may be related to radiation-induced genetic tumor heterogeneity, but also to heterogeneity in post-radiogenic developing rationalization processes constituting a distinct tumor-promoting normative notion.

Therapeutical Impact of Targeting Tumor-Promoting Rationalizations

Tumor-specific and stage-specific therapeutic accessibility of pro-inflammatory processes to induce response in all tumor types indicate a constitutive spin-off of situative rationalization processes constituting tumor-promoting inflammation. Furthermore, this accessibility shows the differential integration of rationalizations into the context-dependent 'living world' of tumor compartments: Inflammation-related activities are communicatively promoted and differentially adapted during tumor evolution. Empirically, differences may be detected in the modalities of developing evolutionary systems and in the acquired functional impact of inflammation-related rationalizations. Biomodulatory therapies, administered as fixed modules, may contribute to the discovery and understanding of novel multi-dimensionally operating regulatory systems in tumor biology. Primarily multi-track 'top-down' approaches including combined transcriptional modulation seem to be an efficacious novel therapeutic option to get regulatory therapeutical access ([25], Chap. 2, 22).

Discussion

Studying rationalization processes for tumor-immanent normative notions is a prerequisite for the efficacious design of combined modularized tumor therapies. Comparative investigations on the therapeutic accessibility of tumor-associated inflammation have shown that rationalizations for distinct normative notions may be rather differentially constituted within different histological tumor types. Combined modularized therapies have to be adjusted correspondingly [1].

Therapeutically all-important is the fact that tumor-associated rationalization processes may be maintained within a patient's tumor disease despite the development of genetic tumor heterogeneity in primary and metastatic tumor sites. Otherwise, specific combined modularized tumor therapies could neither induce long-term tumor control nor continuous complete remission in metastatic tumor

diseases ([25], Chap. 2). Therefore, rationalization processes are important targets, particularly in metastatic tumor diseases for overcoming the obstacle of 'bottom-up' strategies with genetically based tumor heterogeneity (Chap. 22).

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Part VII
Reconstruction of Tumor-Immanent Normative Functions, Structures and Decision Maxims (Hubs)

Chapter 17
Bridging a Diagnostic and Therapeutic Gap: Selecting, Assessing and Categorizing, Tumor-associated Normative Notions

Albrecht Reichle

Abstract How are new communication-based tumor models and novel therapeutic technologies, i.e. combined modularized therapy schedules, best implemented for improving patient care? Tumor evolution draws on modular tumor structures for constituting rationalizations of tumor-associated normative notions. Communication-technically, a systems participator itself as well as its communicative presuppositions may be altered in an evolutionary process so that tumor systems and corresponding rationalizations of normative notions can evolve. The possibility of therapeutically modulating communicative presuppositions of systems participators for tumor evolution highlights the pivotal importance of modules for generating rationalizations of normative notions during cancer development. For clinical use, evolution-adjusted tumor pathophysiology supplies physicians with multifaceted signatures that define the identity and function of systems participators; furthermore, instruments for the cross-validation of tumor-associated rationalization processes are provided. Such cross-validation helps break down complex aggregated normative notions into comparable ubiquitously accessible rationalizations, for instance, for tumor-associated angiogenesis, inflammation, etc., paves the way for therapeutically accessible solutions to assess and redirect tumor-associated normative notions (combined modularized therapies), and provides covariables for novel adaptive trial designs by introducing 'universal' biomarkers and theranostics. The routine applicability of evolution-adjusted tumor pathophysiology allows combined modularized treatment strategies, the repurposing of available drugs, front-line treatment stratifications, and outcome-triggered adaptive trial designs. This way, safety may be established in first-in-human-trials with licensed drugs to maximize the probability of benefitting patients with metastatic tumor disease.

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Introduction

Scientifically assessable tumor-associated normative claims, i.e. normative structures, action norms, and decision maxims including the 'hallmarks' of cancer, are commonly derived from arbitrary tumor systems—either in vitro or in vivo—by applying reductionist observations, i.e. the observer's view. The reductionist perspective offers the opportunity to describe systems participators as objects (in the past tense form) with the aim to formally distinguish one object from other systems objects [1]. At first, communicative presuppositions generating distinct communicative expressions seem to be irrelevant; then, a novel finding, which is denoted by the separation of one systems object from another, is to the fore.

In contrary, the formal-pragmatic communication theory paves the way for considerations about situatively emerging subjects that are integrated into the evolutionary context of biological systems [2].

Evolution theory and the underlying formal-pragmatic communication theory aim at reconstructing communicative interactions of systems participators with respect to their communicative expression. Systems participators are subjected to systems-mediated demands, thereby situatively acquiring novel identities. At that stage, communication-relevant units (modules) are dissectible. These modules are composed of both the systems participators and the respective communication-relevant presuppositions, which finally facilitate a distinct communicative expression at an evolutionarily confined systems stage [3].

Continuous compensation, equilibration, and advancement between inherently provided functions of a systems participator and the claims imposed by the respective tumor systems lead to the perception of modularity. Such modularity is based on the possibility of systems objects of acquiring novel validity and denotation during evolutionary processes in a modularized way. Thereby, the system draws back on the systems participator's modular knowledge. Communication-technically, not the systems objects themselves determine their situative validity and denotation but the modules including the respective communicative presuppositions, thereby facilitating distinctive communicative expression (Fig. 17.1).

Tumor-associated Patterns of Normative Claims

Reproducibly observable characteristic patterns of tumor-associated normative claims among histologically defined tumor types [1] directly impact on the selection of therapy-based interventions, [2] on the applied experimental procedures for specifying clinical observations (patient or physician reporting) and [3] depend on the evolutionarily confined situative constitution of tumor systems (Fig. 17.2).

In a first step, reconstructive investigations aim at assessing correlations between identity and function of systems participators and respective tumor-associated normative notions. Such investigations focus on the reconstruction of rationalization

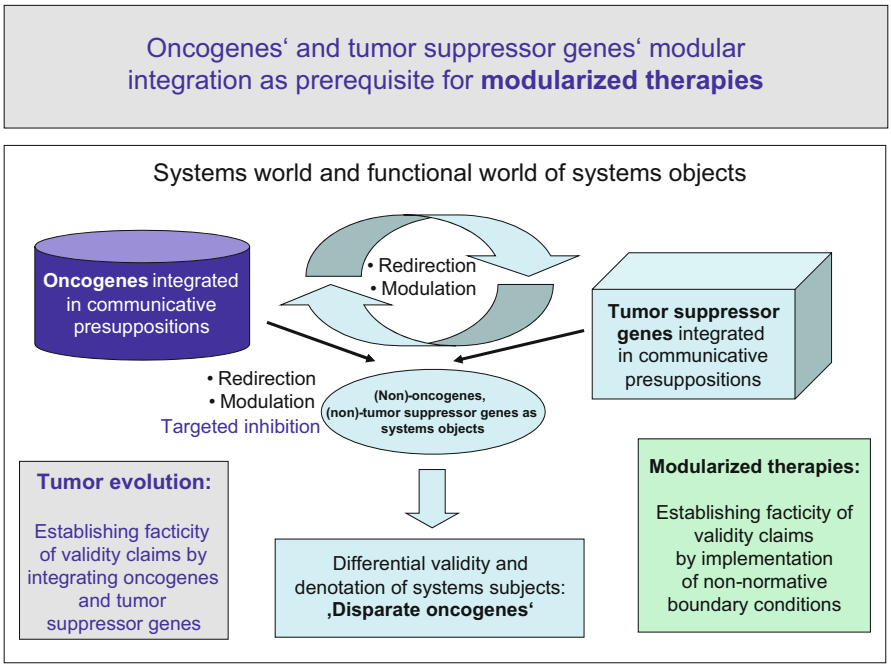

Fig. 17.1 Tumor systems are modularly structured because of the communicative interactions of systems participators: Communicative presuppositions given by the systems context determine the communicative expression of systems participators, i.e. their evolutionary confined validity and denotation. As activated oncogenes and deactivated tumor suppressor genes are modularly organized within rationalization processes of tumor-associated normative notions, their function is accessible for modulation and redirection by combined modularized therapy approaches

processes constituting tumor-associated normative claims (e.g. dysplasia, rapid tumor growth, apoptosis resistance, traditional ‘hallmarks’ of cancer, etc.) either by establishing ‘universal’ signatures, which depict identity and function of systems objects (e.g. by cellular secretome analytics, ‘omics’, epigenetics or molecular imaging), or via physical reconstructions of molecules and pathways inclusively their assignment to respective functional and structural communicative presuppositions that facilitate the constitution of communicative expression: Normative claims linked to systems participators cannot be circumvented and are the basis of every scientific observation (Table 17.1) [3].

Signatures and Tumor-associated Normative Notions

‘Universal’ Signatures Prognostic or predictive signatures do not necessarily admit causal interactions of systems participators. Surrogates, i.e. biomarkers, and particularly signatures derived from ‘omics’ (genomics, metabolomics, etc.) are

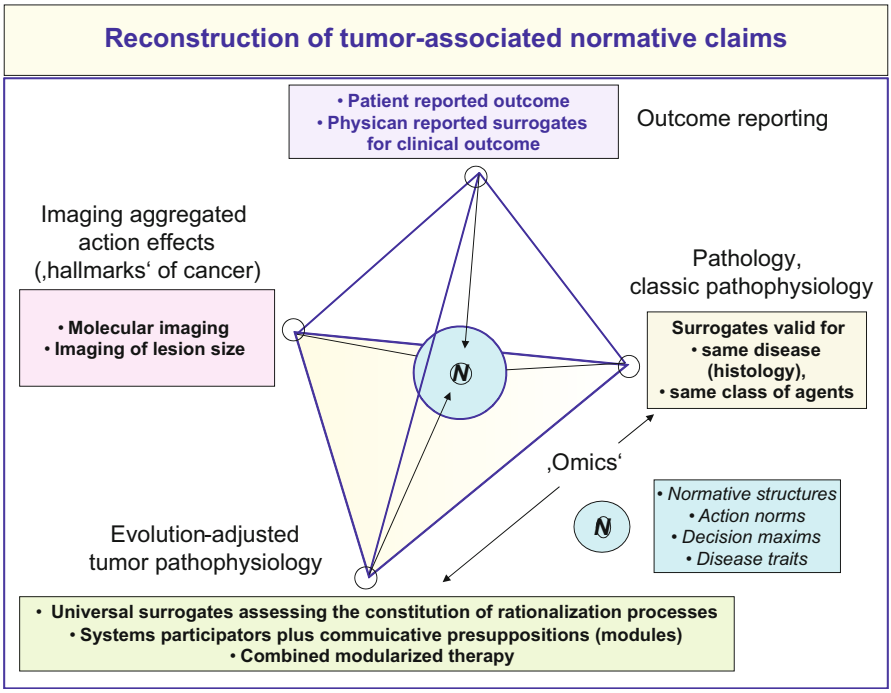

Fig. 17.2 Tumor-associated normative notions may be reconstructed by four methodologically rather different technologies, i.e. clinical outcome reporting, classic pathology and pathophysiology, evolution-adjusted pathophysiology, and molecular imaging. If non-drug specific, ubiquitously available and intersystemically comparable normative notions are selected for systems description, data derived from the four technologies may be cross-validated. In so far, also drug-specific complex normative notions may be broken down into pragmatically manageable tumor-associated normative notions

based on hypothesis-driven considerations and consecutive mathematical work-ups. In the case of evolution-adjusted tumor pathophysiology, 'universal' surrogates (as shown for C-reactive protein) depict communication-derived normative statements and rules. That way, 'universal' surrogates indicate how to therapeutically modulate and redirect tumor systems-related processes, particularly the communicative presuppositions that attribute a distinct communicative expression to tumor systems participators. Surrogates representing the identity and function of, for instance, tumor-associated cell compartments could be the basis of outcome-adaptive trial designs. Knowledge about how normative notions are rationalized in the context of evolutionary systems facilitates patient pre-selection for combined modularized therapies (biomodulation) (Chap. 15).

Table 17.1 Quite different histological tumor types have been used as model systems: Combined modularized therapy may redirect and modulate tumor-associated rationalizations, such as angiogenesis, inflammation and immune response

Tumors as model systems: Combined modularized therapy: Antiangiogenetic/anti-inflammatory/immuno-modulatory trials	
Tumors with high vascular density	Angiosarcoma
	Renal clear cell carcinoma
Tumors with extensive inflammation	Refractory multivisceral Langerhans' cell histiocytosis
	Multiple myeloma
Tumors with inflammation in advanced stage	Sarcoma
	Melanoma
	Cholangiocellular carcinoma
	Castration-resistant prostate cancer
	Gastric cancer

Cross-validation of Normative Notions

In a second step, multifaceted tumor-associated normative levels may be cross-validated by principally different scientific points of view (Fig. 17.3), i.e. (1) outcome reporting (physician-reported outcome, patient preference, quality of life, etc.), (2) classic pathophysiology and pathology, (3) molecular imaging of aggregated action effects, such as the 'hallmarks' of cancer, and (4) evolution-adjusted tumor pathophysiology, which assesses as novel diagnostic step communication-derived pathologies (Chap. 18).

Plurality of Perception Regarding Normative Notions Cross-validation is only feasible if normative notions have been pragmatically selected, are inter-systemically comparable, such as tumor-associated inflammation (Fig. 17.3), angiogenesis, etc., and are accessible by different methodological approaches. General normative notions of tumors and their respective rationalizations may be the basis of generating 'universal' surrogates that provide information about the constitution of rationalization processes and the respective modularly arranged tumor-associated systems participators (Chap. 20). 'Selective' biomarkers or signatures may be selected to predict benefit, treatment failure or adverse reactions.

Bridging Diagnostic Gaps Current surrogate markers are predominantly valid within a distinct histologically defined disease, or for a specified class of agents or targets. These surrogates are supplemented now by surrogates that indicate the communicative expression of tumor systems participators and more comprehensive, even the functional status of rationalization processes constituting tumor-associated normative notions. Depicting rationalization processes and their functional status for monitoring therapeutically induced changes of the tumor's normativity universalizes biomarker signatures and closes a commonly arising diagnostic gap, namely that between the frequently vague validity and denotation of a target and clinical outcome.

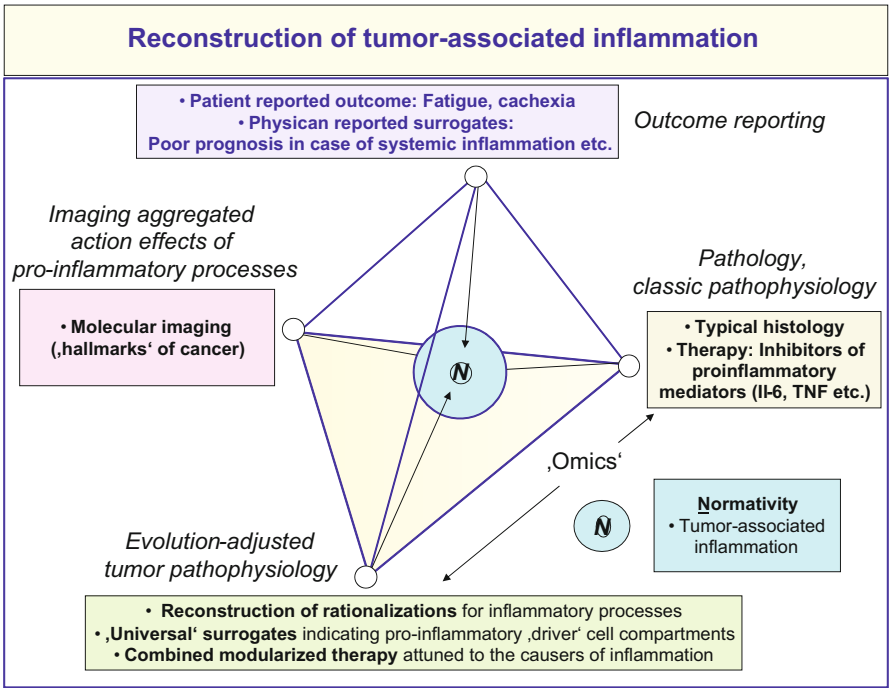

Fig. 17.3 Tumor-associated inflammation may be depicted by reductionist, holistically reconstructive, outcome-orientated, and imaging approaches. Respective results are available for cross-validation

Management of Complex Aggregated Normative Notions

In a third step, complex aggregated normative notions of practical interest are put forward to be reconstructed for facilitating multidimensional therapeutic access via combined modularized therapy approaches. Evolution-adjusted tumor pathophysiology provides data on situative, evolution based rationalization processes. The data are non-drug-specific, inter-systemically comparable, and accessible for cross-validation.

In contrary, reductionist considerations generally generate drug class-specific normative notions, which are described as outcome reports in dependence of a histological tumor type (Chap. 7), or try to clinically correlate complex normative considerations, e.g. polymorphism of drug metabolizing enzymes with prognostic or predictive signatures, e.g. risk of treatment-related complications [4].

For achieving intersystemic comparability among histologically different tumor types, normative notions should be selected and categorized in a pragmatic manner and oriented towards basic tumor-associated features, i.e. normative structures, action norms, and decision maxims, as a common denominator. Differently composed and integrated modules and, on a larger scale, rationalizations may constitute

unique normative notions—as shown in tumor-associated inflammation—and can be assessed in parallel from multifaceted scientific points of view (Fig. 17.3).

Diagnostics and the consecutive assortment of therapeutic interventions is rather restricted if pathophysiology is exclusively described by a drug-target interaction, which principally neglects the evolutionarily restricted communicative expression of a target at an evolutionarily constrained systems stage.

Clinically very important are 'universal' signatures that may be predictive in complex clinical situations, for example, in the occurrence of secondary malignancies in Hodgkin's disease after combined chemo- and radiotherapy. The genetic polymorphism of drug-metabolizing enzymes (pharmacogenomics) represents a drug-specific signature predictive for secondary malignancies in Hodgkin's lymphoma [4]. However, reductionistically derived signatures cannot be linked to ubiquitously accessible non-drug-specific and intersystemically comparable tumor-associated normative notions, i.e. inflammation, angiogenesis, immune response, decision maxims (hubs), etc. Routinely conducted reconstructions of ubiquitously accessible and pragmatically selected normative notions and their corresponding rationalization processes (e.g. 'hallmarks' of cancer), i.e. inflammation, angiogenesis, immune response, etc., would promote intersystemic comparability and facilitate the inclusion of other observation levels for validation. Also intersystemic exchange processes could be uncovered.

Such a work-up of common normative notions would also provide broad access for biomodulatory therapeutic interventions, also in case of developing pre-malignant lesions (tumor prevention, Chap. 15). Currently offered test sets are completely designed in a reductionist manner and do not implement considerations derived from evolution-adjusted tumor pathophysiology, i.e. 'oncotype' and 'recurrence score', 'tissue of origin test', and complex 'omics' data.

Evidence for the Modular Constitution of Tumors

The modular constitution of tumor systems may be delineated from multiple experimental and clinical observations.

1. **The oncogenes' communicative expression:** Many cancers do not have any key mutations that are druggable for tumor control. So far, only 4 % of the identified genetic aberrations in ovarian cancer are druggable and none in head and neck cancer [5, 6]. Alterations in oncogenes are clearly acquired and indicative for a distinct tumor type; moreover, the respective coded proteins are integrated into tumor-specific rationalizations processes. However, the altered oncogenes must obviously not endue key positions, such as hubs [7] (Fig. 17.4). Nevertheless, the multifaceted functions of non-druggable oncogenes are no insuperable barrier to efficaciously facilitate—as shown with combined modularized therapy approaches—the redirecting of normative notions in tumors. Thus, it should be

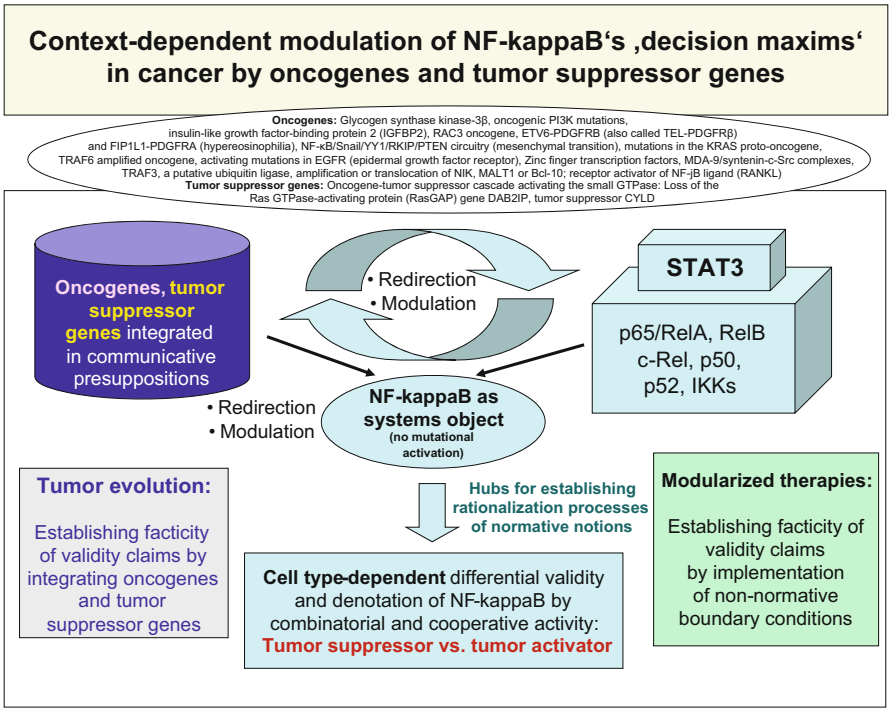

Fig. 17.4 The tumor-associated action norm 'inflammation' is closely linked with tumor activation and progression mediated via the transcription factor NF-kappaB. NF-kappaB activation and linkage to other tumor-promoting processes (e.g. STAT3 pathway) are evolutionarily constrained and rationalized—principally mediated by myriads of oncogenes and tumor suppressor genes. NF-kappaB does not require mutational activation for becoming active as an oncogene. Obviously, NF-kappaB serves as a decisive hub for rationalizing the tumor-associated action norm 'inflammation' by directing decision maxims, i.e. the multifaceted communicative expression of NF-kappaB. The context-dependent and evolutionarily restricted communicative presuppositions, including the newly arising activated oncogenes and tumor suppressor genes, guide the functional impact of NF-kappaB

taken into consideration that cancer-inducing genes are able to change communicative key presuppositions by altering the validity and denotation of systems participators. This explanation would be in accordance with the frequent observation that many oncogenes are not clinically efficacious druggable. In other words, many oncogenes only participate in modules to alter the communicative expression of tumor-associated systems participators [7] (Figs. 17.4 and 17.5). Therefore, the terms oncogene-addiction and non-oncogene-addiction are not very helpful for describing evolution-adjusted tumor pathophysiology.

2. **Evolutionarily confined communicative presuppositions in timely and spatially evolving modular systems:** A further observation on oncogene functions favors the specific spatial and time-related modular integration of oncogenes in cellular rationalizations of tumor-associated normative notion as a prerequisite for

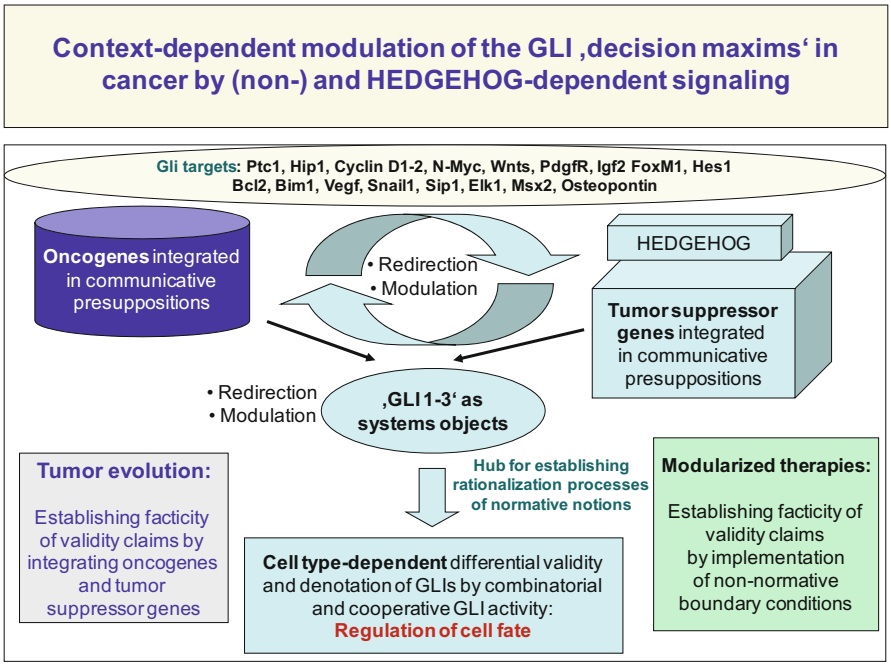

Fig. 17.5 A large number of tumors of the skin, brain, colon, lung, prostate, and pancreas as well as, among others, neoplasia of the blood cells depend on sustained HEDGEHOG-GLI (*GLI* glioma-associated oncogene) signaling for tumor growth. The interactively generated GLI 'decision maxims' represent an important hub within rationalization processes that set the course for cell fate: The central role of the GLI 'decision maxims' is underlined by its context-dependent regulation. The function of oncogenes and tumor suppressors are communication-technically integrated into the evolutionarily confined GLI 'decision maxims'—besides HEDGEHOG-GLI signaling. Both, modularized therapies and tumor evolution-promoting processes establish the facticity of validity claims by drawing on a systems participators' modular knowledge. Natural tumor evolution uses the integration of oncogenes and tumor suppressor genes, whereas modularized therapies simply implement non-normative boundary conditions so that tumor systems for growth control can involve

the development of cancer: The clonal presence of activated oncogenes alone is communication-technically insufficient for explaining cancer initiation, because oncogenes may also be found in benign skin lesions without any malignant potential. Thus, the distinct sequence of events and developing patterns that must occur in a single cell, including oncogene activation and tumor suppressor gene inactivation, is not necessarily sufficient to explain the development of cancer [9, 10]: Delineated from the evolution theory, evolutionarily confined communicative presuppositions in timely and spatially evolving modular systems and rationalizations can also contribute to a process that finally initiates malignant transformation [11]. Thus, non-arbitrary rationalization processes that, in contrast, develop in a digitalized manner within the framework of a cell systems'

'living world' and a systems participator's modular knowledge, strongly influence the communicative expression of proteins derived from oncogene and tumor suppressor genes.

3. **Every cancer genome is unique** [12]. The way to operationalize uniqueness is to prove the validity of an evolution theory: Both systems context and functionality of a systems participator cooperate in establishing facticity of the validity and denotation of systems participators within the framework of situatively redeemable modular knowledge. The term 'modular knowledge' is no virtual conceptualization but determines the range and therefore the communicative modalities on which a genetically altered system may recur to establish novel tumor-characteristic normative notions.
4. **Rationalization processes for tumor-associated normative notions seem to be frequently maintained in metastatic tumors:** The frequent emergence of tumor heterogeneity is confusing, also the current therapeutic methodologies trying to address molecular-genetic tumor heterogeneity [13]: Evolution-adjusted tumor pathophysiology allows the differentiation of the polarization between success-oriented and integration-oriented behaviors, as tumors equip biologically possible validity pegged to a systems object with the strength of facticity (corrupt rationalization) under the conditions of perceivable incompatibility between facticity and validity (Chap. 13). Between these conflicting priorities, the tumor disease unfolds and 'branches' according to rules that require further evaluation by reconstructive activities [14]. Despite the branching processes, which are responsible for frequently occurring tumor heterogeneity, rationalization processes for tumor-associated normative notions seem to be maintained, irrespective of genetic tumor heterogeneity, as indicated by complete remission or long-term tumor response to combined modularized therapy approaches in metastatic tumors (Chap. 2).
5. **Cancer cannot be simply pinned down to a dysregulation of communication processes:** The quote 'Rewiring makes the difference' is frequently too insufficient for describing the redirection of rationalization processes [15, 16].
6. **Clinical trials are often incomprehensively irreproducible**, for instance, the use of bisphosphonates in breast cancer (AZURE, ABCSG-12) [17, 18]: One reason may be found in the methodology and the performance of a phase III trial. But to the same degree, at a first glance non-discernable non-normative boundary conditions (comprising epidemiological influences etc.), may decisively influence outcome parameters.
7. **Efficacy of 'top-down' approaches:** Currently, controversial discussions take place about organ-based or pathway-based therapeutic alternatives, whereas pathway-based therapies that represent a successful part of cancer therapy are rising in number. Rationalization-centric combined modularized therapies, so called 'top-down' approaches, are now shown to be efficacious in histological tumor types with known intrinsic drug resistance.

   A pathway-oriented therapy recurs on the assumption that the validity and denotation of pathways are the same in histologically different tumor types, in all metastatic organ sites, and in molecular-genetically heterogeneous tumors; this assumption, however, may be easily refuted. For example, VEGFR inhibitors

show tumor-dependent activity and toxicity profiles (hepatocellular carcinoma, renal cell carcinoma, and acute myelocytic leukemia), and their activity depends on the metastatic organ site in RCCC (Chap. 7). Alk-positive tumors affect clinical endpoints differently when treated with an Alk-inhibitor [19].

Thus, organ-based and pathway-based therapy decisions are supplementary and share reductionist comprehension, while neglecting the context-dependently changing validity and denotation of potentially tumor-promoting pathways as well as tumor heterogeneity (Chap. 15).

8. **Biomodulatory therapies are modularly structured for simultaneously targeting multiple tumor-associated normative structures, functions, and decision maxims** by implementing non-normative boundary conditions. Thus, such therapies are predestinated to uncover modular tumor systems structures and intersystemic exchange processes. The activity profile of combined modularized therapies has shown long-term tumor control and tumor regression (objective response), even in histological tumor types with modest sensitivity to chemotherapeutic agents [20, 21] (Chap. 2).

Therapeutic Reorganization of Tumor-associated Normative Notions

How can new technologies—provided by evolution-adjusted tumor pathophysiology and combined modularized therapies—improve patient care?

An evolution theory allows the reconstruction of tumor systems by employing ubiquitously available non-drug-specific and intersystemically comparable tumor-associated normative notions; such notions may be scientifically assessed in an adequate manner and even cross-validated by novel up-coming methodologies (evolution-adjusted tumor pathophysiology) [21].

From a therapeutic point of view, the deconstruction of complex and aggregated normative notions derived from daily observations of tumor systems to universal normative and pragmatically manageable claims opens a novel therapeutic window: By applying combined, multifaceted modularized therapy schedules, tumor-associated normative functions may be ‘normalized’ [22, 23]. Normalization means to return to the ‘status ante’, which—from an evolution-theoretical point of view—may be wishful thinking. However, normative functions may be modulated and redirected by therapeutic interventions, this way, tumor systems therapeutically evolved. Selected tumor-associated normative functions can be targeted in a multi-modal manner to enhance anti-tumor efficacy [3].

The combined modularized targeting of normative tumor-associated functions may uncover tumor systems biology, particularly hubs of rationalization processes. The therapeutic availability of—so far—unknown hubs must be supposed, as the broad diversity of acquired genetic aberrations helps establish unique normative notions.

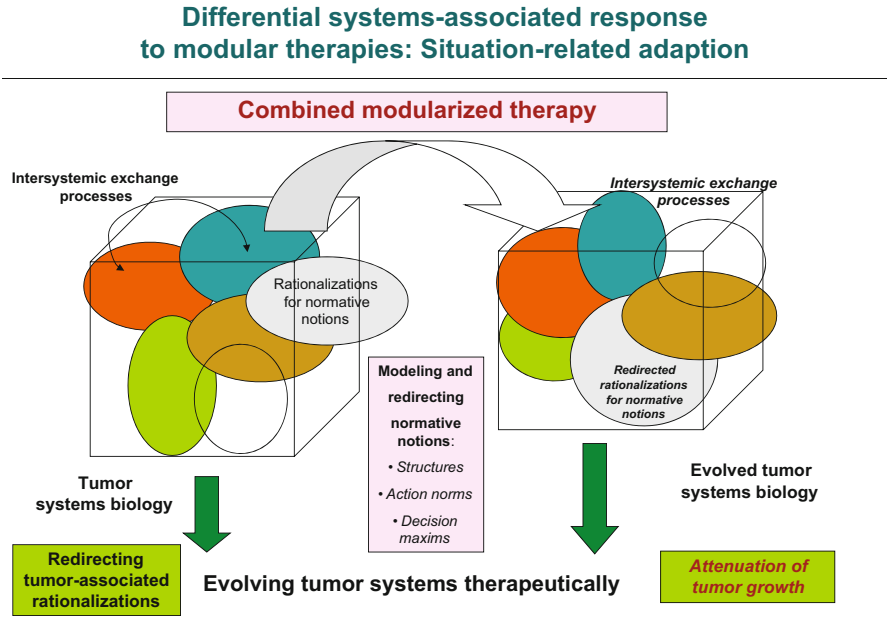

Fig. 17.6 In metastatic melanoma, modularized access to systems biological processes may be obtained—besides metronomic low-dose chemotherapy with trofosfamide—by the module pioglitazone, which has anti-inflammatory effects, in addition to coxib. Significant tumor cell-associated PPARgamma expression may indicate improved progression-free survival and C-reactive protein (CRP) response in serum improved overall survival

Adaptive Randomization by Patient Pre-selection and Outcome-adaptive Design

Combined modularized treatment strategies may schedule drugs with poor or no monoactivity as well as repurposed drugs, probably ab initio in **first-in-human trials** (Chap. 2): The tumors' normativity may be redirected and modulated for achieving attenuation of tumor growth. Via targeting rationalization processes of tumor-immanent normative notions, genetic heterogeneities among histologically defined tumor types and within a metastatic tumor may be overcome as long as tumor-associated rationalization processes are sustained at the metastatic sites. Thus, combined modularized therapies offer the opportunity for specifically targeting rationalizations and for maximizing the probability of benefitting patients with advanced metastatic tumor disease (Fig. 17.6) [24].

In many respects, the risk for patients to receive an inefficacious or toxic therapy may be minimized by including adaptive randomization by patient pre-selection ('universal' surrogates) and outcome-adaptive designs (theranostics):

1. **'All-inclusion' strategy by using 'universal' surrogates:** Generally, cancer patients may be pre-tested for known targeted treatments [25]. If the target is

not available, patients are excluded from the scheduled therapy ('all-exclusion' strategy) (Chap. 22).

Evolution-adjusted tumor pathophysiology, however, provides 'universal' biomarkers or signatures that facilitate adaptive front-line stratification of patient populations with similar rationalizations for tumor-associated normative notions, necessarily overarching tumor histologies.

Knowledge about the way how normative notions are constituted provides specific access for combined modularized therapies. Those 'universal' surrogates do not stratify in 'go-go' or 'no-go' patients ('all-exclusion' strategy) but allow the selection of specific modularized and situatively adapted therapies to redirect ubiquitous tumor-associated normative notions, i.e. angiogenesis, inflammation, etc. ('all-inclusion' strategy). Selection is oriented towards the constitution of underlying rationalization processes (Chap. 22; [23]).

2. **Adaptive trial designs by theranostics:** Challenges in the development of targeted therapies may be addressed by introducing outcome adaptive trails: Selecting the right patient for the right drug, the commonly used procedure, gains a novel facet, namely selecting the right drug combination for the particular patient. Although the use of outcome-triggered trial designs is more problematic, biomodulatory therapies provide theranostics data about the therapeutically sufficient or insufficient redirection of tumor-associated normative notions. Theranostics data again do not aim at separating between 'go-go' and 'no-go' parameters (biomarker present, absent, quantity, changing levels) but provide benchmarks for alternative modularized therapies ('all-inclusion' strategy) (Chap. 22). Surrogates that may specify identity and function of rationalization processes will be available in future to adapt modular therapy schedules. In contrast, common targeted therapies rely on the knock-down of single pathways, irrespective of the pathways' heterogeneous communicative expression in a distinct, and frequently unknown evolutionary context (Chap. 7) [3].
3. **Why remains high clinical efficacy of current targeted therapies an exception?** The exclusive application of reductionist predictors or read-outs for outcome, i.e. the presence or absence of a sufficient number of targets or stereotypically used clinical parameters, such as reduction of tumor load, progression-free survival etc., is double-edged, as it remains a diagnostic gap: Diagnostic efforts do not take account of ubiquitously accessible communication-derived rules, i.e. subjectivity, intersubjectivity and modularity.

   Exceptional targets for knocking down tumor-promoting pathways are, for instance, the chimeric tyrosine kinase in chronic myelocytic leukemia (CML) or receptor tyrosine kinases such as Her-2 in breast cancer as well as an ever growing number of successfully druggable oncogenes [26, 27]. Such targets indeed confirm to proceed with reductionist therapies and outcome validations.

   However, these pivotal examples only indicate that in distinct histological tumor types the respective signaling pathways are often evolutionarily conserved with respect to their communicative expression: Either no additional aberrations are present in the respective malignancy, which could alter validity and denotation of the targets, for example in CML [28], or additional aberrations do not

interfere with rationalization processes, in which the respective target is functionally embedded, or the targeted molecules probably represent hubs in common rationalizations.

'Declination' of tumor-associated normative notions: 'Bottom-up', single-track targeted therapies mostly show a wide range of tumor responses (Chap. 7). Thus, these therapies are more or less sufficient for redirecting tumor-specific normative notions and for attenuating tumor growth, even if they may overall improve progression-free or overall survival, such as bevacizumab [29]. Here, a breakdown of intersystemically comparable tumor-associated normative notions into their respective rationalizations and their 'declination' would be helpful to appreciate the activity profile of a classic targeted therapy on a personalized basis. At least in CML, we know that the occurrence of additional molecular-genetic aberrations significantly alters the validity and denotation of the chimeric tyrosine kinase, as indicated by the insufficient activity profile of tyrosine kinase inhibitors in these cases [30, 31].

4. **Shortcomings of 'bottom-up', single-track strategies:** Anticancer agents directed against pathways have become a focus of clinical trials. Common targeted therapies are on a genome-driven therapeutic course, aiming at targeting driver mutations. In many histological tumor types driver mutations are not directly available or not present at all. Key driver oncogenes are missing in adeno-carcinoma of the lung and in head neck cancer [5, 32].

   An additional problem of current targeted therapy approaches is that each new targeted therapy or class of new drugs may target multifaceted rationalization processes within a tumor dependent on the communicative impact of the target within an evolving tumor system (Chap. 7). The only way to design a 'bottom-up' therapy is to monitor the availability of the target in a tumor biopsy and to select a single track or combined single-track therapy approach (Chap. 22). Thereafter, response and often protracted survival assessments serve to validate therapeutic efficacy (Figs. 17.2 and 17.3).

   By using 'bottom-up' strategies, several important confusing problems remain unsolved: For most targeted therapies no 'universal' surrogates are available indicating early redirection of the tumor's normativity ('monitoring gap'). Further, 'bottom-up' strategies do not meet genetic tumor heterogeneity or the context-dependent changes in the validity and denotation of tumor-promoting pathways.
5. **A monitoring gap:** Outcome reporting remains the only possibility to report clinical activity, as shown by myriads of clinical trials on cytotoxic drugs. Many 'non-targeted' drugs, such as lenalidomide, metronomic low-dose chemotherapy, etc., can be tested in an 'all comers' ('non-marker-selected') fashion because of the lack of specific identifiable biomarkers [33, 34]. The activity profile of 'all-comers' may be 'declinated' and systematically broken down into non-drug-specific and intersystemically comparable tumor-immanent normative notions and their respective rationalizations [3, 35].

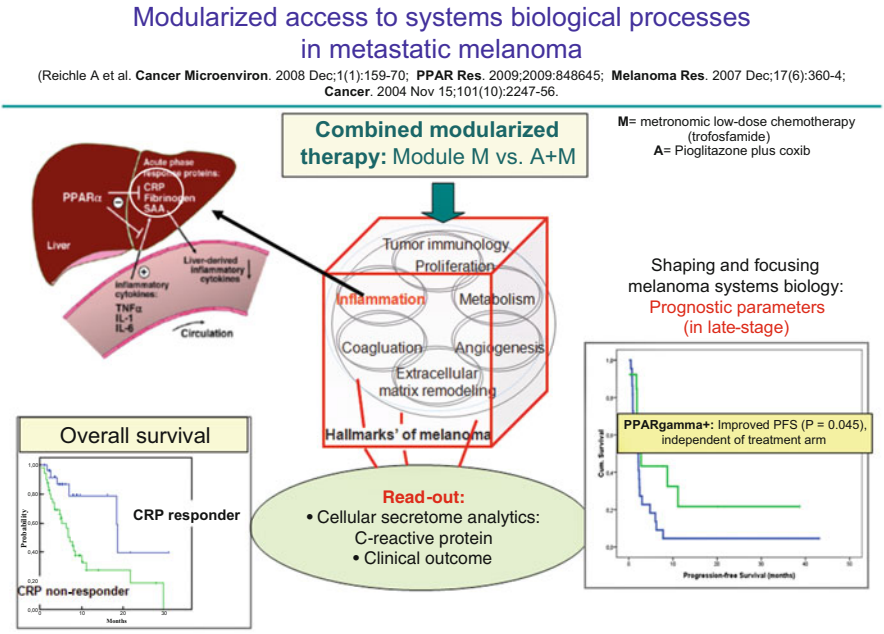

Fig. 17.7 The evolution of tumor-associated systems may be achieved by the therapeutic implementation of non-normative boundary conditions (M versus A + M) for redirecting tumor-associated normative notions. Decreased C-reactive protein (*CRP*) levels in serum of $> 30\,\%$ from baseline ('universal' surrogate for systemic tumor-associated inflammation) is significantly associated with improved survival and PPARgamma expression in melanoma cells (tissue micro-arrays) is associated with improved progression-free survival

6. **Drug repurposing by combined modularized therapies:** Within modularized therapy schedules, drugs may be repurposed, because their biomodulatory capacity is therapeutically decisive, but not their mono-activity. Drugs can be principally administered at low doses (Fig. 17.7). The resulting adverse events and severe adverse events are generally manageable by early dose reduction, already in case of grade 2 toxicity. This way, long-term administration of metronomic therapy becomes possible ([3, 36]; Chap. 2).
7. **'Bottom-up' versus 'top-down' strategies:** Whereas 'bottom-up', i.e. single-track therapies have to be administered at maximal tolerable doses, 'top-down' approaches, primarily multi-track strategies are oriented at the maximal biomodulatory efficacy. Even in mono-therapy inefficacious drugs may be implemented in modular therapy designs.

Discussion

Selecting, assessing, and categorizing intersystemically comparable tumor-associated normative notions, i.e. structures, action norms, and decision maxims, by applying evolution-adjusted tumor pathophysiology has multifaceted clinical implications regarding therapy selection and monitoring.

Tumor Evolution Draws on Modular Tumor Structures Communication-technically both, a systems participator and the communicative presuppositions may be altered in tumors. Correspondingly, tumor systems with completely reorganized rationalizations of normative notions may evolve [3].

To describe tumor evolution in human tumors as a 'continuum model of selection', seems to be a contradiction in itself [37], particularly when the description of evolutionary historical processes is used as an explanation for 'continuously' evolving processes. The 'metabolism' of evolution may only be described by including an evolution theory that allows the provision of communicative rules in form of normative statements [3].

The possibility of modulating communicative presuppositions of systems participators for therapeutic tumor evolution ('top-down' strategy) highlights the pivotal importance of modules for generating rationalizations of normative notions during cancer development (Chap. 19). Excessive numbers of modular functions have been figured out for p53 protein [16]. Attenuating tumor growth may be achieved by the pharmacological uncoupling of an oncogene, for instance, MYC, from its communicative presuppositions, namely from bioenergetic pathways involving glucose or glutamine metabolism [38].

For clinical use, evolution-adjusted tumor pathophysiology supplies physicians with multifaceted signatures that define the identity and function of systems participators and provides instruments for the cross-validation of tumor-associated rationalization processes. Additionally, evolution-adjusted tumor pathophysiology facilitates the 'declination' of complex aggregated normative notions into comparable ubiquitously accessible normative notions (tumor-associated angiogenesis, inflammation etc.), paves the way for therapeutically accessible solutions to reorganize tumor-associated normative notions, and provides covariables for novel adaptive trial designs by introducing 'universal' biomarkers (patient pre-selection) and theranostics (outcome adaptive randomizations) (Chap. 15).

Evolution-adjusted tumor pathophysiology provides the possibility of establishing safety in first-in- human trials with licensed drugs (drug repurposing) to maximize the probability of benefitting patients with metastatic tumor disease. The routine applicability of evolution-adjusted tumor pathophysiology allows the development of combined modularized treatment strategies, the repurposing of available

drugs, as well as front-line treatment stratification and outcome-triggered adaptive trial designs.

Using a communication-technical explanation of tumor evolution, evolution-adjusted tumor pathophysiology provides access for novel tumor therapies: Tumor therapy may be specifically modularized to generate facticity of a particular communicative expression while drawing on and redeeming a distinct modular feature within the particular modular knowledge of systems participators. This way, evolution theory, which may be routinely assessed by evolution-adjusted tumor pathophysiology, directly contributes to improving patient care [39].

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Chapter 18
Personalizing Tumor Pathophysiology by Diagnosing Developmental Problems in Tumors with Imaging Techniques

Fabian Kiessling and Wiltrud Lederle

Abstract Whereas traditional non-invasive imaging was mostly directed to identify disease-associated morphological alterations, the development of functional and molecular imaging techniques has drawn the interest in disease-specific biomarkers as imaging targets in order to gain deeper insight into the mechanisms underlying tumor development as a basis of an improved, personalized medicine. Based on the ten hallmarks of cancer, current non-invasive imaging modalities, methods and contrast agents are presented in this chapter with respect to tumor characterization and treatment monitoring including their potential or already established applications in the clinics.

Today, non-invasive imaging significantly contributes to basic research, connects preclinical and clinical research and is pivotal for many therapeutic decisions in tumor patient care. While non-invasive imaging of the last century was mostly based on assessing morphological alterations, the rise of functional and molecular imaging brought new disease-specific imaging biomarkers into the focus of research and raised the demand for a more mechanistic explanation of imaging findings. In particular, since tumor load and number of metastases do not always correlate with patient survival, it has become clear that other biomarkers with a better prognostic impact have to be elucidated.

Another aspect motivating the development of novel imaging tools is the clinical introduction of molecular therapeutics. On the one hand, enhanced specificity of therapies might increase their effectiveness and reduce their side effects. On the other hand, in comparison to conventional chemotherapeutic agents, the population of patients that will benefit from targeted therapeutics is smaller and has to be selected more carefully. Additionally, early resistance to therapy has to sensitively be detected at an early stage.

Taking these aspects into account, personalized diagnosis is mandatory in addition to personalized tumor therapy.

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In this context, personalization means that imaging should be used to (i) better identify and characterize the tumor, (ii) to pre-select and exclude patients for targeted therapies, (iii) to early identify tumor response to therapy, and (iv) to detect tumor escape from therapy, resistance and relapse.

However, taking into account that the costs for the development of a diagnostic drugs for the clinical use are around 500 million Euros and above and that such diagnostic drug have a limited key market; one can understand that pharmaceutical companies hesitate to invest intensely into their development. Therefore, in the first step it is preferable to focus on imaging strategies and contrast agents that provide insight into general pathophysiological mechanisms of tumors and only in selected cases to develop companion diagnostics to support the therapeutic decision finding.

In the year 2000, Hanahan and Weinberg defined six hallmarks of cancer, which were supplemented in 2011 by four further aspects [1]. These describe the major differences between tumors and healthy tissues and can be seen as an excellent guideline for the development of imaging biomarkers. In detail, these are: (1) sustaining proliferation, (2) resistance to cell death, (3) induction of angiogenesis, (4) evasion from growth suppressors, (5) activating invasion and metastasis and (6) enabling replicative immortality. The four recent ones are: (7) abnormal metabolic pathways, (8) evading the immune system, (9) chromosomal abnormalities/unstable DNA and (10) inflammation.

Taking these hallmarks as guideline, we will present and discuss non-invasive imaging modalities, methods and contrast agents that are available to characterize tumor development and to monitor therapies. Beside their use in preclinical research, chances for clinical translations and the corresponding applications will be highlighted.

Imaging Tumor Metabolism

Several imaging methods are available to assess the increased metabolic activity of tumors. Differences to normal tissue beside the higher quantity of glucose consumption are that anaerobic glycolysis and protein catabolism are markedly increased. In addition, many tumors also show an increased uptake and higher intracellular concentrations of choline, an essential component of cell membranes and an important precursor of the neurotransmitter acetylcholine.

Magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) as well as positron emission tomography (PET) are the most commonly used tools to non-invasively study tissue metabolism.

Magnetic resonance spectroscopy enables the spatially resolved assessment of nuclear magnetic resonance (NMR) spectra displaying the dependence of the nuclear magnetic energy levels on the electronic environment in a molecule (chemical shift). This means that spectral peaks can be assigned to metabolites and thus be used for tissue characterization. With respect to metabolism, the assessment of ^{1}H and ^{31}P spectra is established (Fig. 18.1). The amounts of different metabolites that can

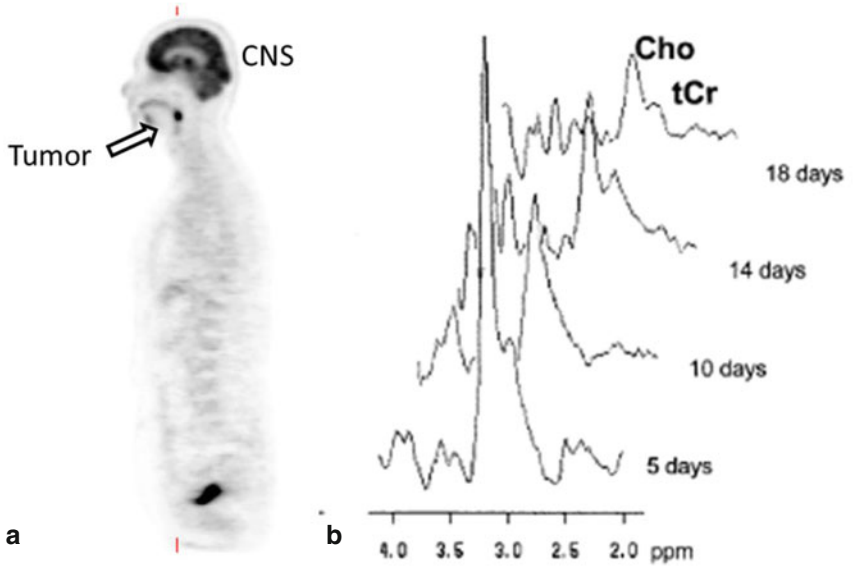

Fig. 18.1 ^{18}F-FDG PET image of a patient with an oropharyngeal carcinoma. **a** The tumor is clearly demarcated from the healthy tissue due to its increased glucose uptake. Only the central nervous system (CNS)—in particular the brain—also shows high glucose turnover (image kindly provided by U. Haberkorn, Univ. Heidelberg). **b** MR spectra of experimental prostate cancers treated with fractionated radiotherapy. The decreasing choline (Cho) concentrations in the tumors over time indicate therapy response. tCr = total creatine (shown with permission of Lippincott Williams and Wilkins; taken from Kiessling et al., Invest Radiol 2004;39:34–44)

be distinguished by proton MRS depend highly on the magnetic field strength. The higher the field strength is, the more metabolites can be distinguished. At 1.5 T and 3 T, which are common field strengths of clinical MRI scanners, choline, creatine and (in the case of brain tumors) N-acetyl asparate and lactate are commonly assessed metabolic markers. ^{31}P-spectroscopy is predominantly used to characterize the energy metabolism of cells and inorganic phosphate (Pi), phosphate mono-esters, phosphate di-esters, creatine-phosphate (P-Crea) as well as adenosine tri-phosphate (ATP) can be investigated. In addition, taking benefit from the dependence of the chemical shift between Pi and P-Crea on the pH value, also the intracellular pH-value can be determined. Another nucleus indicating metabolic aspects is ^{23}Na. In healthy tissues, energy depending sodium pumps keep the intracellular sodium concentration

low. In tissues with disturbed energy metabolism (such as tumors) the intracellular sodium concentration increases, which can be measured by ^{23}Na-MRS [2].

Spectroscopic analyses are also performed for ^{19}F. However, except for the teeth, ^{19}F is found in tissues at negligible concentrations. Therefore, the accumulation and degradation of ^{19}F-labelled pharmaceutics can be studied. For example, Schlemmer at al treated head and neck cancers with the chemotherapeutic drug 5,6-dihydro-5-fluorouracil (DHFU) and could not only show the accumulation of the drug in the tumors but also the appearance of its metabolites with time [3].

A relatively new method to study tumor metabolism is MRI with hyperpolarized substances [4]. Usually MRI suffers from a considerably low sensitivity to contrast agents. However, it is possible to perform ex vivo a nuclear spin polarization of materials far beyond the thermal equilibrium, which in vivo persists for a half life time of several seconds. This increases the sensitivity of MRI by a factor of 1,000 to 10,000. In this context, [1-13C]-pyruvate is used in preclinical and clinical studies to investigate cellular bioenergetics, such as glycolysis, the citric acid cycle, and fatty acid synthesis in prostate, brain and other tumors. Even for the short available time scale, the conversion of [1-13C]-pyruvate to [1-13C]-lactate and [1-13C]-alanine could be assessed.

PET is the clinically most established modality to diagnose and stage tumors as well as to assess therapy response. PET has a high sensitivity to radiotracers and picomolar concentrations can be detected. It bases on the fact that radioisotopes with a beta plus decay will emit a positron and an electron that collide and produce two gamma photons of 511 keV that are emitted in a 180° angle. This annihilation radiation is detected by two opposite detector elements in the PET detector ring. ^{18}F-FDG is the radiotracer of choice for tumor diagnosis outside the brain, which is the healthy organ with the highest glucose consumption under rest [5]. ^{18}F-FDG is internalized via the glucose transporter, phosphorylated (hexokinase-dependent) and then hardly further metabolized due to its D-configuration. Thus, it accumulates in tissues with high glucose uptake (Fig. 18.1a).

In addition to detecting primary and metastatic cancers, ^{18}F-FDG PET has a high potential in the assessment of therapy effects. For example, in several different solid and non-solid tumors, ^{18}F-FDG PET was found to be capable of monitoring already the early tumor response to (radio)chemotherapy, thus significantly impacting the further treatment decisions. Moreover, in non-small cell lung, oesophageal, and cervical cancers, the focal ^{18}F-FDG uptake after the end of chemoradiotherapy correlated with a poor prognosis and decreased survival.

Similarly, prognosis was found to be worse in Hodgkin and non-Hodgkin lymphomas if focal FDG uptake was observed.

Since increased protein catabolism in malignant tumors is often associated with an up-regulation of amino acid transporters, radiolabeled amino acids, such as methionine or tyrosine, can be used to indicate increased protein metabolism. In this context, one well established example is L-[methyl-^{11}C]methionine (^{11}C-MET), which has shown high sensitivity and specificity for the detection of malignant glioma. In addition, it has been shown that ^{11}C-MET PET is highly suited to differentiate recurrent tumors from radiation-induced necrotic areas and to accurately delineate the extent

of brain tumors. Brain tumors are among the preferred pathologies for amino acid PET since ^{18}F-FDG PET is difficult in the brain due to its naturally high glucose metabolism and thus high background signal.

Imaging Tumor Cell Proliferation

Cell proliferation can be assessed indirectly by measuring tumor volumes over time. In this context, several imaging modalities can provide accurate results. It has also been shown that the accuracy in tumor size determination significantly increases when a third diameter can be measured (external calliper measurements provide only two diameters) or when segmented tumor volumes are determined. For size measurements, CT and MRI are the methods of choice providing 3D data of the entire tumor volume. Ultrasound imaging can also be used to accurately assess tumor sizes, however, identification of lesions deep in the body can be difficult and reproducibility is limited due to the user-dependent image acquisition. The latter can be overcome by motor-driven transducer holders that slowly move the transducer over the tissue of interest and thus produce 3D images. These are currently tested in clinical studies on breast tumor detection and characterization.

Besides indirectly assessing tumor cell proliferation by determining the changes in tumor volume, PET radiotracers are available that can directly indicate the proliferative nature of a tissue. These radiotracers usually are nucleic acids labeled with ^{18}F. In this context, an established radiotracer is [^{18}F]fluorothymidine (^{18}F-FLT) [6]. ^{18}F-FLT is taken up by cells via passive diffusion and via transport by Na+-dependent carriers. After phosphorylation by the thymidine kinase 1, which is significantly up-regulated in proliferating cells, ^{18}F-FLT is trapped inside the cell and thus this radiotracer accumulates over time. However, since ^{18}F-FLT only reflects the uptake of thymidine but not its integration into DNA, it can fail to indicate decreased proliferation after treatment with chemotherapeutic agents like 5-fluorouracil and methotrexate that induce cell arrest in the S-phase, where the thymidine kinase 1 activity is still high or even increased. Nevertheless, cytostatic drugs like cisplatin, adriamycin and also radiotherapy were shown to significantly reduce the uptake of ^{18}F-FLT. Also in patients with breast cancer, ^{18}F-FLT was shown to be an excellent indicator of therapy response.

In brain tumors, initial results suggest that ^{18}F-FLT might be well suited to distinguish low and high grade gliomas and to distinguish tumor recurrence from radiation effects. In particular in brain malignancies, similarly to ^{11}C-MET, ^{18}F-FLT has advantages over ^{18}F-FDG due to its lower background signal.

Indirect information of cellular proliferation can be obtained from MRI/MRS data. MR-spectroscopy can assess the increased choline metabolism of tumors, which is associated with increased membrane synthesis, and thus also with cell proliferation. This imaging biomarker has already been discussed above.

Another MR biomarker being linked to increased cellular proliferation is diffusion weighted MRI (DWI) [7]. Here the Brownian motion, or in other words the diffusion

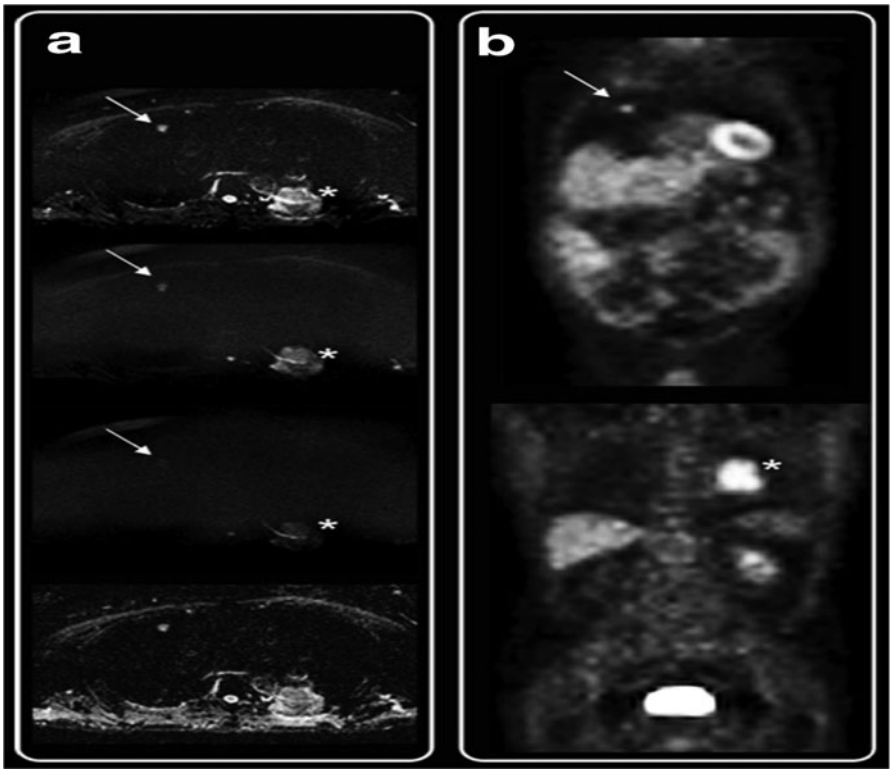

Fig. 18.2 DWI and PET images of a male patient with multifocal bronchial carcinoma; the original b-value images with b-values of 0,400, and 1,000 s/mm ^{2} and ADC-map are given from the bottom-up in (**a**) and the coronal PET with ^{18}F-FDG are given in (**b**). Both methods highly sensitively depict the small tumor in the lung (shown with permission of Lippincott Williams and Wilkins; taken from Lichy et al., Invest Radiol 2007; 42: 605–613)

of water within the tissue, is assessed. In the case of many diffusion barriers, the impaired diffusion leads to higher MR signals. The most relevant diffusion barriers in tissues are cell membranes. Thus, the higher the cellularity of tissues, the more restricted water diffusion will be. For some solid tumors including prostate and breast cancer, DWI has been shown to improve sensitivity and specificity for tumor detection. In particular, for whole body tumor staging and the detection of metastasis, DWI can be used as an efficient screening method since most normal tissues only provide little background signals (Fig. 18.2).

In addition, the predictive potential of DWI was shown for several malignant tumor types where low pre-treatment ADC values (indicating lower cellularity) indicated a better prognosis. DWI can also assess the response of tumors to systemic and regional treatments, for example bone metastases of prostate cancer cells in nude mice showed an increase in ADC when being treated with docetaxel [8]. On the other hand, when treating experimental bone metastasis of breast cancer in rats with an

antiangiogenic agent (bevacizumab), no significant change in diffusion was observed at the early stage of therapy [9]. This might be explained by the predominant effect of the therapy on the endothelial cells and the retarded effect on tumor cells due to oxygen and nutrient deprivation. Therefore, most cellular membranes and thus diffusion barriers might still have been intact at the time of examination.

Beside these imaging methods that can be used in both, preclinical and clinical research, reporter gene imaging can be applied to study cell proliferation in preclinical models. In this context, tumor cells that express green fluorescent protein (GFP) or red fluorescent protein (RFP) depending on the expression of cyclin D1 or "Nuclear Factor-Y" have been published. Nuclear Factor-Y activates the basal transcription of regulatory genes responsible for cell cycle progression (including mitotic cyclin complexes). While hardly any in vivo imaging results with these reporter genes have been published so far, at least for superficial tumors this concept is promising.

Imaging Invasion and Metastasis

The malignant growth of tumors is usually accompanied by vascular activation and sprouting. This leads to an increased vascularization and blood vessel permeability in the direct tumor neighborhood. When characterizing breast tumors, it has been shown that even in situ carcinomas often show increased vascularization around the tumor lesion that can be detected by dynamic contrast enhanced (DCE) MRI.

The increased vessel permeability of invasive tumors leads to an enhanced extravasation of plasma proteins and thus to edema. The increased water content appears bright on T_2-weighted MR images, leading to a bright rim around a tumorous lesion which makes it suspicious for being malignant. In particular, for the differentiation between malignant and benign breast tumors, edema detection by MRI appears to have diagnostic impact.

Another tumor entity where edema detection by MRI is of significant importance is glioblastoma multiforme, which is characterized by single cell invasion. In this case, not the visible solid tumor mass but the entire edema area is assumed to contain tumor cells and thus defined as tumor infiltrated tissue.

Besides edema formation, general signs of invasive growth that can be detected by ultrasound, MRI, CT as well as by optical techniques like optical coherence tomography are blurred tumor margins and the interruption of the tissue architecture (e.g., interruption of fibrous bundles).

Another indication of malignant stroma conversion is the upregulation of matrix-metallo proteinases. These enzymes can be visualized with MMP-specific radiotracers using PET or with activatable probes using optical imaging. The MMP activatable optical probes contain fluorescent dyes that are coupled in close proximity to each other by an MMP substrate. In this close proximity of the dyes, the fluorescence is quenched, meaning that no fluorescent signal is released after excitation. An MMP-dependent cleavage of the substrate leads to the release of the dyes and thus to fluorescence. Bremer and co-workers were among the first groups who used this

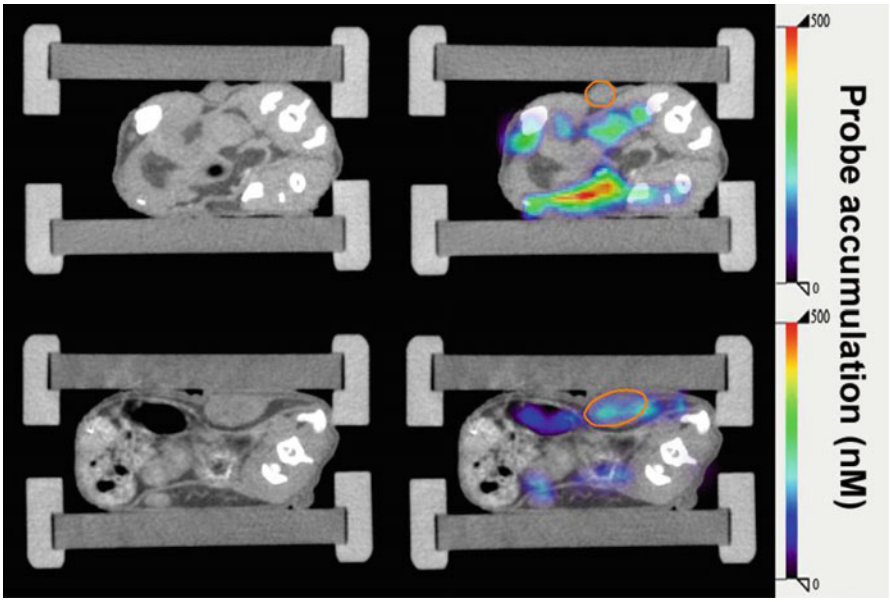

Fig. 18.3 μCT (*left* panels) and μCT-FMT fusion images (*right* panels) of representative HaCaT-ras A-5RT3 tumors (*red* circles) of untreated animals (*lower* panels) and animals treated for 1 week with the angiogenesis inhibitor sunitinib (*upper* panels). Whereas a strong accumulation of MMPSense 750 FAST (optical probe for active MMPs, Perkin Elmer) is observed in the untreated control tumor, almost no active MMPs can be detected in the tumor treated with sunitinib (Sutent®, Pfizer)

concept for in vivo imaging. In this context, they showed a significantly decreased MMP activity in tumor xenografts of mice that were treated with the MMP-inhibitor prinamostat [10]. In a recent study in our group, a significant reduction in MMP-activity was observed in squamous cell carcinoma xenografts in response to treatment with the angiogenesis inhibitor sunitinib (Fig. 18.3).

It is hardly possible to detect micrometastases with currently available non-invasive imaging methods. However, metastases with sizes above 2–3 mm can often be diagnosed. Except for PET and SPECT, the limited spatial resolution of the imaging modality is mostly not the limiting factor but the insufficient tissue contrast and the high background noise. Generally, CT is very well suited for the detection of lung and bone metastases and when applying contrast agents, also liver and larger lymph node metastases can easily be detected. Further tools being highly suited for whole body staging for metastases are MRI including DWI and ^{18}F-FDG PET. Since the information obtained by PET and MRI/CT are complementary and since their combined use increases sensitivity and specificity for tumor detection, hybrid PET-CT or PET-MRI are the clinical standard today [5].

For intraoperative metastasis screening, a clinical study on optical imaging has recently been published [11]. By spectral unmixing, the optical imaging device enabled the visualization of near infrared fluorescence at daylight conditions, thus facilitating its use for the surgeon. A folate receptor-targeted probe was injected to label peritoneal metastases of ovarian cancer and significantly more metastases were detected using fluorescence-guided visualization than by intraoperative visual inspection.

The last paragraph of this section is dedicated to the detection of lymph node metastases. PET and SPECT hybrid imaging with CT or MRI are the methods of choice to screen for lymph node metastases. In this context, besides the use of ^{18}F-FDG in many solid and non-solid tumor types, ^{11}C-choline PET is an excellent method to detect lymph node metastases of prostate cancers [12]. Also ultrasound can depict metastatically infiltrated lymph nodes based on their altered shape, architecture and vascularisation and it is frequently used in head and neck cancer metastasis screening. Ultrasmall superparamagentic iron oxide nanoparticle (USPIO)-enhanced MRI has also shown promising results in the detection of lymph node metastases, in particular for those from prostate cancer. The principle is that macrophages in lymph nodes accumulate USPIO, thus turning normal lymph node tissue black on T_2-weighted and T_2*-weighted images. Metastatically infiltrated areas in the lymph nodes lack high macrophage densities and thus appear brighter [13]. However, this method could not be established in clinical routine so far since a significant training of the physicians seems to be necessary to adequately apply this method.

Imaging Cell Death

Different modes of cell death are described in literature, including extrinsic or intrinsic apoptosis (programmed cell death), regulated necrosis, autophagic cell death and mitotic catastrophe [14]. Apoptosis plays a fundamental role in the maintenance of tissue homeostasis and occurs frequently during embryonic development and aging. It has been addressed by different imaging approaches targeting characteristic molecular markers [15]. Apoptosis is characterized by distinct morphological and biochemical alterations leading to the collapse of the transmembrane electrochemical potential, to the release of cytochrome c to the cytosol, to chromatin condensation, segregation of nucleoli, nuclear fragmentation and plasma membrane blebbing. On the other hand, apoptosis is accompanied by the activation of characteristic enzymes like caspases and endonucleases [15]. Apoptosis is dysregulated in various pathological conditions, e.g., cancer cells show a decrease or complete failure in programmed cell death [15]. On the other hand, since cancer therapies (e.g., chemotherapy, radiation, antiangiogenic treatment) induce a marked apoptotic response in the tumor tissue, the detection of apoptosis can be beneficial for treatment monitoring. Therefore, non-invasive apoptosis imaging has received great attention for cancer diagnosis and monitoring of the disease course or of therapy effects. During the early stage of apoptosis, phosphatidylserines (PS) are externalized at the plasma membrane. The

multi-functional protein annexin V binds with high affinity to PS, thus representing an interesting tool for apoptosis imaging [15]. In consequence, annexin V based radiotracers or imaging probes have been developed for different modalities such as PET, SPECT, optical imaging and MRI [15].

The most widely used tools for apoptosis imaging are the SPECT-tracers ^{99m}Tc-Annexin V and ^{99m}Tc-HYNIC-Annexin V. ^{99m}Tc-HYNIC-Annexin V has been tested in phase II/III clinical trials for assessing the efficacy of chemotherapeutics in cancer patients [15]. For the use in pre-clincial studies, annexin V has been also labeled with NIRF-dyes, e.g., Cy5.5 for pre-clinical optical imaging, including the monitoring of anti-tumorigenic therapies [15]. However, it has recently become evident that the use of annexin V probes or tracers for investigating the therapy response in cancer can lead to misleading results. If the therapeutics, e.g., antiangiogenic drugs, severely affect the tumor vasculature, the delivery of the probe can be strongly impaired, resulting in a low accumulation of the probe/tracer despite a high apoptosis in the tissue [16].

An alternative approach to detect apoptosis in vivo is based on caspase imaging. Activation of caspases also occurs relatively early during apoptosis and has been considered as one hallmark for a long time, though also caspase-independent apoptotic processes have been discovered in the meantime [14]. Prominent candidates for imaging are the so-called execution caspases, e.g., caspase-3 and −7 that activate cytoplasmic endonucleases and proteases, resulting in DNA as well as nuclear and cytoskeletal protein degradation.

Direct caspase imaging has been achieved using the caspase-3/7 inhibitor 1-[4-(2–^{18}F-fluoroethoxy)-benzyl]-5-(2-phenoxymethyl-pyrrolidine-1-sulfonyl)-1H-indole-2,3-dione (^{18}F-WC-II-89) as PET-tracer. This tracer showed a high accumulation in the liver of a cycloheximide-treated rat [17]. For optical imaging, activated caspases can by monitored by using so-called “smart probes”. A caspase-activatable probe (TcapQ(647)) comprising a caspase recognition sequence, a far-red quencher and a fluorophore, was successfully used for imaging parasite-induced apoptosis in different mouse models in vivo [17]. Caspase-imaging has additionally been achieved using cell-permeable nanoparticles to which NIR-fluorochromes were coupled in close proximity in order to enable quenching in the inactivated state. In a theranostic approach, a caspase-activatable imaging moiety was coupled to a photodynamic drug in order to perform and simultaneously monitor photodynamic therapy in vivo [17]. Additionally, reporter gene monitoring of caspase activity has been achieved in bioluminescence imaging. A fusion protein comprising three different reporter proteins (red fluorescent protein, firefly luciferase, HSV1-sr39 truncated thymidine kinase) linked through a caspase-3 recognizable polypeptide has been generated and successfully been used for imaging staurosporine-induced apoptosis [18]. In the meantime, a soluble substrate for caspase-3/7 (DEVD-aminoluciferin) is commercially available for apoptosis imaging [17].

An alternative approach of apoptosis imaging is based on the collapse of the mitochondrial membrane potential. In the case of a normally high mitochondrial membrane potential, lipophilic phosphonium cations accumulate within the mitochondria. However, the accumulation of phosphonium cations is reduced when the

mitochondrial membrane potential has collapsed. A decrease in the accumulation of ^{18}F-fluorobenzyl triphenylphosphonium has been already observed in an orthotopic prostate cancer model 48 h after docetaxel treatment, whereas at this time point, the ^{18}F-FDG uptake was much less reduced [19].

Imaging of Angiogenesis

Angiogenesis is the dynamic process of vessel outgrowth from pre-existing vessels. Directly imaging tumor angiogenesis in vivo can be achieved by intravital microscopy (including two photon microscopy) using window chambers or skin flap models. Also few papers report on the use of micro CT to longitudinally monitor the angiogenic switch and the generation of a vascular network within tumors [20]. Photoacoustic imaging is another powerful technique at least for superficial tumors which provides a sufficiently high spatial resolution to visualize small angiogenic blood vessels. The principle of photoacoustic blood imaging is the absorption of near infrared light by hemoglobin and the subsequent thermoelastic expansion leading to an acoustic pulse, which is detected by ultrasound transducers [21].

Most other methods that are able to visualize microvessels like cast preparations (Fig. 18.4b) or supermicroscopy only work ex vivo and thus are not suited for longitudinal observations.

Many non-invasive imaging methods, however, allow monitoring indirect effects of tumor angiogenesis by assessing functional and molecular parameters. Functional vascular characteristics assessable by imaging include the relative blood volume (rBV), blood flow and perfusion, vessel permeability, vessel functionality, oxygenation and mean vessel size [22, 23]. Most frequently, a tomographic imaging method is used to record signal- or concentration-to-time courses after injection of a contrast agent. First pass methods basing on the indicator dilution theory or the model of Miles are robust and provide data of the rBV and perfusion (Fig. 18.4a), respectively the mean transit time. For the assessment of vessel permeability respectively the separation between perfusion and permeability, compartment models are required. In this context, two compartment models (e.g., the models of Brix and Tofts) are best established in the clinics which describe the exchange of the contrast agent between an intravascular and an extravascular extracellular compartment. Using MRI and CT, numerous preclinical and clinical studies have shown that this kind of dynamic contrast enhanced (DCE) analysis can provide valuable information for tumor characterization and therapy response evaluation. For example, in clinical MR mammography, the information of the contrast agent dynamics is one of the major evaluation criteria to decide whether a lesion is malignant or not. Also for many therapies, including standard chemotherapy but particularly antiangiogenic therapies, it has been shown that rBV and perfusion decrease significantly before the tumor starts to decrease in size.

Also ultrasound imaging is favorably suited to assess vascular characteristics [24]. Without the need of contrast agent injection, blood vessels can be visualized

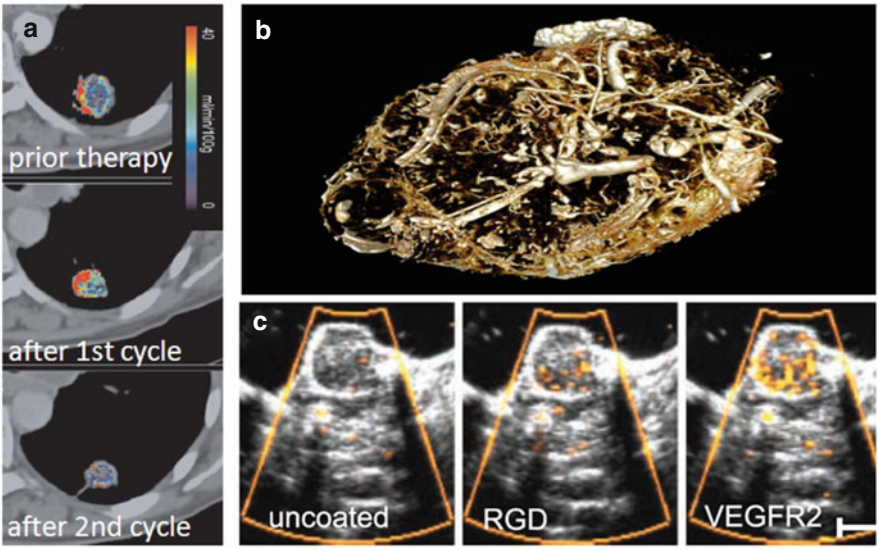

Fig. 18.4 **a** CT images of the *left* chest with a brochial carcinoma recorded before and after the first and second cycle of chemotherapy. Tumor perfusion obtained with dynamic contrast-enhanced CT is presented as a pixelwise overlay on the tumor and decreases markedly under therapy thereby indicating therapy response (Image reproduced from: Kiessling F, Boese J, Corvinus C et al. (2004) Perfusion CT in patients with advanced bronchial carcinoma: a novel chance for characterization and treatment monitoring. Eur Radiol 14:1226–1233). **b** 3D reconstruction of μCT data of an ovarian cancer xenograft after vascular casting. **c** Example for molecular ultrasound imaging of a squamous cell carcinoma xenograft, several minutes after injection of uncoated, alpha-v-beta3 integrin-targeted and VEGFR2-targeted microbubbles. Only the targeted microbubbles adhere to the angiogenic tumor vasculature (shown with permission of the American Association for Cancer Research; taken from Palmowski et al. (2008) (Mol Cancer Ther 7:101–109)

and blood flow can be quantified by Doppler imaging. When the acoustic pulse is reflected by a moving blood cell, it is reflected with a frequency shift that depends on the direction and speed of this cell. The sensitivity of Doppler ultrasound for slow blood velocities increases with increasing ultrasound frequency. However, since tissue penetration decreases with increasing frequencies its capabilities for tumor imaging are limited and very small angiogenic or capillary vessels can hardly be captured. Nevertheless, several studies proved that effects of antiangiogenic therapies can be assessed using Doppler ultrasound imaging. The sensitivity of ultrasound to microvessels can be increased when microbubbles are used as contrast agent. In this context, Palmowski and co-workers showed that the combined use of non-contrast-enhanced Doppler (visualizing more mature blood vessels with considerably high flow) and contrast-enhanced Doppler (visualizing all vessels) enables the assessment of vessel maturation in squamous cell carcinomas treated with a multispecific tyrosine kinase inhibitor [25].

Since ultrasound microbubbles strictly remain intravascular, vessel permeability cannot be analyzed. The assessment of tumor perfusion in contrast enhanced ultrasound imaging is usually based on the destruction replenishment method, which was initially published by Wei and coworkers [26]. When having reached a stable blood concentration of microbubbles, a microbubble-destructive ultrasound pulse is given and the replenishment is analyzed afterwards by fitting the up-slope of the intensity time curve.

Antiangiogenic therapies are known to result in a higher degree of mature vessels. The induction of vascular maturation can be part of the therapy since it leads to better tissue oxygenation and thus a better response of tumor cells to radiotherapy and better accumulation of intravenously injected pharmaceuticals. Therefore, imaging vessel functionality and maturation in general are further important vascular features worth to be imaged in order to assess early antiangiogenic therapy response and to optimize and personalize chemo-/radiotherapy schedules that include antiangiogenic drugs.

Two MRI methods will be discussed that can be used to characterize vessel functionality. Bold (Blood Oxygenation Dependent) MRI characterizes the tissue vascularization by measuring differences in signal intensity between oxygenated and deoxygenated blood. Since functional blood vessels respond to the oxygen and carbogen inhalation by vasoconstriction respectively vasodilatation, the functionality of vessels can be assessed [27]. A more indirect MRI approach, similar to the combined use of contrast enhanced and non-contrast-enhanced Doppler imaging, is vessel size imaging (VSI). VSI uses the fact that after injection of a blood pool contrast agent, the change in T_2-relaxation time depend on vessel diameters and the vessel environment, while the change in T_2*-relaxation time mainly depends on the amount of contrast agent (meaning the rBV). Thus, combining these parameters enables to determine the mean vessel diameter of the tissue [22]. Anti-angiogenic therapies mostly destroy angiogenic immature vessels. These usually are small and thus the mean vessel diameters reach higher values during maturation. However, in some tumors immature vessels form lacunas and here, the effect can be opposite.

Photoacoustic imaging (or optoacoustic imaging) also uses the endogenous contrast of hemoglobin to characterize tissue vascularization. The principle is that hemoglobin absorbs near infrared light, which then leads to a thermoelastic expansion of the molecule. Since the light absorption depends on the oxygenation of hemoglobin the blood oxygenation status of tissues can be assessed [28].

While all approaches described so far provide insight into the effects of angiogenesis, molecular imaging can monitor angiogenesis, inflammation and sprouting. For this purpose, antibodies, peptides or other targeting moieties are bound to signaling molecules that bind to markers at the endothelial cell surface. Ultrasound imaging is highly suited for vascular molecular imaging since targeted microbubbles do not extravasate (no unspecific accumulation in the interstitial space occurs) and thus can sensitively and specifically be detected by ultrasound [24, 29]. In addition, morphologic and functional information about the vascularization can easily be obtained. Microbubbles targeting VEGFR2 were used in many preclinical studies to characterize tumors and to assess therapy effects including antiangiogenic treatments and radiation therapy (Fig. 18.4c). In addition, first VEGFR2-targeted microbubbles were

developed for clinical use and tested for prostate cancer detection. Since preclinical studies recently indicated that for distinguishing benign and malignant breast tumors, molecular ultrasound imaging of VEGFR2 is superior to functional rBV assessment using non-targeted microbubbles, another indication field is currently appearing.

Beside VEGFR2, many other targets such as alpha-v-beta3 integrin, E-selectin, endoglin and ICAM-1 were successfully targeted by molecular ultrasound imaging, but microbubbles have not yet been developed for clinical use.

Besides ultrasound imaging, PET, SPECT, optical imaging, photoacoustic imaging as well as MRI were used to characterize angiogenesis and to monitor tumor response to therapy [30]. PET and SPECT have the advantage of being highly sensitive and better suited for tumors localized deeper in the body. Also the reproducibility is better since these modalities are less user dependent. However, spatial resolution is much lower and it is more complex to obtain functional information within the same imaging session. Functional information, however, in particular those of the rBV is important in order to normalize the molecular information and to clarify whether the change in targeted probe accumulation during therapy is due to a change in vessel number at a constant target level at the endothelial cell or due to a change in target expression by the endothelial cell (e.g., during maturation).

Nevertheless, for all targets being localized outside the vasculature and in particular for those with an intracellular localization, PET and SPECT are the modalities of choice. With respect to angiogenesis ^{18}F-MISO, ^{18}F-AZA and ^{64}CuATMS are important PET tracers being able to indicate tumor hypoxia. These radiotracers enter the cells, are subsequently reduced in hypoxic tissues and thus are trapped within the cells due to binding to intracellular proteins. These radiotracers have significant potential for individualizing radiotherapy and were successfully tested in many preclinical and clinical tumor studies with respect to the assessment of chemotherapy and anti-angiogenic therapy effects. For example, in a rat glioma model, the response to a multispecific tyrosine kinase inhibitor could be sensitively monitored by ^{18}F-MISO [31]. Also in colorectal cancer xenograft, the effects of the antivascular compound 5,6-dimethylxanthenone-4-acetic acid (DMXAA) could be assessed. In a clinical study, the ^{18}F-MISO uptake in head and neck squamous cell carcinomas indicated a worse outcome of radiotherapy [32].

Besides contrast agent and radiotracer-based molecular imaging, molecular targets can be studied using reporter genes, which are very helpful in preclinical research. In this context, tumor cell lines where the expression of angiogenic factors coincides with the expression of luciferase and fluorescent proteins have been established. Prominent examples for reporter gene concepts in this regard are HIF-1 alpha-dependent GFP or luciferase expression and VEGF promoter luciferase reporter chimeric constructs that were used in various cancer cell lines including glioma, colon cancer and breast tumor cells [33]. These cell lines were predominantly used for in vitro studies and the few in vivo optical imaging studies reported mostly on basic mechanistic research and rarely on the monitoring of novel cancer therapies.

Imaging Tumor Inflammation and Immunological Response

Inflammation is considered as an important mediator of tumor initiation, progression and invasion. It is also supporting tumor angiogenesis. Thus, markers of angiogenesis, vessel activation and tumor inflammation are sometimes identical. Typical markers of vascular inflammation are e.g., ICAM-1 or alpha-v-beta3 integrin, a marker of inflammation and angiogenesis. Using targeted microbubbles, an early upregulation of these markers in tumor vessels after heavy ion radiotherapy was demonstrated [24].

Inflammation goes along with the migration and invasion of immune cells, particularly macrophages, into the tumor tissue. Tumor-associated macrophages intensely internalize iron oxide nanoparticles that are used as MR contrast agent. Therefore, these contrast agents have been suggested for characterizing tumor inflammation and the immunological response [34].

During their migration to the tumor site, immune cells interact with endothelial cells. Surface molecules like the integrin lymphocyte function-associated antigen (LFA-1) on leukocytes mediate binding to ICAM-1 on the endothelial cells. In this context, fluorescent superparamagnetic iron oxide nanoparticles carrying a LFA-1 domain were developed, which thus mimicked leucocytes and were shown to significantly accumulate in the vasculature of experimental tumors [35].

Within the tumor tissue, immune cells release lysosomal enzymes like cathepsin D and β-glucuronidase being responsible for matrix remodeling in inflammatory states. Activatable optical probes sensitive to cathepsin have been developed and proved promising for detecting small experimental pancreatic cancers using in vivo NIRF imaging [36]. β-glucuronidase was targeted using a PET radiotracer ([^{18}F]FEAnGA), which strongly accumulated in the viable part of C6 gliomas in rats [37].

The modulation of the immunological response against tumors is a promising therapeutic strategy. For example, in clinical studies, activated dendritic cells are injected into patients to stimulate the immunological response against metastasized malignant melanoma. However, these cells only fulfill their function if they migrate into lymph nodes. Labeling these cells with USPIO and radiotracers enabled the non-invasive monitoring of cell implantation and migration by SPECT and MRI [38].

In another study, cytotoxic T-cells were sensitized against the MHC class I epitope of ovalbumin (OVA) [39]. Labeling these cells with USPIO enabled MRI monitoring of their selective migration into xenograft tumors that were genetically modified to over-express OVA, while only few cells accumulated in non-transfected control tumors. This T-cell therapy resulted in a significant decrease in tumor size that could faithfully be monitored by MRI.

Imaging Chromosome Abnormalities and Genome Instability

The direct non-invasive assessment of alterations in the genome and the imaging of genome instability are difficult and hardly possible today. Fluorescent reporter genes as mentioned previously can be used to visualize activation and deactivation of tumor

Table 18.1 Overview on imaging biomarkers and the used imaging modalities

Hallmark: Metabolism		
Imaging biomarker	Imaging modality	Clinically applicable
Choline, Creatine, ATP, lactate, pH. . .	Magnetic resonance spectroscopy (MRS)	Yes
Pyruvate metabolism	Hyperpolarised MRI	Yes
Glucose metabolism/hexokinase activity	PET (^{18}F-FDG)	Yes
Amino acid transporters	PET (e.g., ^{11}C-MET)	Yes
Hallmark: Increased proliferation		
Thymidine kinase 1 activity	PET (^{18}F-FLT)	Yes
Cellularity	DWI	Yes
Cyclin D1, nuclear factor-Y	Optical imaging using reporter genes (fluorescent proteins)	No
Hallmark: Imaging invasion and metastasis		
Edema	MRI	Yes
Unsharp tumor borders, destruction of regular tissue architecture	MRI, CT, US	
Enhanced MMP activity	PET	Yes
	Optical imaging	No
Metastasis detection	CT, MRI, ultrasound, PET, SPECT, PET-CT, PET-MRI	Yes
Peritoneal metastases during surgery	Near infrared optical imaging using folate receptor-targeted probes	Yes
Lymph node metastases	PET-CT, PET-MRI, SPECT-CT	Yes
	USPIO-enhanced MRI	Yes
Hallmark: Imaging cell death		
Externalized phosphatidylserines	SPECT-CT (^{99m}Tc-HYNIC-Annexin V), PET-CT, near infrared optical imaging, MRI	(Yes)
Caspases	PET-CT, near infrared optical imaging, bioluminescence imaging	No
Collapsed mitochondrial membrane potential	PET	No
Hallmark: Angiogenesis		
Microvessel morphology	μCT (casts), photoacoustic imaging, microscopy (e.g., two photon microscopy, OEFDI, supermicroscopy)	No
rBV, perfusion, blood velocities	DCE MRI, (ce) ultrasound, DCE CT, (optical imaging, PET, SPECT)	Yes
Vessel permeability	DCE MRI, DCE CT, (optical imaging, PET, SPECT)	Yes
Blood oxygenation	BOLD MRI	Yes
	Photoacoustic imaging	Starting to be used
Mean vessel diameter	VSI (MRI)	Yes
Vessel functionality	BOLD MRI, ultrasound	Yes

Table 18.1 (continued)

Molecular target.g., VEGFR2, alpha-v-beta3 integrin)	PET, SPECT, ultrasound	Yes
	Optical imaging, MRI, photoacoustic imaging	No (lack of applicable probes)
Hypoxia	PET (e.g., ^{18}F-MISO, ^{18}F-AZA and ^{64}CuATMS)	Yes
HIF-1 alpha, VEGF	Optical reporter gene imaging (luciferase, fluorescent proteins)	No
Hallmark: Imaging tumor inflammation and immunological response		
ICAM-1, alpha-v-beta3 integrin	Ultrasound	No
	PET	Yes
	MRI	No
Cathepsin D	Optical imaging (activatable probe)	No
β-Glucuronidase	PET	No
Dendritic cell tracking	MRI, SPECT	Yes
T-cells tracking	MRI, SPECT	No
Hallmark: Imaging chromosome abnormalities and genome instability		
Oncogenes	Optical reporter gene imaging (luciferase, fluorescent proteins)	No

suppressor and oncogenes. Furthermore, indirect assessment of altered protein expression succeeds by targeted imaging as discussed previously.

Conclusion

In conclusion, non-invasive imaging can contribute to the investigation of several hallmarks of cancer, including sustaining proliferation, apoptosis, angiogenesis, invasion, metastasis, and abnormal metabolism. Also inflammation and immune-reactions can be studied. In contrast, micro-metastases, DNA instability and replicative immortality can hardly be visualized so far. Table 18.1 gives an overview on biomarkers for non-invasive imaging and on preferred imaging modalities sorted according to the hallmarks of cancer. Many of these biomarkers have shown their potential in improving diagnosis by better detecting and characterizing tumors as well as in therapy monitoring. In future, it is expected that these imaging biomarkers will significantly support the conventional morphological imaging and will provide an essential contribution to the translation of personalized medicine.

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Chapter 19
Biologic Memory: Induction by Metronomically Administered Combined Modularized Therapy

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Abstract Can temporally limited and metronomically administered combined modularized therapies facilitate a biological memory in tumor systems to sustain long-term tumor growth control? We report on the follow-up of patients with either castration-resistant prostate cancer (CRPC) or multiple myeloma (MM) who had to discontinue their study medication because of medical indications. These patients had achieved an objective response to metronomically administered low-dose chemotherapy with treosulfan (250 mg twice daily) combined with transcriptional modulation including dexamethasone (1 mg once daily) and pioglitazone (60 mg once daily). Patients with CRPC (phase I/II trial for CRPC, first-line therapy) had also received metronomically administered imatinib (400 mg once daily) and etoricoxib (60 mg once daily), and patients with MM (third-line therapy, phase I, ongoing phase II trial) lenalidomide (10 mg or 15 mg once daily). After discontinuing the study medication, 3 patients with CRPC had stable disease for more than 1 year (12.5–15 months) and responded to retreatment with a PSA response of $> 50\,\%$; 1 patient with a contraindication to classic chemotherapy responded to the addition of a GnRH analogue after the second progression. One patient with MM achieved the first complete remission after third-line therapy and remained in very good partial remission (VGPR) with detectable light chains only in urine; the very good partial remission period lasted 13 months, which constituted the longest therapy-free interval since diagnosis. After retreatment, the patient responded again with VGPR. None of the 4 patients received any tumor-specific therapy after treatment discontinuation. The clinical observations derived from combined modularized therapy indicate that seemingly stable tumor-associated epigenetic benchmarks of gene activity – which are responsible for promoting tumor growth – can be redirected by combined modularized therapies and dynamically respond with a biological memory for growth control. The epigenetic redirection of cellular functions by transcriptional regulation could be a major therapeutic approach in the treatment of numerous diseases, from neurodegenerative diseases to metastatic cancer, and in tumor prevention. Finally, the possibility of therapeutically inducing a biologic memory is a strong hint for a practically applicable and reliable evolution theory.

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Introduction

Therapy concepts for metastatic tumors underlie paradigmatic normative notions. Correspondingly, the primary therapeutic aim is tumor eradication. Alternatively, the tumor burden should be reduced to a level that is only detectable by molecular biological examination; this tumor level may then be further stabilized by continuous maintenance therapy. A bulk of clinical data supports this traditional notion: Imatinib therapy in gastrointestinal stroma tumors or in chronic myelocytic leukemia, CD20 monoclonal antibody in non-Hodgkin's lymphoma, Her-2 monoclonal antibody in breast cancer, maintenance therapy in multiple myeloma, and chronic graft versus host reaction in multiple myeloma [1–6].

The inability to achieve tumor eradication or at least to lessen the tumor burden to minimal residual disease rises the question whether metastatic tumors should be treated intermittently or continuously with classic pulsed schedules in a palliative setting or alternatively with metronomic low-dose therapy until final uncontrolled tumor progression [7].

The possibility of systematically creating therapy schedules that establish a biological memory for tumor growth control after therapy discontinuation has not yet been taken into consideration because of the lack of theoretical prerequisites for therapeutically processing a biological memory for growth control.

The recently developed evolution theory provides a theoretical basis for therapeutically evolving tumor systems [8]: Data derived from metronomically administered combined modularized therapies have shown the capacity to evolve tumor systems by implementing multi-side regulatory effects that target principally modularly structured tumor systems: Thereby, 'corrupt' rationalizations of tumor-associated normative notions may be redirected in a therapeutically meaningful way [9].

However, the following question remains unanswered: May temporally limited combined modularized therapies, such as 'induction' therapies, create a biological memory for sustaining long-term growth-control of tumor systems? A therapeutically established biologic memory supporting and promoting growth attenuation should explicit allow the discontinuation of combined modularized therapies without the risk of rapid tumor progression, although the tumor disease itself is still present.

Metronomically administered combined modularized therapies have been originally conceptualized for the treatment of final stage metastatic tumor disease [9]. After years of further development, growing evidence suggests that this metronomic concept has the capacity for controlling even extremely chemoresistant tumor diseases ([10], Chap. 2). The principle of biomodulatory therapies is the modular targeting of tumor-associated rationalization processes that establish growth-promoting normative notions, which represent the pathophysiologic basis of tumor systems for maintaining tumor progression.

Our 4 patients had been treated with similar metronomically administered therapy modules, i.e., metronomic low-dose chemotherapy with treosulfan and combined transcriptional modulation with dexamethasone and pioglitazone, for rather different malignant diseases (CRPC in first-line and MM in third-line therapy) and had achieved an objective tumor response [10, 11]. In all 4 patients, the combined modularized therapies had to be discontinued because of non-tumor-related medical

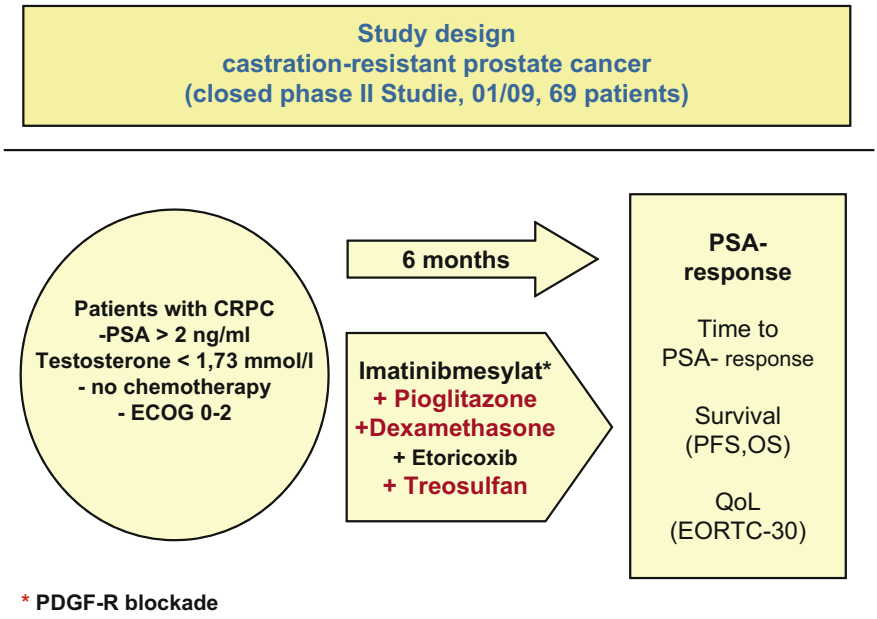

Fig. 19.1 Combined modularized therapy for castration-resistant prostate cancer with metronomically administered low-dose chemotherapy (treosulfan 250 mg twice daily), transcriptional modulation with dexamethasone (1 mg once daily) and pioglitazone (60 mg once daily), etoricoxib (60 mg once daily) and imatinib (400 mg once daily)

indications: Surprisingly, the therapy breaks did not lead to rapid tumor progression as expected. On the contrary, the therapy-induced biologic memory for continuously attenuating tumor growth resulted in the long-term stabilization of the disease without any tumor-specific therapy.

Patients and Methods

Here, we report on the follow-up of 4 patients who had to discontinue their study medication because of medical indications. These patients had achieved an objective response to metronomically administered low-dose chemotherapy with treosulfan (250 mg twice daily) combined with transcriptional modulation including dexamethasone (1 mg once daily) and pioglitazone (60 mg once daily). Patients with CRPC (phase I/II trial for CRPC, first-line therapy) (Fig. 19.1) [10] had also received metronomically administered imatinib (400 mg once daily) and etoricoxib (60 mg once daily), and patients with MM (third-line therapy for MM, phase I, ongoing phase II trial) (ClinicalTrials.gov Identifier: NCT01010243) (Fig. 19.2) lenalidomide (10 mg or 15 mg once daily).

PSA and paraprotein response were the primary endpoints of the trials, respectively.

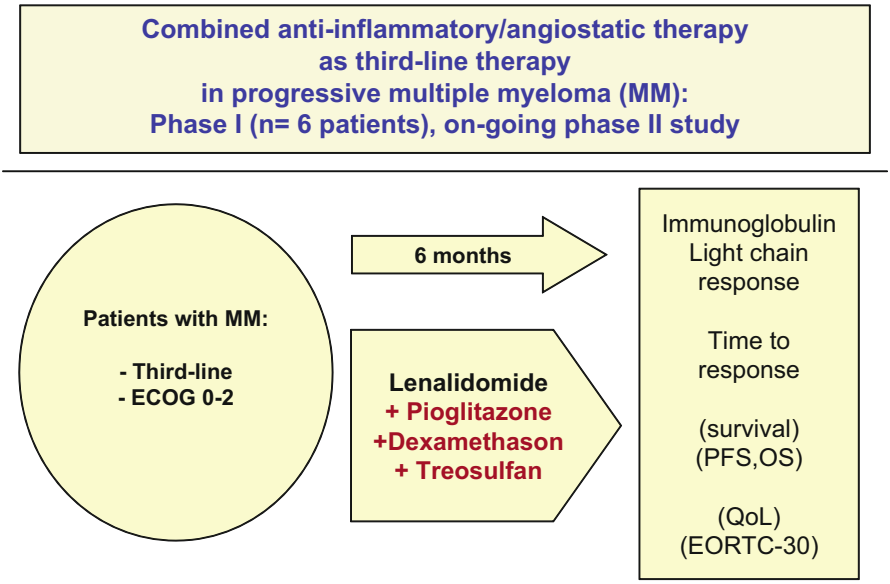

Fig. 19.2 Combined modularized therapy for multiple myeloma (third-line) with metronomically administered low-dose chemotherapy (treosulfan 250 mg twice daily), transcriptional modulation with dexamethasone (1 mg once daily) and pioglitazone (60 mg once daily), and lenalidomide (10 mg or 15 mg daily) (phase I/II trial)

In both trials, therapy discontinuation for more than 4 weeks led to trial termination, as did the achievement of complete remission in the multiple myeloma trial. In the CRPC trial, tumor progression was assumed during follow-up if the PSA level had doubled at the time of study discontinuation or, in the case of MM, if paraprotein was again detectable in the serum.

Within the CRPC trial, patients with histologically confirmed progressive CRPC were continuously treated in 11 German centers during the 6-month core phase until PSA progression. Patients responsive to study medication were allowed to enter the extension phase until disease progression or presence of intolerable toxicity levels.

In the core phase of the trial on the third-line therapy of multiple myeloma, 2 cohorts with 3 patients each were continuously treated with daily lenalidomide 10 mg (cohort 1) and 15 mg (cohort 2) respectively, pioglitazone 60 mg, treosulfan 250 mg twice daily, and dexamethasone 1 mg for 1 month. Patients suffered from osteolytic multiple myeloma and had proven progression with detectable serum paraprotein levels. Patients responsive to study medication were allowed to enter the extension phase until disease progression or presence of intolerable toxicity levels.

In both trials, we monitored serum C-reactive protein response (decline of $> 30\,\%$ from base-line). In the MM trial, we also monitored hemoglobin in serum and measured paraprotein (Figs. 19.3 and 19.4) [12–15]. In the CRPC trial, we used bone scans or imaging techniques (MRT, CT scan) to assess response, if clinically indicated, and measured PSA levels.

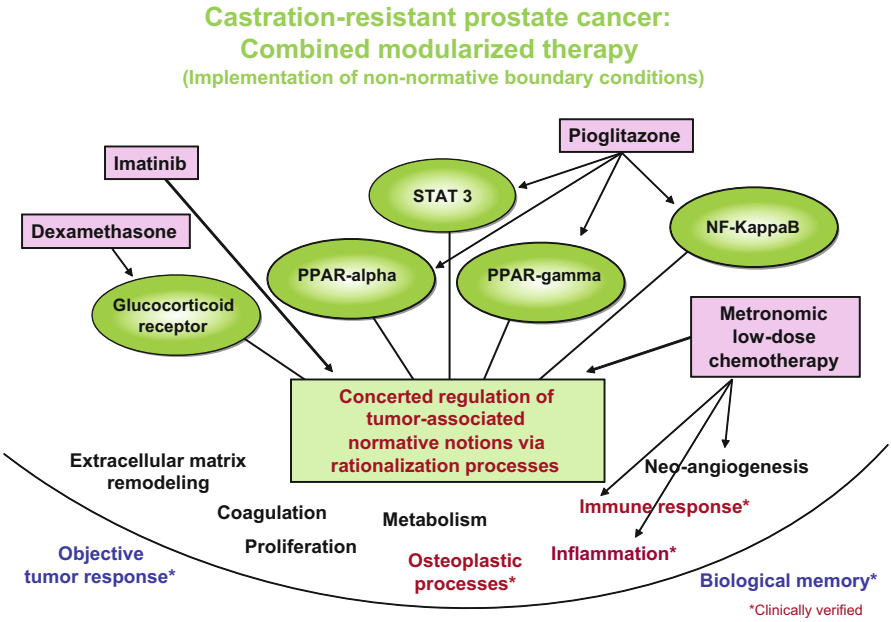

Fig. 19.3 Concerted regulation of tumor-associated normative notions by means of targeting rationalization processes with combined modularized therapy approaches. Metronomic low-dose chemotherapy exerts pleiotropic and tumor type-dependent activity on tumor-related normative notions. Redirected and modulated normative notions (e.g., hallmarks of cancer) serve as read-outs as well as a biological memory and an objective tumor response

Results

Persisting therapy response without any maintenance therapy

After the discontinuation of their study medication (Fig. 19.5), 3 patients (62–73 years old) with metastatic CRPC (bone lesions n = 3, lymph node metastases, n = 1) maintained tumor response for more than 1 year from the time of therapy interruption without any tumor-specific therapy (12.5–15 months). Moreover, all patients responded to retreatment with a PSA response of > 50 %; 1 patient with a contraindication to classic chemotherapy responded to the addition of a GnRH analogue in the second progression [6–10]. Corresponding to a PSA doubling time at base line (study inclusion) of less than 3 months, the PSA doubling time of 2 patients was up to 10-times longer after the discontinuation of the study medication.

One patient with MM achieved the first complete remission in the third line and remained in VGPR for 13 months with light chains detectable in urine only. The overall progression-free survival was 31 months, his longest therapy-free interval since the initial diagnosis of stage IIIA MM. After retreatment, the patient responded again with VGPR. None of the 4 patients received any tumor-specific therapy after therapy discontinuation.

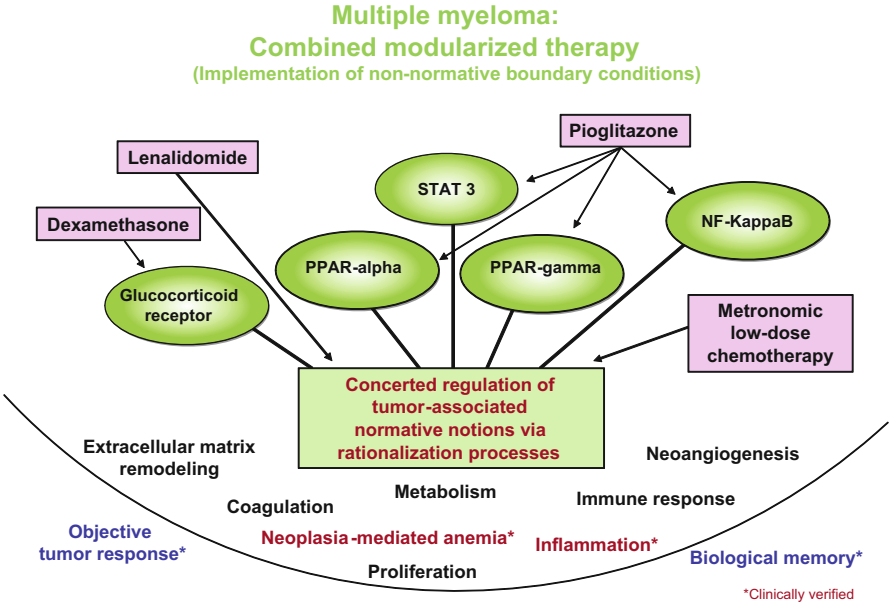

Fig. 19.4 Concerted regulation of tumor-associated normative notions by means of targeting rationalization processes with combined modularized therapy approaches in multiple myeloma. Read-outs are redirected and modulated normative notions, including the hallmarks of cancer as well as a biological memory and an objective tumor response

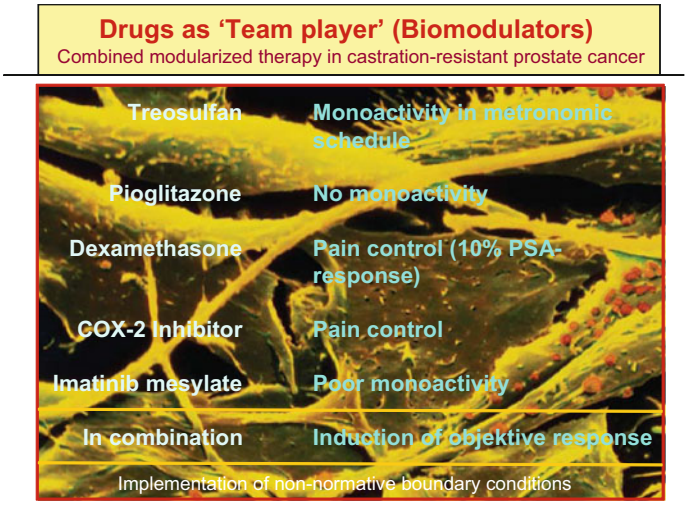

Fig. 19.5 Combined modularized therapy may include therapy modules with poor or no monoactivity, because therapy elements are targeted at altering the communicative presuppositions of tumor-promoting systems objects, aimed at redirecting their validity and denotation and at achieving tumor growth control

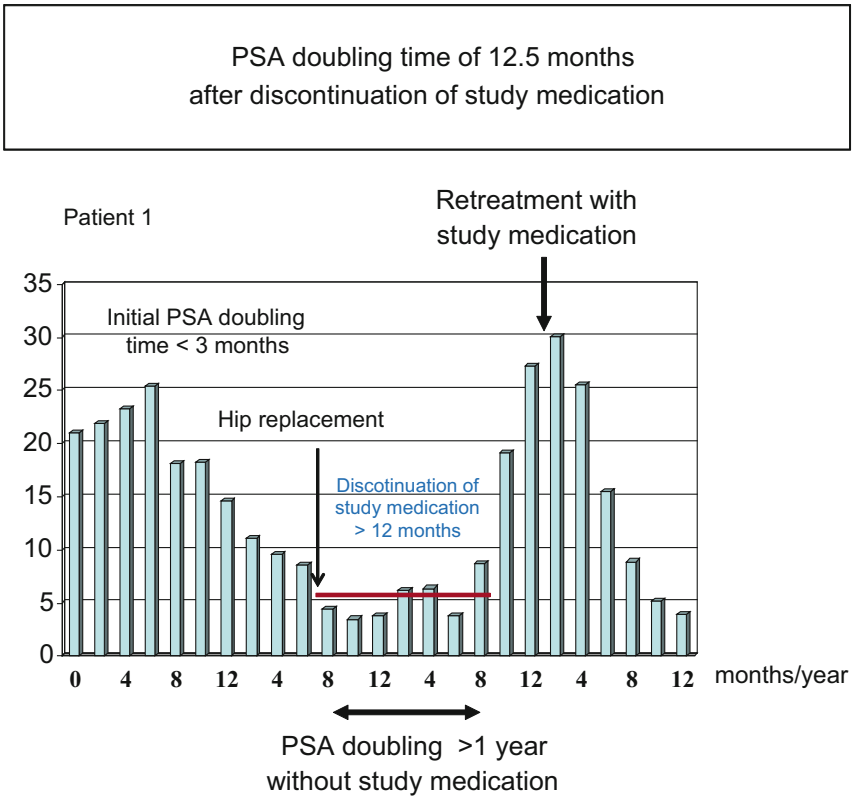

Fig. 19.6 Combined modularized therapy induces a biological memory for long-term tumor control, despite the presence of residual tumor disease

Study termination (CRPC multi-center trial, recruitment between February 2007 and October 2010, 61 patients) was decided by the physicians of the patients with CRPC due to non-tumor-related surgery. In the MM trial (phase I trial, 6 patients, on-going phase II trial), 1 patient achieved CR; thus, the study medication was discontinued according to protocol.

One patient with multiple myeloma discontinued therapy because of the first complete remission in third-line therapy. CR, which was achieved transiently, was followed by long-term stable very good partial remission (13 months), constituting the longest treatment-free interval since the start of the systemic therapy for stage IIIA multiple myeloma (ClinicalTrials.gov Identifier: NCT01010243).

At the time of therapy discontinuation, all 3 patients with CRPC still had a tumor burden as indicated by PSA levels (Figs. 19.6–19.8). The monoclonal light chain levels detectable in urine only were present 2 months after the diagnosis of CR in MM but remained stable.

After the progression-free interval of > 1 year, the 3 patients with CRPC receiving re-treatment by means of the original therapy schedule, but without imatinib, showed

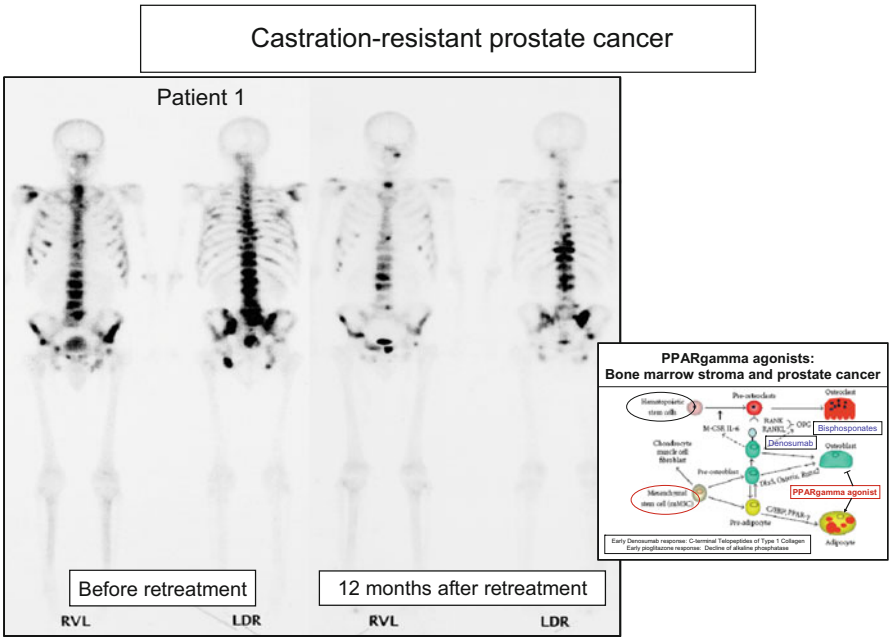

Fig. 19.7 Re-treatment with study medication (Fig. 19.5) leads to a significantly decreased technetium up-take in bone scan that is paralleled by a PSA response of > 50 %

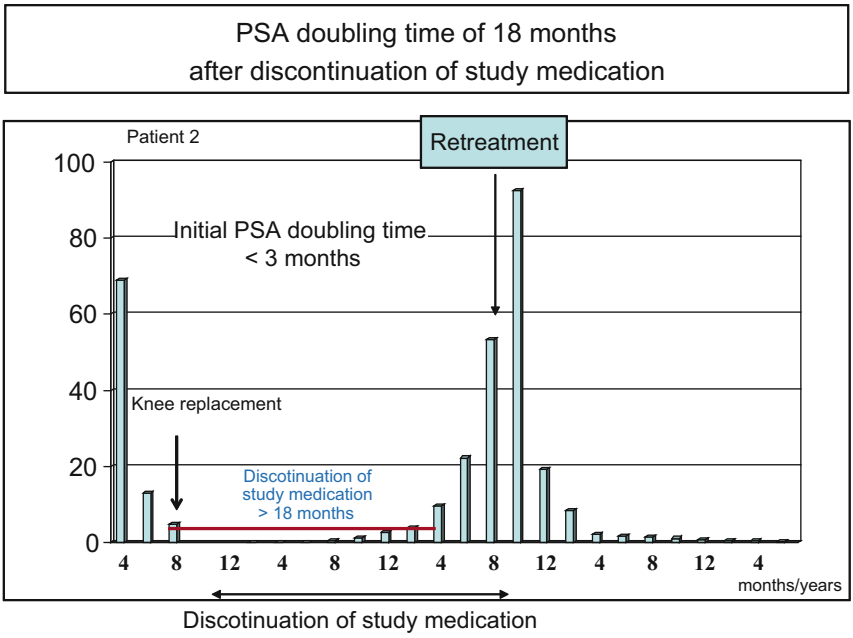

Fig. 19.8 Re-treatment with the study medication after tumor progression led again to a PSA response of > 50 % in the castration-resistant stage

again a > 50 % PSA response. One patient with a contraindication to chemotherapy received a GnRH analogue during the second progression in addition to the combined modularized therapy. The PSA response after the addition of a GnRH analogue indicated the recovery of hormone-sensitivity. The patient with MM again responded with a very good partial remission to re-treatment by means of the original therapy schedule.

In our trial, we could show that the implementation of novel normative boundary conditions by combined modularized therapies facilitated to redirect and modulate tumor-associated normative notions by control of systemic inflammation, immune response in CRPC and MM, anti-osteoplastic activity, recovery of hormone-sensitivity in CRPC, and amelioration of myeloma-mediated anemia (Figs. 19.3, 19.4 and 19.9–19.11) [10–12, 16].

Response Despite Frequent Dose Reductions

As doses were already reduced in the case of grade 2 toxicities with the aim of keeping patients on study medication in the long term, none of the patients with an objective response had to discontinue the study medication because of side effects. All 4 patients had dose reductions according to protocol.

The therapeutic schedules reported did not include any classic cytotoxic agents; thus, drug-related toxicities of standard pulsed chemotherapy regimens could be avoided [10]. Although all patients experienced at least one adverse event, drug-related toxicity was generally manageable after prompt dose modifications for events of grade 1 or 2 toxicity. These changes did not appear to markedly limit the efficacy of the regimen: although 77 % of the patients with CRPC and 100 % of the patients with MM required some type of dose modification or a temporary interruption of the study drug, over 60 % of the study population showed either a PSA response or maintained a stable disease course in the case of CRPC. In the MM population, worst response was stable disease. In addition, quality of life could be maintained throughout the study [10].

Discussion

Transcriptional Modulation and Combined Modularized Therapy

Termination of the study medication because of medical indications during a phase of objective tumor response facilitated to detect the phenomenon that similar combined modularized therapies may implement a long-term biological memory for attenuating tumor growth in biologically rather heterogeneous tumor diseases, such as multiple myeloma and castration-resistant prostate cancer.

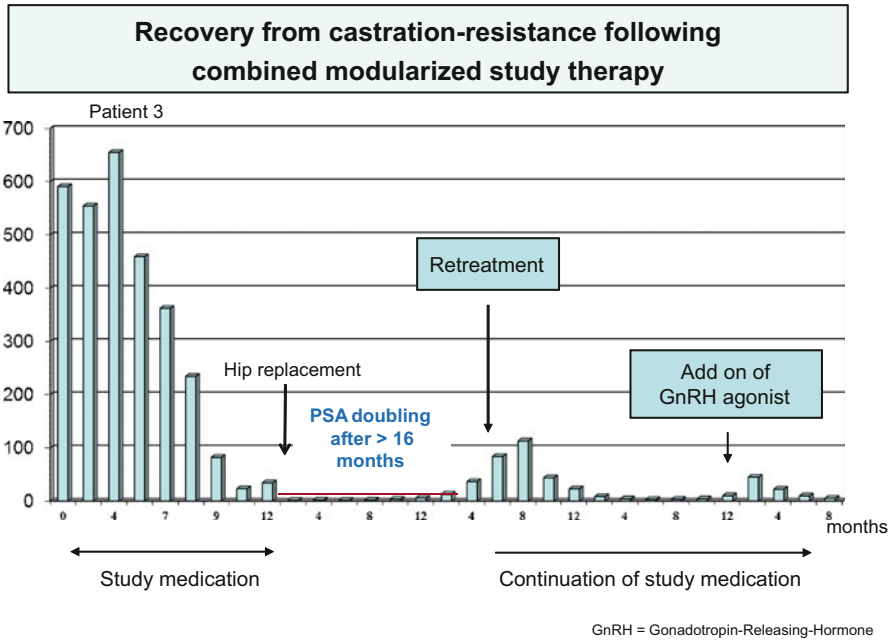

Fig. 19.9 Recovery from castration-resistance after combined modularized study therapy

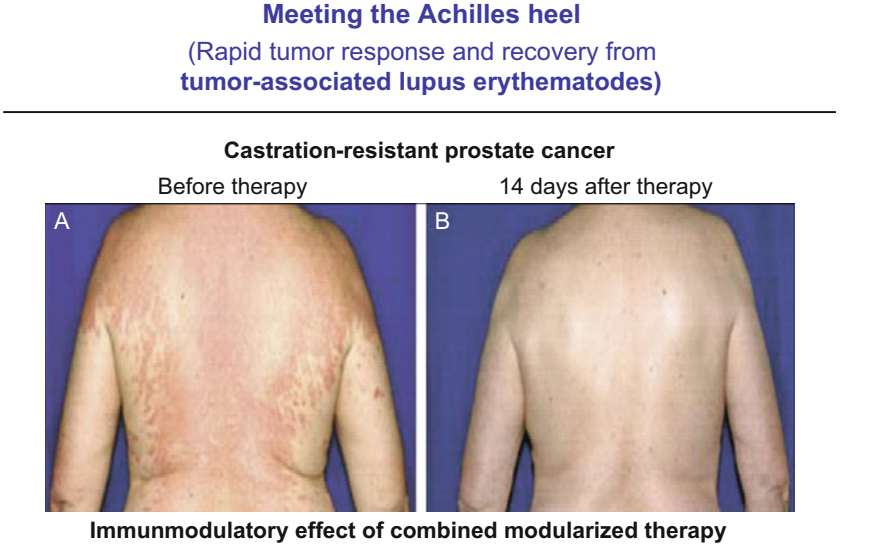

Fig. 19.10 Rapid tumor response and recovery from tumor-associated lupus erythematodes is indicative for the immuno-modulatory capacity of combined modularized therapy

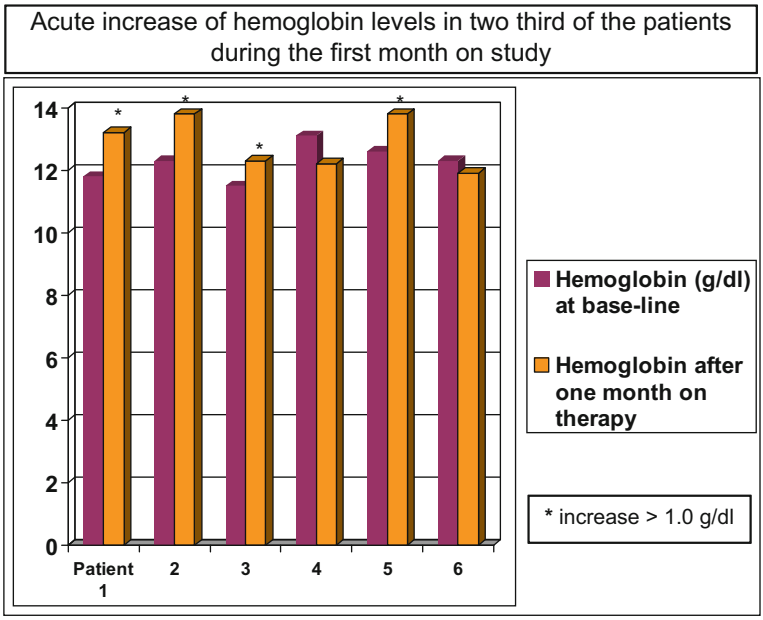

Fig. 19.11 The acute increase of hemoglobin levels in two thirds of the patients during the first month on study (multiple myeloma trial) indicates a direct suppression of hematopoiesis via myeloma cells

These observations indirectly argue in favor of inducing a biological memory as a main cause of tumor control during the therapy with biomodulatory therapy regimens including combined transcriptional modulation.

Long-term stable tumor disease in CRPC and after third-line therapy for multiple myeloma for more than 1 year is uncommon and should attract further interest: In none of the patients did the level of clinically detectable residual tumor disease indicate rapid tumor progression after the discontinuation of the study medication.

Achieving disease stability at still clinically detectable tumor levels with 'induction' therapies is no unique characteristic for metronomically administered combined modularized therapies. Stable therapy phases, particularly for short time intervals, are routinely observed in palliative care [17–19]. However, no systematic approaches are available addressing the question of how to induce a biological memory for attenuating tumor growth over longer periods or how to explain the natural history of metastatic tumor diseases with smoldering courses, which rarely occur among different histological tumor types.

Therapy-mediated redirections of tumor-associated growth-promoting normative notions could be clinically shown after combined modularized therapy of CRPC and MM: Most importantly, implementing non-normative boundary conditions in growth-promoting normative notions of histologically rather different tumor systems induced an objective response without compromising quality of life [10]. Furthermore, the redirection of selected 'hallmarks' of cancer, the control of systemic inflammation (> 30 % decline of serum C-reactive protein levels from base-line), and

immuno-modulation could be clinically monitored besides anti-osteoplastic activity in CRPC and amelioration of myeloma-mediated anemia [10, 12, 14, 15].

Epigenetic Modulation for Overcoming Resistance to Hormonal Therapy

The epigenetic regulation of gene expression has emerged as a major contribution to hormone resistance in breast and prostate cancer [20]. Epigenetic alterations coupled with castration-resistance provide a basis for pharmaco-epigenomic therapy approaches [21].

In 1 patient, we could show recovery of hormone-sensitivity due to the administered combined modularized therapy. Epigenetic modulation by the combined transcriptional regulators applied may play a central role in reconstituting hormone-sensitivity and should be further investigated.

Targeting histone demethylases and other androgen receptor coactivators may represent alternative therapeutic options, but their efficacy needs to be evaluated in further clinical trials [22].

Biological Memory and Epigenetic Regulation

Biological memory can be implemented by epigenetic regulatory processes. Ligand-mediated activation of transcription factors alters methylation and acetylation of histones aimed at modifying differential chromatin accessibility. Nuclear receptors, such as the targeted glucocorticoid and PPAalpha/gamma receptors, mediate chromatin modification by recruiting further regulatory factors that determine the validity and denotation of the transcription factors in an evolutionary context [23]. This recruitment follows rather different evolutionarily confined cell-specific long-term and short-term timelines for constituting epigenetic signatures that are characteristic for the gene activity profile of specific cellular phenotypes. Despite the multifaceted influences on gene expression and cellular phenotype, the epigenetic molecular players and mechanisms in the PPARγ signaling have to be defined in more detail.

Modifications in the chromatin structure are highly dynamic and are paralleled by the transcriptional output induced by the ligand activation of receptors: Ligand-mediated transcriptional activation of PPARgamma targets adhesive interactions of MM cells with the bone marrow microenvironment [24, 25]. Thereby, PPARgamma negatively controls multiple myeloma growth and viability, partly through bone marrow stroma cells inhibiting interleukin-6 production [26]. PPARgamma is also frequently overexpressed in castration-resistant prostate cancer [12, 27] but, for example, epigenetically silenced in colon cancer [28].

Epigenetic signatures may be modified via the ligands of nuclear receptors, such as glucocorticoids and pioglitazone, in a cell-type specific manner: In present therapy schedules, the regulation of genes is induced by the ligand-mediated activation of

transcription factors, i.e., glucocorticoid and PPARalpha/gamma receptors. These nuclear receptors belong to a class of ligand-stimulated transcription factors. To unfold activity, these receptors must overcome the condensed chromatin to get access to DNA: PPARgamma agonists significantly inhibit DNA binding and transactivation of STAT3 bound to the promoter of target genes in chromatin [24, 25].

Currently, scientific discussion is mainly focused on the potential of epigenetic therapy in MM: Histone deacetylase inhibitors also show promising results in clinical trials and particularly good therapeutic outcome when administered in combination with other standard chemotherapeutic agents [29].

Altering the Biological Memory

Clinical observations indicate that seemingly stably established epigenetic benchmarks of gene activity that promote tumor growth in a tumor system can be modulated by combined modularized therapies. Such epigenetic benchmarks dynamically respond to combined transcriptional modulation with a biological memory for tumor growth control by modifying epigenetic processes.

Epigenetic benchmarks are redirected by the modified access of transcription factors to DNA. The modified access contributes to the alternation of the availability of promoter sequences for activation or inhibition by transcription factors that critically determine the validity and denotation of cellular functions.

Modulating Long-Term and Short-Term Memory

Generally, the triggering of nuclear receptors has been shown to mediate more rapid and 'transient' epigenetic changes [30–32].

Therefore, our data clinically give hints that epigenetic modulation may be therapeutically accessed to stably maintain therapy-induced rationalization processes of tumor-associated normative notions within a clinically meaningful interval. Currently, we cannot specify which kinds of rationalizations are predominantly involved to sustain biological memory.

Targeting the 'Metabolism' of Evolution

As shown, combined modularized therapies directly interfere with the 'metabolism' of evolution [33]. Thereby, these therapies use the inherent robustness of systems by modulating normativity and respective tumor-associated rationalizations, subjectivity by modulating the systems interpretation via hubs, the orientation of actions, intentions, or motivations by instigating signals and intersubjectivity by modulating validities of communication lines. Modularity is the communication-technical prerequisite for implementing the evolutionarily constrained communicative expression of systems objects.

The possibility to stably redirect tumor-associated normative notions from the initially growth-promoting intention to attenuating growth means that rationalization processes of normative notions are fundamentally reorganized in a digitalized manner. This reorganization is possible due to the availability of the evolutionarily confined modular knowledge of systems objects, which is situatively available and therapeutically redeemable: The reorganization of rationalization processes can only take place within robust and digitalized organized systems—a fact that does not exclude analogously working subsystems.

Biomodulatory therapy approaches do not primarily intend to destabilize robust systems, but – as shown – may implement a novel and therapeutically relevant robust systems status, which may be principally realized in the range of evolutionarily constrained degrees of freedom within a tumor system.

Constitution of Tumor-Associated Normative Notions

Myeloma and castration-resistant prostate cancer are rather different tumor diseases, both genetically and phenotypically. The most prominent difference is the presence of osteolytic lesions in MM and of osteoplastic bone metastases in PC. Nevertheless, very similar combined modularized therapies promote long-term growth attenuation. Thus, response to therapy in the respective study populations suggests that rationalizations of tumor-promoting normative notions provide common hubs within rationalization processes (Figs. 19.12 and 19.13) that are therapeutically accessible by implementing non-normative boundary conditions.

Meeting Tumor Heterogeneity

In metastatic tumor disease, tumor heterogeneity has to be suggested on a genetic and molecular-genetic level. Combined modularized therapies meet the problem of tumor heterogeneity [34]: Metastatic tumor disease may be successfully controlled by redirecting tumor-associated normative notions, i.e., the hallmarks of cancer, or, more generally defined, by modulating tumor-associated decision maxims, action norms, and normative structures. Successful control indicates that (1) targeting tumor-associated normative notions provides access for tumor growth control, and that (2) presumably genetically heterogeneous tumor cells in CRPC or MM may provide common rationalizations or hubs within rationalization processes generated to support particular tumor-associated normative notions. A common morphological denominator could be osteoblasts, which support tumor growth in multiple myeloma and bone metastases in CRPC (Chap. 6).

Advanced tumor disease, even in third-line or in the castration-resistant stage of prostate cancer, could not be controlled with uniform combined modularized therapies, as therapeutic ‘top down’ technology, without the availability of common hubs

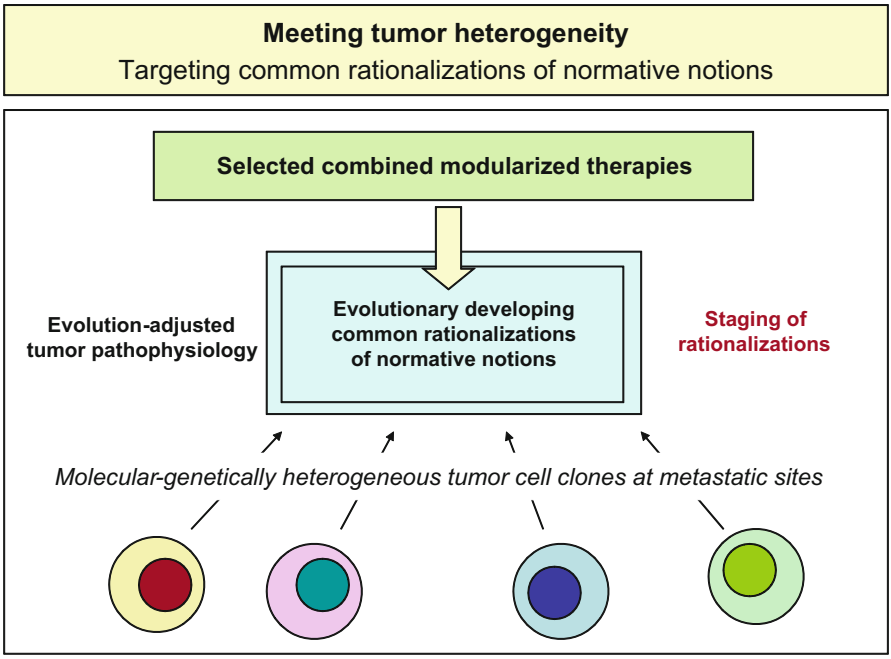

Fig. 19.12 Evolutionarily developing common rationalizations of normative notions or hubs of rationalizations provide the common denominator for therapeutic approaches to overcome tumor heterogeneity

in rationalization processes or unique rationalizations of tumor-associated normative notions.

The development of unique hubs in rationalizations within an individual tumor disease could be based in the common evolutionary origin of tumor cells and on constrained normative structures for shaping tumor-immanent normative notions. These observations underline the necessity to exploit rationalization processes as therapeutic targets independent of genome-oriented and histology based approaches.

Vice versa, external tumor promoters, cellular stress, carcinogens, radiation etc. can be considered as non-normative boundary conditions inducing chromosomal or molecular-genetic aberrations, and additionally, heterogeneous rationalizations for identical normative notions ([35]; Chap. 2): Radio-therapeutically pretreated metastatic lesions are frequently progressive, despite disease stabilization or response to combined modularized therapies at other metastatic tumor sites.

To specifically meet genetically based tumor heterogeneity at the time of tumor staging; therapy-relevant questions should be answered (Chap. 15): What are inter-systemically comparable tumor-associated normative notions, and what notions are promoted by similar rationalization processes or hubs among molecular-genetically heterogeneous tumor probes?

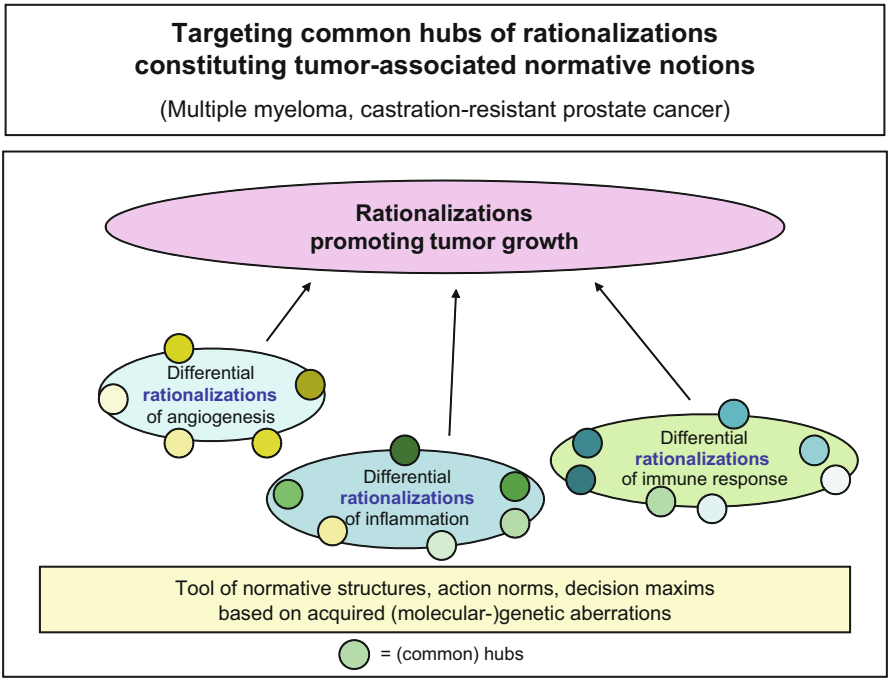

Fig. 19.13 The tool of normative structures, action norms, and decision maxims based on acquired (molecular-) genetic aberrations provides common hubs even in heterologous cell systems, i.e., multiple myeloma cells and castration-resistant prostate cancer cells as well as in their associated neighboring stroma cells. The activity profile of identical therapy regimen, combined transcriptional modulation, and low-dose metronomic chemotherapy cannot be explained by identical rationalizations of tumor-promoting normative notions in both tumor types but by the therapeutic use of common hubs within heterologous rationalizations for identical normative notions (inflammation, angiogenesis, etc.)

Conclusions

Long-term stable disease at clinically detectable tumor levels may be induced with modularized therapies including metronomic low-dose chemotherapy with treosulfan and combined transcriptional modulation with dexamethasone and pioglitazone in addition to etoricoxib and imatinib in CRPC and lenalidomide in multiple myeloma. Despite initial disease progression, these schedules may induce an objective response as well as a memory for long-term tumor growth attenuation.

Epigenetic modifications are closely linked with a biological memory: The capacity of similar combined modularized therapies for inducing long-term tumor growth control in rather different tumor diseases indicates that growth attenuation in CRPC and MM is mediated by common rationalization processes or hubs for rationalization processes. These results underline the necessity to systemize rationalizations of tumor-promoting normative notions in an evolution-adjusted tumor pathophysiology.

Translating combined modulation of nuclear receptors, in the present cases ligand-mediated activation of the PPAalpha/gamma and glucocorticoid receptors, on an epigenetic level and consecutively on the description of redirected tumor-associated normative notions may provide more specific therapeutic instruments for guiding long-term tumor growth control or recovery of cell functions (hormone-sensitivity) by combined transcriptional modulation.

Molecular-genetic and genetic tumor heterogeneity is a central obstacle for the systemic therapy of metastatic tumors. Targeting rationalizations or hubs of rationalization processes constituting tumor-associated normative notions could be a independent way to therapeutically meet molecular-genetically or genetically based tumor heterogeneity [36].

Regaining hormone-sensitivity seems to be a central therapeutic project, because worldwide about 180,000 men are predicted to be diagnosed with prostate cancer and 27,000 men to die of castration-resistant prostate cancer every year.

Keeping in mind the potency of transcriptional modulators to simultaneously regulate tumor and stroma cell epigenetics, combined transcriptional modulation could generally be an important approach for implementing a biological memory [36]: The epigenetic redirection of cellular functions by transcriptional regulation could be a major therapeutic approach in the treatment of numerous diseases, from neurodegenerative diseases to metastatic cancer, as well as in tumor prevention (Chap. 15).

For palliative tumor control, metronomically administered combined modularized therapies should be evaluated in schedules that provide therapy breaks. Such schedules including combined modularized therapies could be a realistic and presumably less toxic alternative to the currently favored induction and maintenance schedules, particularly in palliative care.

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Part VIII
The Tool of Rationalizations Constituting Tumor-Associated Normative Notions: The Non-Genomic Counterpart of the Tumor Genome and Therefore, an Important Therapeutic Target for Diversifying Palliative Care

Chapter 20
Diversifying and Specifying Palliative Care for Patients with Metastatic Cancer by Therapeutic Implementation of Non-Normative Boundary Conditions

Albrecht Reichle and Gerhard C. Hildebrandt

Abstract Clinical scientific progress is the result of three main factors, i.e., a yet unmet medical need (systemically pretreated patients with metastatic tumors), a hypothesis-driven vision (a formal pragmatic communication theory), and technological advances to pursue that vision (biomodulatory therapy approaches, clinical proteomics, epigenetics and molecular imaging techniques). The therapeutic relevance of situative validity claims of tumor systems objects (cells, modules, oncogene-addicted targets, etc.) may be substantiated by the replicability of a formal pragmatic communication theory. Such a theory needs to be based on reconstructive activities that integrate clinical and laboratory surrogate parameters, which are derived from the long-term implementation of non-normative boundary conditions, into a metastatic tumor's holistic and normatively structured communicative system (i.e., morphological structures, the hallmarks of cancer as action norms and decision maxims). The implementation of non-normative boundary conditions may be achieved via non-oncogene-addicted therapeutic targets (biomodulatory therapies). Therapy-derived clinical and laboratory surrogates, whose validity has been shown in histologically rather different metastatic tumor types (e.g., C-reactive protein, ECOG status, etc.), confirm the usefulness of reconstructive activities for exploring 'universal' response parameters that indicate the changes in a tumor's normative systems structures. Such reconstructive activities allow the depiction and specific targeting of a tumor's normative systems structures, defined as cellular therapy in situ. Tumor-associated disease traits may now be targeted to substantially attenuate tumor growth or even to induce continuous complete remission. This way, communication-derived tumor pathophysiology decisively broadens the therapeutic options in the palliative care of patients with metastatic tumor diseases.

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Introduction

In 2030, one third of the European population will be older than 65 years, and one third of this age group will be dying of cancer [1, 2]. This scenario presents a huge challenge because the treatment of such a large number of patients represents a yet unmet medical need. Therefore, age-appropriate therapies for patients with metastatic malignancies should be designed without any further delay [3–5].

Such therapies must meet ambitious demands. Multiple and partially unrelated factors affecting the therapeutic outcome have to be considered, for instance, age-related changes in the pharmacokinetics of drugs, the likelihood of comorbidities of elderly patients, age-dependent characteristic cytogenetic aberrations in distinct neoplasias (e.g., myelodysplasia, acute myelocytic leukemia), and the fact that tumor-associated disease traits compromise the quality of life as well as the social and psychical well-being of patients [6, 7]. First of all, therapies must be accessible and affordable for both patients and social communities.

Before any decision on a therapy for metastatic tumors can be made, all uncertainties need to be considered that may influence the conditions of decision-making (Table 20.1). Some conditions are very familiar, but most conditions may not be sufficiently anticipated at the time. Study endpoints as well as appropriate surrogates are often not adequately discussed with regard to **communicative and purposive concerns**—that are directly derived from a tumor's 'biology' and its normatively structured systems—for answering the all-important question: What is the most successful way to prolong overall survival under the aspect of optimized palliative care?

One novel option is the attenuation of tumor growth via the **resolution of tumor-associated disease traits** [8, 9] by redirecting and modulating tumor-immanent normative notions (normative functions, structures and decision maxims) with biomodulatory therapy approaches ('top-down', primarily multi-track approaches) ([10], Chap. 2, 22). By contrast, the most common approach is repetitive remission induction with cytotoxic agents or classic targeted therapies that directly induce apoptosis ('bottom-up' strategy, mostly single-track approaches) ([11, 12], Chap. 2). Beyond palliative care, 'bottom-up' or 'top-down' approaches may mediate cure or long-term tumor control via redirection and modulation of tumor-associated rationalization processes, which concertedly constitute the tumors' normativity ([13–16], Chap. 10).

Meeting endogenous communicative and purposive concerns of the tumor's systems biology requires estimating the communicative expression of communication lines or respective communication mediums (information transmitters) in an evolutionary context [10]. Usually, researchers tend to be diagnostically satisfied when tumor-specific aberrations are detected as potential therapeutic targets, particularly in case of so-called 'driver' mutations [17]. However, not only the presence or absence

Table 20.1 Therapy of metastatic tumors: Decision-making conditions of uncertainty

• **Drug selection on the background of uncertainty about validity and denotation of systems objects**
• **Aims of treatment**
• **Life expectancy (survival time)**
• **Quality of life**
• **Control of disease traits**
• **Treatment tolerance**
• **Long-term treatment compliance**
• **Availability of caregiver**

of (oncogene-addicted) targets in a tumor is therapeutically decisive, but also the tumor systems' communicative expression that is based on validity claims of communication acts in the framework of the evolutionary systems context [10, 17, 18]. The situative meaning of communication acts may significantly differ in the presence of various numbers of additional molecular-genetic aberrations and is finally crucial for attenuating tumor growth via therapeutic control of tumor-associated disease traits [19, 20].

In individual pre-therapeutic situations, the situative validity and denotation of tumor systems objects (proteins, pathways, cells, functional arrangements, etc.) is usually unknown, particularly in large mutation-loaded, molecular-genetically heterogeneous tumor diseases, such as pancreatic cancer, glioblastoma, NSCLC, malignant melanoma, acute myelocytic leukemia, etc. [21–25]. A well-studied example is acute myelocytic leukemia: Here, multiple chromosomal and molecular-genetic aberrations can be frequently observed, particularly in elderly patients. However, little is known about how the functional impact of a distinct 'driver' mutation changes dependent on the actual pattern of additionally acquired chromosomal aberrations (Chap. 13). Communication-technically, mutations cannot be invariantly valued as 'dominant' or 'bystanders' because they may context-dependently change their validity and denotation [10, 18, 26].

Currently, scientists do not routinely use available methodologies to diagnostically overcome uncertainties about validity and denotation of therapeutically targeted systems participators. Tumor systems participators, however, are inevitably communicatively integrated in primarily unknown, novel evolutionary systems contexts on the background of varying numbers of additionally acquired chromosomal and molecular-genetic aberrations [10, 27].

In the present paper, we conceptually discuss diagnostic and therapeutic aspects with the aim to further personalize therapies for metastatic cancer by simultaneously targeting multiple, but purposively selected facets of the tumors' normativity:

- How can we diagnostically assess the validity and denotation of systems objects besides the physical constitution of rationalization processes?
- What kind of therapeutic implications arise from targeting endogenous communicative and/or purposive activities of tumor systems by redirecting and redeeming the validity and denotation of tumor-associated systems participators?
- How can we specifically redirect and modulate selected tumor-immanent rationalizations for attenuating tumor growth?

The Implementation of Non-Normative Boundary Conditions in Normatively Structured Tumor Systems: Cellular Therapy in Situ

Validity claims of the tumors' systems objects shape the constitutional background of communicative processes and contribute to the evolvability of tumor systems. Validity claims of systems participators are unbiased and do not necessarily implicate a tumor-promoting or tumor-attenuating attitude [10]: Validity claims of systems objects represent universally available communicative laws that guide the evolution of tumor and neighboring stroma cells against the background of their evolutionarily developing normative systems structures.

The targets of purposive-rational tumor therapies are communicatively linked intentional activities of tumor systems comprising all cellular and extracellular components of the tumor environment [10, 27]. Purposive-rational tumor therapies aim at redeeming and redirecting the tumor-specific situative tool of validity claims by implementing non-normative boundary conditions into a tumor's world of normative structures, functions and hubs. Biomodulatory, combined modularized therapies are able to persistently establish non-normative boundary conditions for inhibiting tumor growth, irrespectively of the multiplicity of mutation-associated 'driver' mutations [8]. By targeting the tumors' normativity, molecular-genetic heterogeneity as the major therapeutic obstacle of the classic targeted therapies, may be overcome (Chap. 2)

The tool of validity claims is evolutionarily constrained and situates tumor-specifically rationalized tumor systems. The therapeutic frame for purposive-rational therapies is constituted by the holistic communicative systems context, i.e., the tumor's living world [18].

Validity claims are prerequisite for the possibility to specifically implement non-normative boundary conditions by physicians ([28], Chap. 2). This way, biomodulatory therapies may establish therapeutically relevant changes in a tumor's normative systems structures. Functional changes in normative systems structures induced by biomodulatory therapies represent situational tumor-specific response features that mirror evolutionarily restricted tumor-associated expressive patterns of communication lines, rationalization processes, and normatively organized systems structures [8].

Biomodulatory therapeutic activities are focusing on the resolution of tumor-associated disease traits—that are often the clinical display of the 'hallmarks' of cancer [29]—and finally aim at achieving attenuation of tumor growth. Biomodulation can be understood as an attempt to decouple evolving 'corrupt' rationalizations of tumor systems from their communicative presuppositions: Biomodulatory therapy strategies have the capacity to therapeutically exploit evolutionarily restricted tools of modular systems structures by redeeming validity claims of tumor systems objects: Non-normative boundary conditions may be implemented towards the suggested normatively structured systems world of a tumor.

Normative biological systems structures include (1) cellular structures (morphological cellular and extracellular structures including molecular-genetic or genetic aberrations and modules), (2) compartmentalized action norms (different structures promoting angiogenesis, inflammation, immune response, robustness, cell death inducing mechanisms, evasion of immune surveillance, glycolytic production of ATP also under aerobic conditions, the Warburg effect, compromised cell death programs, self-sufficiency in growth signals, tissue invasion, metastatic potential, limitless proliferation, stress phenotypes, i.e., metabolic, oxidative, and mitotic, DNA damage stress, etc.), and (3) decision maxims (nodes and hubs) ([10, 29, 30], Chap. 17).

Examples for clinically well-studied tumor-associated normative notions are tumor pathologies triggered by tumor cell proliferation (e.g., acute leukemias), accumulation of differentiated malignant cells (apoptosis resistance in chronic lymphatic or myelocytic leukemias), dysplasias (myelodysplastic syndromes) [17, 31], local tumor expansion versus systemic tumor expansion, and organ tropisms, etc. [32].

Molecular targets for biomodulatory therapies include non-oncogene-addicted regulatory active structures (Chap. 2), but also oncogene-addicted ligand sites, for instance actionable 'driver' mutations [33]. The individual components of a primarily multi-track purposive-rational therapy must not necessarily exert mono-activity in the respective tumor type [8].

Independent of the selected drug or drug combination, purposive-rational tumor therapy must meet a tumor's normative systems structures to induce phenotypically accessible clinical and/or laboratory responses (surrogate markers). From a communication-technical point of view, the underlying operational conditions to achieve a therapy-relevant modulation of normative systems structures with biomodulatory therapies may be extremely heterogeneous. Either single drugs (tyrosine kinase inhibitors, lenalidomide, dexamethasone, metronomic low-dose chemotherapy etc.) or drug combinations (multi-track approaches) are able to implement non-normative boundary conditions over long time periods, e.g., by metronomic drug administration of multiple drugs at low dose levels (Chap. 2, 22). Aim of biomodulatory therapies is to modulate tumor-promoting normative systems structures in a clinically meaningful way [8, 14, 34] to achieve optimized palliation, i.e., simultaneous tumor and symptom control.

Identical components of modular therapy approaches differently hit evolutionary rationalized and normatively structured tumor systems as indicated by multifaceted response patterns [10]. Combined biomodulatory activities are characterized by poor single agent activity towards non-oncogene-addicted tumor promoting targets [35].

Purposive-rational therapies indirectly mediate access to multimode cell death-inducing mechanisms: As shown, such therapies have the capacity to induce continuous complete remission in renal clear cell carcinoma, Langerhans' cell histiocytosis and angiosarcomas ([8], Chap. 2). In contrast, the overwhelmingly applied therapeutic principles, i.e., cytotoxic therapy, and classic targeted therapy approaches, aim at facilitating the direct induction of cell death triggering processes [36].

Molecular targets for modulating the tumors' normative systems structures decisively differ from commonly used reductionist-derived targets [37, 38]: For purposive-rational tumor therapies targets predominantly comprise non-oncogene-addicted molecular structures ([8, 10, 27, 34], Chap. 2). Reductionist approaches, however, frequently focus on acquired molecular-genetic aberrations of a tumor [39]. These tumor-specific 'aberrant' targets (mutation-associated 'drivers') are commonly used on the presupposition that acquired aberrations are always linked to the same expressive status, irrespectively of the number of additional aberrations within in a distinct tumor disease [21–25].

The therapeutically decisive factor remains the context-related validity and denotation of a targeted communication line or communication medium and its relation to normative systems structures and to apoptosis-inducing pathways:

1. Altering the signal transmission of a single communication line may suffice to sustainably disturb the holistic communicative system and to induce life-long leukemia control in chronic myelocytic leukemia (CML) [40]. Targeting a single 'dominant' mutation in CML substantially involves normative systems structures and restricts the recourse on rationalization processes for maintaining the original leukemia promoting systems functions.
2. The selection and combination of multiple therapeutic targets, derived from individually proven 'driver' mutations, may be clinically successful [41] as long as their validities and denotations are evolutionarily conserved. Such conservation cannot be suggested as a matter of course in novelly evolving tumor systems. More frequently, drug assortments clinically fail, if a large additional mutation load is present, and if the operative links between the targeted communication lines and the expected normative systems structures are missing [41–43]. Therapeutic success is often compromised by a missing link via intersystemic exchange processes to cell death-inducing pathways [36, 44]. Functioning intersystemic exchange processes between purposively modulated normative systems structures are therapeutically relevant for attenuating tumor growth with biomodulatory approaches [10, 45].
3. Cytotoxic drugs or drugs targeting oncogene-addicted molecular structures seek to mediate apoptosis by by-passing modulation and redirection of tumor-associated normative systems structures and directly aim at immediate and dose-dependent cytotoxicity [36].
4. The long-term metronomic implementation of non-normative boundary conditions into a tumor's normative systems world with primarily multi-track biomodulatory therapies meet a fundamental therapeutic problem in metastatic cancer diseases with a large mutation load and multiple 'driver' mutations: Such

'top-down' approaches may overcome tumor heterogeneity by simultaneously targeting selected tumor-immanent normative notions. Simultaneous targeting via the respective rationalizations also overcomes the problem that in clinical practice, the evolutionary constrained expressive status of acquired chromosomal and molecular-genetic aberrations cannot be sufficiently interpreted despite the availability of characteristic recurrent molecular-genetic features [19].

5. Biomodulatory therapies may be considered as cellular therapies in situ, which intentionally deal with locally available and even complexly structured cellular and molecular communication-related resources of a tumor in order to alter the validity and denotation of systems objects for therapeutic purposes, to resolve tumor-associated disease traits, and to attenuate tumor growth. By redirecting the tumors' normativity, surrogate markers may be generated that reflect changes in normative systems functions ('universal' response parameters), exemplarily shown for inflammation control (C-reactive protein) and the attenuation of clinically manifest disease traits ([8, 10, 20], Chap. 22). In future, adaptive trial designs could be implemented to successfully modulate individually available normative systems structures and functions (purposive-rational therapy) [46].

'**Universal**' **surrogate parameters** indicating modulation of distinct normative systems structures and functions will be available to therapeutically monitor a broad spectrum of biomodulatory therapy approaches, e.g., by analysis of cellular secretomes derived from tumor-associated cell compartments (in serum or plasma), or molecular imaging data, etc. 'Universal' surrogate parameters may be implemented into study concepts to guide adaptive trial designs (Chap. 15).

The Reconstructive Method of Normative Systems Structures: 'Universal' Response Parameters

Tumors are communicatively evolving systems, constituted by systems objects with communicative competence. The communicative competence is aimed at maintaining normative notions and at constituting robustness [47]: The possibility to assign meaning to systems objects (communication lines, modules, cells, etc.), and the capacity of systems objects to act intentionally and to maintain intersubjective relations in the tumor-specific frame of a holistic communication system (the tumor's 'living world') represents the backbone of a formal pragmatic communication theory for biological systems [47]. The scientifically accessible communication-based references of systems objects constitute the difference between biological systems objects and those systems objects considered in natural science [47].

As long as observable events are supported by a normative background that structures the meaning of communication lines in biological systems, reconstructive activities offer the opportunity for comprehending the status of communicative expression. In parallel normative systems structures may be uncovered. Communication-derived rules (validity claims of systems objects) are a prerequisite for scientifically detecting rationalization processes and background knowledge of systems objects [47].

Rationalization processes describe (1) how normative systems functions and structures are contextually organized [35, 48], and [2] how the tumors' normativity is situatively attributed to distinct cell compartments on the background of the frequently observed redundancy of cellular functions [28]: Different tumor-associated normative notions—integrating functions of oncogenes and tumor suppressor genes—are constituted by distinct rationalizations, including 'corrupt' rationalizations.

Thus, the physical constitution of rationalization processes strongly indicates the particular pathophysiological character of a specific tumor disease, which should be presented in an evolution-adjusted tumor pathophysiology [17]. The equivalent of tumor-associated rationalizations on genetic basis represents the tumor genome.

Modularity is a feature of all communicative processes in biologic systems. Consequently, normative systems structures may be supported by modularily arranged systems rationalizations. Thereby, **novel interfaces** between systems structures may develop: The 'individual' arrangements of tumor systems contexts allow us to comprehend ex post interfaces between tumor-associated normative notions and corresponding intersystemic exchange processes. Knowledge about the rationalization of normative systems structures or about their therapy-associated behavior, e.g., indicated by cellular secretome analyses, molecular imaging techniques, etc., may be used to describe distinctive genetic patterns among the bulk of genetic aberrations (whole genome analyses), which are supporting tumor-immanent normative notions (Fig. 20.1).

Universal communication-derived rules govern complex biological systems. These rules are of increasing therapeutic interest, especially during evolutionary systems phases, i.e., in evolving tumor systems. The communicative impact of single tumor systems objects, involved in tumor promotion, depends on highly varying, additionally acquired chromosomal and molecular-genetic aberrations. Heterogeneous chromosomal aberrations within a histologically defined tumor disease necessitate the reconstruction of a tumor's differently developing rationalization processes, the communicative expression of communication lines, and the novel compartmentalization of systems functions (Table 20.1). Therefore, in evolving systems, communicative expression of systems objects cannot be adequately anticipated—as commonly suggested—without previous evaluating steps, for instance, correlations of rationalization processes with patterns of acquired molecular-genetic aberrations in the tumor and stromal cells (Table 20.2).

Communication is responsible for irreplaceable modes of cellular and molecular integration in tumors: Communication-derived rules serve as therapeutically available targets, as shown by the successful implementation of biomodulatory therapies for the treatment of metastatic tumors ([8], Chap. 2). The therapeutic relevance of validity claims of systems objects may be confirmed by the replicability of a recently developed formal-pragmatic communication theory in many different tumor systems (Chap. 2). The theory is based on reconstructive activities that integrate clinical and laboratory surrogate parameters, which are derived from long-term implementation of non-normative boundary conditions (biomodulatory therapies) into a metastatic tumor's holistic and normatively structured communicative system. Furthermore, therapy-derived clinical and laboratory surrogates that are valid in histologically

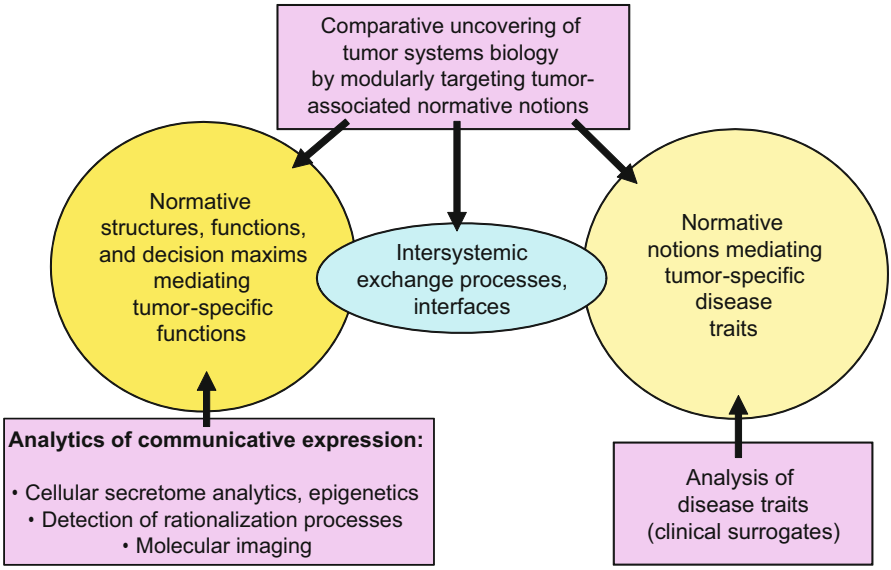

Fig. 20.1 Analysis of communicative expression: Tumor-specifically rationalized and modularized normative structures

Table 20.2 Communication-derived pathophysiological evaluation of tumor systems

- ***Basic communicative activites (communication lines, mediums)***
  - *Presence of distinct communication lines*
  - *Altered information transmission*
  - *Enhanced or mitigated signals*
- ***Tumor-inherent purposive-rational activities***
  - *Communicative expression of communication lines*
  - *Novel rationalization processes*
  - *Novel compartimentalizations of systems functions*
  - *Alterations of normative systems structures*

quite different metastatic tumors (for example, C-reactive protein) confirm the usefulness of those reconstructive activities that indicate changes in the tumors' normative systems structures ('universal' response parameters) [8, 28].

'Universal' response parameters facilitate operative access to communication-related rules in tumor systems and the exploitation of 'universal' prepositions for generating distinct meanings of communication lines (**evolutionary conserved communicative meaning**) (Chap. 22).

Table 20.3 Overcoming uncertainty about validity and denotation of systems objects in a novel evolutionary context

- **Comprehension and assessment of the context-dependent functional status** of systems objects within a tumor (e.g. compartmentalization of functions, assignment of functions to cellular compartments, validity and denotation of systems participators)
- **Alignment of cytogenetic and molecular-genetic patterns with** corresponding rationalization processes constituting tumor-immanent normative notions
- **'Universal' surrogates:** Generated by monitoring the redirection and modulation of the tumors' normativity during purposive-rational therapies; prerequisite for novel adaptive trial designs
- ***Evaluation of the originating impulse for communication acts***

Communication-associated rules are accomplished through the binding principle of shared holistic communication and understanding: Communication integrates demands of the functional world of systems participators and their imposed systems' world.

Reconstruction-based tumor models gain facticity, if single reconstructive steps can be reproduced with adequate scientific methods. By applying suitable methodological tools (Table 20.3), the following pragmatic aims may be obtained:

- Appreciation of evolution-based normative systems structures before initiating systemic therapy
- Prediction of the efficacy of biomodulatory therapy approaches via '**universal**' **response parameters**
- Selection of therapeutic resources, i.e., tumor-specific rationalizations of normative notions, with the aim of targeting stage-specific, multifaceted communicative structures accountable for tumor-associated disease traits.

Therapeutic instruments for guiding tumor-specific communication tools, i.e., biomodulatory therapies, give rise to therapeutically exploit both tumor-immanent communicative and endogenous purposive activities [10].

The attempt to reconstruct normative systems structures and to demonstrate how to uncover and monitor these processes for therapeutic purposes cannot lead to a concept for comprehending a tumor's normativity 'per se' [10]. Normative notions cannot be summarized and depicted as cohesive tumor-associated systems networks. Tumor-immanent rationalizations are a concrete 'utopia' realized in a normative notion (Chap. 10). The timely, structural, and topographical '**vagueness**' of normative systems structures in evolving tumor systems raise special issues resulting in specific reconstructive activities.

What is at issue here, is to discuss the daily diagnostic and therapeutic challenges generated by evolutionary developing novel communicative presuppositions and aimed at broadening the therapeutic instruments. The communicative background of tumor-promoting communication lines has been shown to be inevitable for the continuous and non-circumventable process of reaching communicative understanding as well as for strategic, i.e., therapeutic communicative interventions.

Communicative Action Systems

Communication-technically, tumors may be considered as **therapy-relevant communicative action systems** that provide the basis for maintaining homeostasis, robustness and tumor-immanent normative notions. On top, endogenous intentional activities, i.e., purposive-rational processes are imposed that promote changes in normative tumor structures (localized tumor expansion, metastatic spread, proliferation, etc.).

Therefore, in a tumor, two communicative action systems may be separated that provide targets for purposive-rational therapy approaches:

- For maintaining normative structures, communicative action is the primary mode of intercellular and molecular interaction. Communicative action is oriented towards a reciprocal understanding within a tumor's living world, namely the holistic communicative context. Both strategic and communicative tumor activities involve the communicative resources of a tumor's living world. Such tumor activities are operated by the expressive action of individual tumor-specific communication lines and mediums or by normative structures that are inducible and tractable by purposive-rational therapy approaches.
- The basic communicative activity serves as therapeutic target in order to disturb tumor-promoting communication lines, to alter information transmission, to enhance or mitigate signals, and to promote or attenuate available rationalizations.
- The endogenous strategic, intentional activities of a tumor system (instigation, contingency programming, education, tumor-promoting communication) [28] may be redirected by the therapeutic implementation of non-normative boundary conditions. Biomodulatory therapies are aimed at modulating normative systems structures by inducing novel topographical or textual rationalizations of systems functions and novel functional compartmentalization of systems features.

Rationalization processes allow intersystemic exchange processes, and are suggested to be convergent organized via hubs, starting from quite heterogeneous aberrant cytogenetic patterns: A huge variety of chromosomal aberrations may organize similar rationalization processes and normative notions (Chap. 13).

Missing, severely restricted or disturbed communication might induce cell death without using specific cell death pathways. Communicative exchange processes could provide the opportunity at linking therapeutically induced attenuation of clinical disease traits (represented by clinical or laboratory surrogates) with still functioning cell death-inducing pathways. Rapid therapy response to combined modularized therapies is indicative that communicative Achilles' heels have been hit [8].

Pragmatic Functions of Communicative Expressions

Therapeutically employed purposive-rational communicative actions are characterized by the missing intention to reach a reciprocal understanding within a tumor's characteristic living world—in contrast to constitutive systems-related pathophysiologic interactions.

Corresponding to the pragmatic functions of communicative expressions, four relevant topics involved in communicative expression may be therapeutically modified,

- a tumor's relation to its external reality (microenvironment, host organ),
- the internal reality, characterized by tumor-specific and stage-specific rationalizations of tumor functions and situational redeemable background knowledge,
- the intersubjective reality comprising the intentions of systems objects,
- and normative systems structures.

Targeting External and Internal 'Reality'

Tumor-related complex activities are operationally separable by the division of function. Normative functions may be simultaneously attributed to the external and internal reality, such as inflammation, neoangiogenesis, immune response, etc. Therefore, tumor-immanent normative notions are characterized as multidimensional processes, which are rationalized in a tumor-specific way [10]. Multidimensionality has to be taken into account by respective therapy designs: Tumor systems-directed therapies have the capability to use aggregated action effects as adjustable sizes to therapeutically modulate a tumor systems' stability, homeostasis, and robustness within the frame of evolutionarily accessible holistic communicative background knowledge (purposive rationality) [8].

The internal communicative 'reality' of a tumor is maintained by reciprocal communicative activities with the external 'reality', the microenvironment, and the host's organs. Normative systems structures of the external 'reality' sustain tumor-associated normative structures on the basis of the context-dependent redeemable validity claims of the systems [49–51].

Targeting communication-derived rules by implementing non-normative boundary conditions into both the external and the internal reality offers the opportunity of therapeutically modulating pathophysiological relevant communication processes.

The implementation of non-normative boundary conditions may inhibit the cells' recourse on rationalization processes for maintaining normative notions (robustness). Robustness-repressing biomodulatory therapies focus on generating novel and normatively relevant rationalizations within the tumor's living world by inducing novel configurations of coherences between validity and denotation of systems objects [8].

Long-term disease control with consecutive tumor regression and induction of complete remission in multiple and histologically rather different tumor types highlight robustness-directed approaches as therapy-relevant, particularly in neoplasias with known intrinsic apoptosis resistance (melanoma, castration-resistant prostate cancer) [34, 52]: The therapeutically impeded recourse on multifaceted systems processes for maintaining normative notions involved in promoting tumor growth, may therapeutically destabilize tumor systems, which finally leads to objective

tumor responses, even though with extreme delay in some cases of renal clear cell carcinoma [8, 10].

- In castration-resistant prostate cancer, calcification of lymph nodes could be observed in response to biomodulatory therapies ([34], Chap. 5). This observation is indicative for tumor necrosis and saponification of fatty acids: Ideally, the therapeutic implementation of non-normative boundary conditions may link redirected normative systems structures via intersystemic exchange processes with available apoptosis-inducing pathways. The multifold possibilities to synergistically couple normatively altered tumor-associated disease traits, such as redirected and modulated inflammation, angiogenesis, etc. with cell death (pathways) via biomodulatory therapies indicates the therapeutic availability of a broad spectrum of normative systems structures as a starting point for tumor control [53]. Simultaneously, the resolution of tumor-associated disease traits may be accomplished. Because cell death pathways are in a narrow cross-link [53, 54] with multifaceted features of communication-associated pathologies, biomodulatory therapies might foster alternative cell death pathways.
- Efficacious shielding from growth-promoting input (microenvironment or the host's organs) with biomodulatory therapies could be shown in castration-resistant prostate cancer: Attenuation of osteoplastic activity (bone scan) was closely related with PSA response [34, 55].
- Implementation of biological memory has been shown in castration-resistant prostate cancer (CRPC) and multiple myeloma: Non-tumor-related discontinuation of biomodulatory study medication (e.g., surgery) was frequently associated with disease stability for more than one year, if the tumor responded previously [34].

Targeting Structures of Intersubjectivity

If we adjudge communication and action competence to communication participators in a tumor, then these participators share features of intersubjectivity, i.e., competences, such as the interpretation of a situation (depending on the propositions for a communicative action). Furthermore, these participators may direct their actions, and they act with both, intention and motivation (instigation, education, contingency programming).

Targeting a holistic communicative system, i.e., the aggregated action effects, means to influence the multifaceted intersubjective exchange processes, which first and foremost attribute references to the systems objects.

A prominent and frequently practiced therapy approach entails compromising signal quality of oncogenic-addicted communication lines. The so-called targeted therapies, which are frequently directed against oncogene-addicted targets, try to interrupt the signal transfer by blocking agents [56]. By now, legendary approaches are the clinically successful use of tyrosine kinase inhibitors in chronic myelocytic leukemia or Her-2 inhibitors in breast cancer.

Mostly, this kind of targeted therapies is based on reductionistically-derived therapy approaches. The validity of those approaches is commonly restricted to the presence of one single aberration serving as a therapeutic target. However, additional acquired aberrations frequently alter the validity and denotation of the respective target in such a way that therapeutic efficacy may be significantly compromised: A communication act has symbolic character and should be context-dependently evaluated for its communicative expression in evolving systems [10].

Therefore, intersubjective communicative expression—even though identical communication lines are operated—may unfold multifold facets of rationalizations, which are available to be targeted with biomodulatory therapies (Chap. 11). The situative rationalization of the tumors' living world by tumor-associated normative and non-normative boundary conditions decisively influences the validity of a communication act and highlights the 'symbolic' character of communication lines. The one-target-one-drug approach neglects the fact that classically targeted therapies are marked by context-dependent therapeutic efficacy (Chap. 7)!

Targeting Structures of Communicative Expression

Tumors constitute normative systems structures and functions that are concertedly maintained and promoted by the environment and the tumor cells [49–51]. **Non-normative boundary events**, may occur endogenously, but can be also therapeutically mediated by biomodulatory therapies and **noxa** during long-term exposition [57]. Tumors may either react with robustness (recruitment of alternative pathways, repair mechanisms, etc.), perturbations or cell death, or by further tumor evolution, either by tumor promotion or attenuation of tumor growth (e.g., induction of biologic memory by epigenetic changes) ([58–60], Chap. 2, [19]).

Molecular-biological studies working out differential gene expressions of mammalian cells upon exposition with noxa (epistatic miniarray profiling), show that novel normative systems structures may evolve during treatment: Many genes are not directly related to DNA damage response but are involved in exposition-specific novel genetic networks [61, 62]. These specifically inducible networks without direct oncogene addiction [63–65] are constituted by genes that are not necessarily tumor-specific. Only their contextual interrelation generates functional specificity that may be comprehended by communication-technical evaluation steps and targeted by implementation of non-normative boundary conditions.

The peroxisome proliferator-activated receptor gamma (PPARgamma) represents a nuclear transcription factor without direct oncogene addiction. As shown, its expression is specific for a histological tumor type, but additionally stage-specific. In metastatic melanoma it may serve as late-stage biomarker ([64], Chap. 8, 22).

Biomodulatory therapies provide significant evidence that **evolving non-oncogene-addicted communicative networks** are highly specific targets for drugs that implement non-normative boundary conditions ([8, 34, 64], Chap. [2]).

Novel non-normative boundary conditions may be therapeutically imposed on tumor-promoting normative systems structures. Pathophysiologically, the following factors may play an important therapeutic role:

- The decoupling of the identity of tumor systems objects (theme-dependent context knowledge) by evolutionary developing biological stages. That is identical with the decoupling of compartmentalized knowledge by the communicative arrangement of the systems objects' validity and denotation in a novel communicative background.
- The evolutionary constrained inhibition of the implementation of internally-derived or externally-derived modular knowledge according to rules present in modularly arranged and rationalized systems textures.

Targeting Communication-Derived Pathophysiological Resources with Biomodulatory Therapy

Pathological tumor-inherent communicative processes can be understood as a separation from physiological and homeostasis-maintaining communicative processes by redirection of the systems participators' validity claims. Their redirection may be intentionally triggered by implementing non-normative boundary conditions. The tool of accessible validity claims within a tumor's 'living world' forms the framework for therapy-inducible rationalization processes and possible modular rearrangements.

- The **meaning of tumor-immanent communication lines** may be therapeutically decoupled from the communication-relevant generalization of particular prepositions that establish generally valid norms within a tumor system. The availability of generalized prepositions is the prerequisite for acquiring reductionistically-derived knowledge and for successfully transferring the systems objects' meaning into novel evolving biologic systems (Chap. 2).
- **Compromising the understanding of a communication act** (contingency programming, education, instigation, time-sensitivity) by tumor-associated systems objects has influence on the
  - Reciprocity of the communication act's immanent obligations
  - Clarification of an intersubjective use of communication paths to gain an understanding for propagating intentional activities
  - Universalization of action-associated norms by the availability of generalized prepositions
  - Intersubjective commonality in the communicative exchange
  - Actions and intersubjective mandatory expectations of behavior: What are the prerequisites for cells to start the communication act?

Normative structures are pacemakers of evolution. 'Corrupt' rationalization processes evolving in a tumor may lead to decoupling from physiologic normative systems structures by establishing novel normative contexts. Communicative activities, i.e., the basic communicative patterns and endogenous purposive activities,

obviate the need to distinguish between structures and functions of tumor systems objects by introducing and describing scientifically accessible communication-associated validity claims that represent 'universal' communication rules given by the communicative context and by the physical-chemical or biologic constitution of systems objects. Validity claims in biological systems may be not primarily delineated from physical-chemical interactions but have to be systematically evaluated, particularly in evolving systems [51, 66].

- In biological systems, structure and function are basically mutually dependent. Normative structures connect structures and functions of systems objects in a time-dependent and space-dependent manner and in a distinct evolutionary systems context.
- The concurrence of the functional status of systems objects (i.e., nodes, pathways, etc.) and the requirements of the systems world leads to characteristic communication-derived pathologies [28]. The decoupling of the functional status of a systems object and the systems status is symbolized by
  - Deformations
  - Inconsistencies
  - Achilles' heels
  - Disturbed intersystemic exchange processes
  - Aggregated action effects
  - Altered intersystemic exchange [35]

Communication-derived evolutionarily developing pathologies are efficacious therapeutic targets for biomodulatory therapies (Chap. 2).

Evaluation of Communicative Expression

Communicative expression is an inherent biologic feature of a communication line or a tumor-associated communication tool. The availability of (1) universal presuppositions for the distinct communicative expression of a communication line or medium, (2) the universal reciprocity of the communication act's immanent obligations, (3) universal clarifications of an intersubjective use of communication paths, (4) the possible universalization of action-associated norms, and (5) the intersubjective commonality in the communicative exchange does not necessitate to evaluate communicative expression in a novel evolutionary context.

However, many years of experience with classically targeted and oncogene-addicted therapies have shown that multifold communicative expressions may be linked with identical communication lines and transmitters of information. Obviously, the acquired genetic background of a tumor decisively alters communicative expressions and rationalizations of normative notions [63, 64]. A novel communicative expression of an otherwise familiar communication line requires innovative diagnostic steps to keep close to the demands of personalized tumor therapy.

Table 20.4 Technical instruments for purposive-rational therapy

- ***Molecular imaging techniques***
- ***Epigenetic analyses from mononuclear peripheral blood cells***
- ***Cellular secretome analytics from serum or plasma:*** *Evaluation of identity and function of tumor-associated cellular compartments*
- ***Mapping and monitoring of tumor-associated disease traits***
- ***Multi-faceted clinical response analyses***
- ***Functional genomics:*** *Correlation of genetic patterns derived from whole genome analysis with rationalizations of tumor-immanent normative systems functions*
- ***Adaptive trial designs***

The present issue is to uncover communication technical obligations of tumor systems objects, which may cause novel tumor-immanent communicative expression and endogenous purposive-rational activities (Table 20.4). These obligations may situate the redirected communicative expression of systems objects in a novel arrangement of normative systems structures. Now, reductionist analysis steps should be supplemented by methodologies depicting tumor-immanent normative notions and their corresponding rationalizations via 'universal' surrogate parameters.

A further supplementary approach for discovering cancer therapeutics is emerging: Genomic analysis of tumor and stroma cells provides the key for understanding oncogenic interactions and for classifying tumor diseases molecular-biologically. To functionally interpret aberrant genetic and molecular-genetic patterns, the tumor systems' normativity and the respective rationalization processes have to be analyzed and compared with respective genetic patterns derived from total genome analysis. The next step requires correlating the molecular-genetic features of a histologically defined tumor type with arising rationalizations of tumor-immanent normative notions, evolutionary established interphases and with the non-oncogene-addicted networks. With this correlation step, the therapeutic repertoire broadens from originally single-track 'bottom-up' approaches identifying small-molecule and antibody therapeutics to primarily multi-track approaches targeting holistic communicative structures (rationalizations) by implementing non-normative boundary conditions ('top-down' approaches) (Chap. 2, 22).

Multiple evaluation strategies and methodologies may be implemented to uncover communication-derived pathophysiological processes in a clinical context:

- **Comparative uncovering of normative biological systems structures by modularly targeting tumor-associated systems structures**: (1) Cellular structures, (2) compartmentalized action norms, (3) decision maxims (nodes, hubs) may be analyzed by modularly targeting tumor-associated normative notions (i.e., inflammation, etc.)
- **Cellular secretome analytics** (in serum, plasma) may monitor identity and function of cellular compartments in the tumor before and during biomodulatory therapy [67]

- **Epigenetic analytics** (biological memory)
- **Clinical and laboratory monitoring of tumor-associated disease traits from patient and physicians' side**
- **Genetic patterns derived from whole genome analysis** must be correlated with rationalization processes for distinct tumor-immanent normative notions
- **Molecular imaging techniques**: Evaluation of rationalization processes, modular structures, and therapeutically redeemable tools of validity claims of systems objects [68]
- **Classic histology** may identify e.g., late-stage biomarkers [64]

Only by adequately understanding the multidimensionality of communicative systems structures, we are able to estimate the tool of rationalization processes and modular rearrangements, which may be redeemed by implementing non-normative boundary conditions (biomodulatory therapies) into an evolutionary developing systems context (Chap. 17). The novel technologies, i.e., cellular secretome analytics, molecular imaging techniques, and epigenetics, allow uncovering normative systems structures and their topological allocations against the background of distinct patterns of acquired chromosomal or molecular-genetic aberrations in both tumor and stroma cells.

Pre-therapeutic pathophysiological studies estimating communicative expression of communication lines, rationalization processes for distinct tumor-immanent normative notions should be routinely established in addition to the pathological and molecular-pathological studies already conducted (Chap. 15).

The attempt to curb tumor growth with purposive-rational therapies presupposes properly functioning (pathologic) systems and **intersystemic exchange programs**, which may be specifically redirected and redeemed on the basis of validity claims (rules). The availability of validity claims and knowledge about communicative structures invariably allow purposive-rational therapies. The success of biomodulatory therapies depends on the knowledge about the tumor systems objects' communicative expression, the modular knowledge of systems participators, the restrictions of the tumors' 'living world' and matching therapeutic resources.

Biomodulatory therapies implementing non-normative boundary conditions evolve the tumors' normativity. For therapeutic purposes tumor-immanent normative notions may be pragmatically selected. The selected normative notions must not coincide with conventional perceptions about normative structures and functions, the so-called 'hallmarks' of cancer [69, 70]. As shown, also osteoplastic processes in castration-resistant prostate cancer may be targeted.

Biomodulatory therapies, administered as fixed modules, contribute to the discovery and understanding of novel regulatory systems in tumor biology. Tumor-specific and subtype-specific rationalization processes indicate that e.g., inflammation-related activities are communicatively promoted and differentially adapted during tumor evolution [10].

Molecular imaging techniques frequently depict complex biologic systems behaviors, particularly the multifaceted tumor-immanent normative notions and respective rationalization processes. Respective surrogate parameters exemplarily

demonstrate the therapeutic accessibility to tumor-immanent normative functions, as shown e.g., by positron emission tomography (PET) ([71], Chap. 18).

The novel 'universal' surrogates mirroring the tumors' multifaceted normativity serve as starting points for stratifying and monitoring biomodulatory therapy approaches (Chap. 15). Biomodulatory therapies, which target selected normative systems structures by implementing non-normative boundary conditions, may place new molecular imaging technologies in a novel tumor-biological and tumor-therapeutic context.

Diverse biological, biochemical, molecular imaging techniques and therapeutic strategies may be applied to reconstruct the situative meaning of systems objects and their functions within rationalization processes. On the basis of the new pathophysiological knowledge, purposive-rational, primarily multi-track therapies may be designed for controlling tumor-associated disease traits. Validity claims of systems participators within an evolutionary constrained systems context and corresponding multifold novel intersystemic exchange processes among specifically rationalized normative notions are the basis for purposive-rational, primarily multi-track therapy approaches, their biomodulatory activity and therapeutic specificity.

Biomodulatory Activity for Diversifying Tumor Systems' Behavior: Pragmatic Function of a Communication-Derived Pathophysiology

Expressive attitudes of communication processes or normative systems structures may be recorded during endogenous purposive activities or external communicative interventions (non-normative boundary conditions). To design **purposive-rational therapies**, knowledge about the communicative expression of tumor-associated structures and functions is necessary beyond the simple appreciation of the presence or absence of (aberrant) communication lines and systems structures: One aim of biomodulatory therapies is to evolve systems for resolving tumor-associated disease traits and consecutively—via intersystemic exchange processes—to attenuate tumor growth.

The 'Present'

The **therapeutic purpose** of managing metastatic tumors usually conceals a unique intention, namely objective tumor response. Imaging techniques unveiling tumor size are commonly the backbone for monitoring the efficacy of tumor therapies: Our expectations in tumor therapies are minted by available technologies depicting tumor size. Tumor response is equated with tumor shrinkage. Necessarily, unique established response parameters are used that are slightly adapted to histological tumor types, i.e., the RECIST criteria [72].

Particularly small molecules and their multifold response patterns, as depicted by molecular imaging techniques (which do not primarily focus on measuring tumor size), have mixed up classical response parameters [73]: Tumor control must not be necessarily associated with simultaneous tumor shrinkage.

Biological response characteristics may be related to

- the drug or drug combination administered,
- to the way how drugs induce perturbations of tumor systems or
- how drugs evolve a tumor's normative systems (e.g., biological memory),
- and to the available cell death pathways.

The Future: Purposive Rationality in Cancer Care for Improving Outcome

Clinical and laboratory results derived from biomodulatory therapies necessitate developing tumor models, which are able to integrate normative systems structures, functions and decision maxims (hubs) as novel therapeutic targets and which depict these tumor-associated normative notions with clinically accessible patterns of biomarkers ('universal' surrogates).

Therapeutically induced functional changes of pathophysiologically relevant tumor-associated normative notions may be monitored by 'universal' surrogates for tumor response. '**Universal**' **surrogates** could serve as potential clinical or laboratory endpoints, which can be utilized to some degree independently of the administered drug or drug combination [10].

Physicians' purposive-rational therapy activities continue the tumors' steadily on-going evolution-promoting experiments Tumor systems aim at implementing novel rationalization processes to propagate tumor expansion. Therapeutically oriented approaches use the same available patterns of validity claims of tumor systems objects. However, these approaches focus on the redirection of tumor-associated systems features (robustness, rationalization processes, etc.) and now, may be comprehended as cellular therapies in situ.

Systems actors are subjected to constrains, which again restrict them to adapt attitudes facilitating distinct normative notions with respectively available communication lines. Attitudes for communicative actions are obviously more loaded with presuppositions (an indication of robustness) than the objectifying attitudes of strategic actors ('knowing that'), i.e., physicians administering a therapy that interferes with a holistic communicative tumor system (biomodulatory therapy). On the other hand, communicative interactions mediated through acts for reaching understanding within tumor systems' participators show a multi-facetted, but more restricted structure than strategically intended biomodulatory designed actions.

Clinically accessible, communication-derived surrogates (Chap. 2, 22) are important prerequisites for successfully establishing **adaptive trial designs**, which

avail communication-derived tumor pathophysiology [46]: Surrogates for successful modulation of normative systems structures and functions as well as adaptive trial designs allow the implementation of tumor stage-specific and tumor systems-specific therapeutic resources [47].

When therapy-relevant normative structures, functions and hubs are scientifically separated in ever-growing numbers for facilitating tumor control by disturbing or evolving normative systems structures, novel systems-directed therapies may be designed to significantly broaden and personalize therapeutic options.

Physicians must not primarily draw any more on the myriad of **target-specific surrogates** (reductionist, 'bottom-up' therapy approaches), which are supplemented by the small tool of clinical surrogates (Chap. 22). Also the paradigm can be relativized that tumor shrinkage at best represents tumor response.

By contrast, we advance changes of tumor systems-related surrogates and their possible link to cell death-inducing pathways as valid surrogates for response [8, 10, 20, 74]. For establishing purposive-rational therapies, we may desist from the currently established ontological thinking, which tethers response evaluation exclusively at available morphometric imaging technologies.

As shown, selected 'purposive-rational' targets for tumor therapy, corresponding to selected rationalizations of tumor-associated normative notions, can be directly linked now with phenomenological and clinically accessible tumor-associated disease traits (Fig. 20.2). For therapeutical issues, scientists are not any more compelled to exclusively conduct 'ontology graph mapping' or to trace the 'logic' of tumor cells [75]. These approaches would be equivalent with efforts for conclusively generating the tumors' normativity from single normative notions (Chap. 10)!

Besides uncovering rules of physical-chemical interactions of the tumors' systems participators, the task of scientists is to promote the reconstruction of principle generative conditions (idealized presuppositions of communication) that constitute universal communicative structures accounting for compelling reciprocal communication and understanding by introducing novel technologies, e.g., cellular secretome analytics, biomodulatory therapies, molecular imaging techniques, epigenetics, etc. [67, 68].

Study Endpoints

The important therapeutic turn entails the decoupling of study endpoints from virtual normative expectations of the physicians that tend to focus on tumor shrinkage and the induction of complete remission [76, 77]. The value of these study endpoints is frequently overestimated, while other factors, such as toxicity, quality of life, costs, and emancipatory interests propagating reductionist therapy approaches, are being ignored [35, 78]. For most metastatic tumor diseases, the all-important composite endpoint remains improved overall survival at a high level of quality of life [78, 79]: The introduction of purposive rationality in therapy of metastatic tumors, tumor control via selected redirection and modulation of tumor-associated disease traits, adequately meets both surrogate endpoints ([8, 34], Chap. 2).

The novel therapy strategy necessitates the functional evaluation and therapeutic appreciation of the situational endogenous purposive activities of systems objects within rationalization processes and consecutive, the clinical use of an evolution-adjusted tumor pathophysiology. The reconstruction of the communication-derived pathophysiological status of tumor systems participators facilitates biomodulatory therapies as well as the study of therapy-mediated changes of the tumors' normativity. Probably, also novel links to still available or restored apoptosis-inducing pathways may be uncovered [80, 81].

Differentially employed strategic interactions (implementation of non-normative boundary conditions) offer the possibility to therapeutically exploit the situational communicative background knowledge of tumor systems participators on the basis of validity claims. Clinical or laboratory parameters, 'universal' study surrogates, reflecting changes in tumor-associated disease traits may be selected [20].

Selection of purposive-rational therapy approaches takes into consideration normative tumor-immanent resources, their rationalizations, topology and functionality, predominant clinical disease traits and corresponding laboratory surrogates, links to tumor growth promoting attitudes, as well as toxicity profiles of biomodulatory drug designs (multi-track approaches).

Multi-facetted ethical requirements, particularly differential palliative aspects, can be met by establishing therapies adapted to tumors' stage-related communicative pathophysiology and by modulating situationally arising tumor-associated disease traits for attenuating tumor growth (Fig. 20.1, 20.2).

Discussion

Results from multiple phase II trials implementing non-normative boundary conditions to redirect and modulate selected tumor-immanent normative notions, also disease traits, demonstrate that these approaches have the capacity to impressively diversify the treatment aims for palliative care (Table 20.5, Fig. 20.3). Exploiting tumor-immanent communication-derived pathologies allows multifaceted ways of tumor control: Growth control can be achieved by selected combined modulation of tumor-associated inflammation, immunmodulation, angiogenesis, and osteoplastic activities etc. (Chap. 2).

Therapeutically efficacious access to a broad variety of metastatic tumors via purposive-rational therapy approaches shows that the current meaning of the term 'biological system' is often rather narrowly conceived: Biologic systems should be extended by their communicative substance, which may be presented by communication-derived tumor pathophysiology. Applying communication-based tumor models facilitates the implementation of validity claims of systems participators [48, 51], rationalization processes [35], and normative systems structures [28] as biomodulatory accessible tumor targets. Now, the term 'biological system' may be understood in a novel pragmatic and therapeutically relevant way.

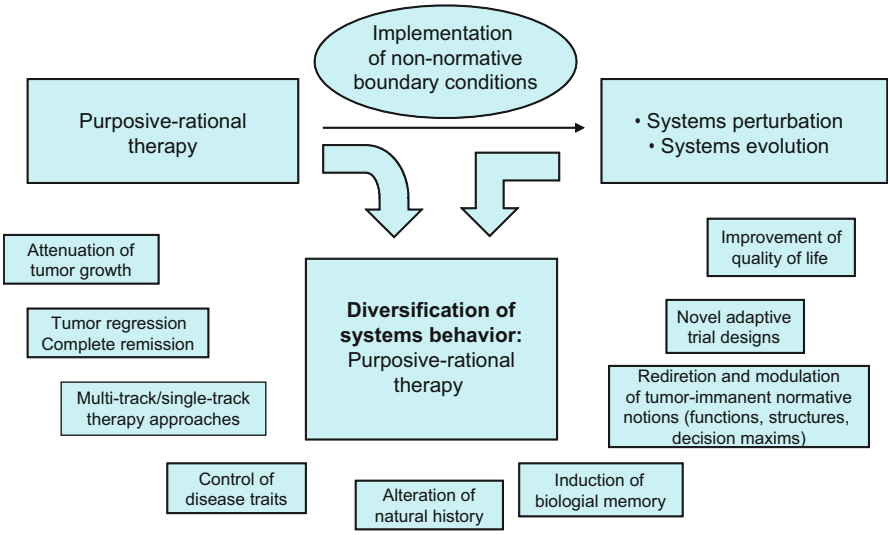

Fig. 20.2 Purposive-rational therapy: Readapting therapy endpoints

Table 20.5 Purposive-rational therapy

- *Addressing the meaning of a systems objects: From theme-dependent to evolution-adjusted therapy*
- *No monoactivity of the single drugs necessary (primarily multi-track approaches)*
- *Implementation of*
  - *non-normative boundary conditions (biomodulatory active drugs in combination)*
  - *modular knowledge (on tumor site)*

Therapeutically verifiable tumor models implementing communicative rules (**cellular therapy in situ**) are helpful to answer questions about pre-therapeutic personalized tumor evaluation (Chap. 15). Diagnostic evaluation of evolutionary accessible communication tools impacts the selection of molecular targets and the planning of so-called 'biology-driven' clinical trials, which aim at achieving release of tumor-associated disease traits followed by tumor control (Fig. 20.2): Currently, the designs of clinical trials focus more and more on meaningful patient-oriented outcomes because of strained health care systems, the availability of insufficient clinical trial options, and the increasing number of elderly patients suffering from metastatic cancer. Therefore, tumor models and perceptions of tumor systems biology should be specified to further improve options for personalized tumor therapy,

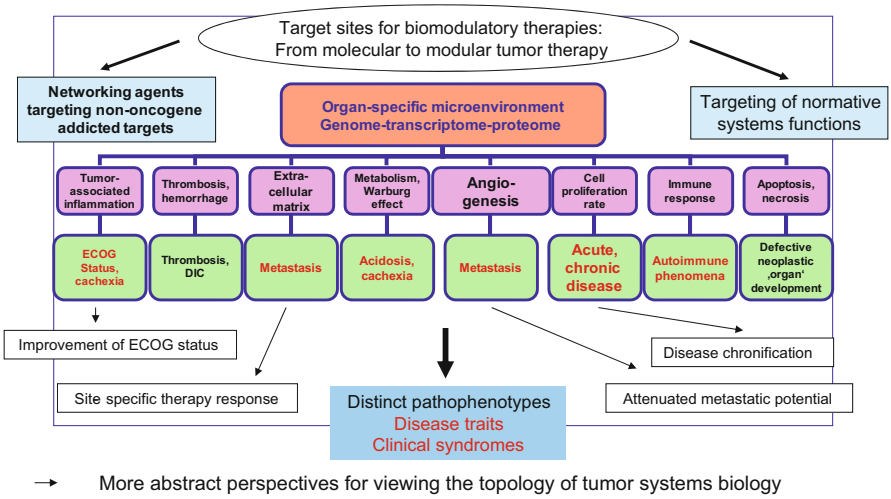

Fig. 20.3 Implementation of non-normative boundary conditions in normatively structured tumor systems for attenuation of tumor-associated disease traits and tumor growth

particularly to diversify palliative care in order to simultaneously improve symptom control and overall survival (Fig. 20.3).

'**Among the many consequences that follow from what has been said'**—also in the present paper—'this in particular can be underscored, that knowledge only exists (is for real) and may be presented as science **or** as system', Georg Wilhelm Friedrich Hegel claimed in his introduction of 'The Phenomenology of the Spirit'. In a current interpretation, the reductionist scientific world is seen to be opposed by a philosophical and ontological systems world that, in Hegel's views, is based on particular stages of consciousness.

The systems world and the scientific world, which seemed to be concurrent and imposed to one another in the 19th century, are now consolidated—also in the biologic setting—by means of reconstructive activities on the action of biomodulatory therapies. For tumor control, systems-directed therapies may, on purpose, metronomically implement non-normative boundary conditions into a tumor system and its host over long time periods [8]. Now, reconstructive activities on communication acts—originally methodologically applied in their respective field by later philosophers, social scientists, and linguists—are appropriate to analyze the constitution of tumor-associated communicative processes (normative structures) and to elucidate the meaning of communication lines within a situative tumor-related communication tool, i.e., a tumor's living world.

A plethora of tumor-associated disease traits are associated with pro-inflammatory processes, i.e., ECOG status, cachexia, fatigue, depression, anxiety, cognitive impairment. Combined modular therapeutic access, that means concerted redirection and modulation of tumor promoting normative notions, has shown to

decisively improve palliative care in two respects, inflammation-related symptom control (attenuation of disease traits) and tumor control ([8, 10], Chap. 2). Inconsistencies of outcome data with regard to drugs commonly used for inflammation control in metastatic tumors, i.e., glucocorticoids, NSAIDs, anti-Il-6 monoclonal antibodies etc., may be caused by (1) a tumor type- and stage-dependent differential constitution of rationalization processes supporting inflammation [10, 82] and (2) a currently one-dimensional consideration of tumor-related pro-inflammatory processes.

Our data have shown a multifaceted and multi-dimensional constitution of tumor-related pro-inflammatory processes and differential therapeutic accessibility with combined modularized therapies ([8, 10], Chap. 2). Therefore, we conclude that rationalization processes are principally important targets for tumor and symptom control, and not only the traditionally targeted pro-inflammatory communication lines and transcription factors, i.e., Il-6, Il-1, TNFalpha, NFkappaB, STAT3 etc. ([83], Chap. 17). Rationalization processes of tumors are preferably accessed for therapeutic purposes in a primarily 'multi-track' manner ([10], Chap. 22). Combined modularized approaches are highly specific and should be adapted to the constitution of rationalization processes. These 'top-down' approaches, including (combined) transcriptional modulation, seem to be an efficacious novel therapeutic option, even in tumor types with known intrinsic resistance to chemotherapy ([27, 34, 52], Chap. 2–5, 22).

Further, selective therapeutic accessibility of tumor-promoting normative notions and developing disease traits via respective rationalization processes indicates that we can broaden the therapeutic repertoire in a novel way, to cope with the claims for a personalized therapy of metastatic tumor disease. Mostly all-oral, combined modularized therapies meet important therapeutic aims: Quality of life can be maintained or improved, and long-term disease control is possible also in elderly and medically non-fit patients (Table 20.1) (Chap. 2–5, 19).

The task of scientists is to employ their expertise in the interest of personalized tumor therapies with the dual aim of appropriately isolating communication-derived pathophysiological resources for purposive-rational biomodulatory therapies and of situating these normative systems structures for therapeutic purposes. Then, a most obvious therapeutic aim may be achieved, i.e., stemming 'corrupt' rationalization processes in a tumor for (1) protecting non-regenerative tissue resources and for (2) attenuating tumor-associated disease traits (purposive-rational palliative care), before the ever desirable aim of (3) objective tumor response can be accomplished.

The intricate problem of achieving relief of tumor diseases is closely linked to the evolutionary development of a tumor and the missing consistent evaluation of communication-pathologic systems stages, which are useful for guiding therapy strategies and for the selection of therapy resources. The quasi ontological motivation for using reductionistically-derived therapeutic approaches is challenged now by systems-directed and purposive-rational therapies.

Scientific studies on the expressive status of communication tools in tumors should be promoted in order to more successfully configure scientific interventions for inhibiting tumor growth. Themes and questions in connection with appropriate

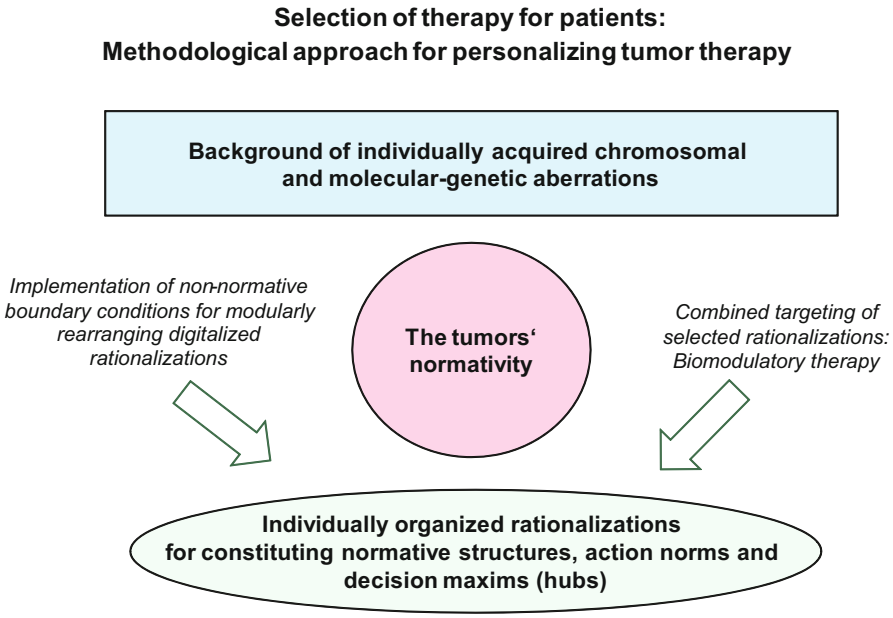

Fig. 20.4 'Top down' approaches: Organizing principle are tumor-immanent normative notions (structures, functions, hubs), which may be specifically targeted with combined modularized therapy approaches

biomodulatory therapy strategies should be elaborated by novel methods or should foster the hypothesis-triggered utilization of available methods.

On the basis of methodological-driven clinical trial designs, a novel purposive-rational study endpoint discussion ('universal' surrogates) may be encouraged, which is oriented at (1) evolutionarily developing and scientifically accessible tumor systems features, (2) the facticity of propositional aspects of communication, (3) the therapeutic specificity of therapies implementing non-normative boundary conditions into tumor systems.

Then, scientists may accomplish a shift of paradigms, namely from understanding intentional reductionist systems, which focus on the presence or absence of targets and communication lines (single-track approaches), to the evaluation and therapeutic utilization of normative systems structures (how to control systems-associated processes with therapy modules to achieve response); from object-associated to situation-associated systems interpretations (biomodulatory therapies in metastatic tumors); and from an intentional (reductionist) to an evolution-based systems explanation (systems behavior and response) [35] (Fig. 20.4).

The work of scientists entails the task of describing what they can see; here, it is tumor control via non-oncogene-addicted targets initiated by biomodulatory therapies [8]. Scientists with predominant emancipatory interests are endeavored to care for respective justifications, about what they have done or intend to do [35].

If scientists also happen to be experts, they try to simultaneously find the ideological packing and marketing strategy to demonstrate that their strategies are right so that any doubts are dispelled from the beginning: Commonly, therapeutic purposes are aligned to virtual, often technology-guided paradigms based on simple inter-subjective agreements. Thereby, science and the systems world are dissociated in Hegel's sense by imposing theoretical, quasi ontological systems (classic study endpoints) on top of the arduously accessed scientific world.

From oncologists to clinical biologists Researchers should facilitate the scientific evaluation of tumor-inherent, stage-related normative structures by experimental or therapeutic (long-term) implementation and adaption of appropriate non-normative boundary conditions. The aim remains to therapeutically meet situational communicative tumor pathophysiology, which basically suggests that systems objects are frequently subjected to novel validity claims in evolving tumor systems. When, similar to (molecular)-pathology, communication-based tumor pathophysiology is really clinically established, further progress will be made in personalizing therapies for patients with metastatic tumor disease and in more frequently converting cancer into a purposively manageable (extension of palliative care resources) or curable disease: Biomarker-driven adaption of therapy according to normative tumor systems response (adaptive trial designs on the basis of 'universal' surrogates) will change oncologists into clinical biologists: In future, therapies will be selected for patients and not patients for therapy (Table 20.4) (Fig. 20.4).

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Chapter 21
Targeting a Hallmark of Cancer: Simultaneous Inflammation and Tumor Control for Palliative Care in Metastatic Cancer

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Abstract Clinical attenuation of the inflammation-related symptom complex, comprising cachexia, anorexia, fatigue, depression, neuropathic pain, anxiety, cognitive impairment, sleep disorder and delirium, is of extreme importance to improve palliative care in metastatic cancer. Laboratory data provide strong evidence that pro-inflammatory processes are related to multifold clinical disease traits of metastatic cancer and that pro-inflammatory processes are even drivers of tumor progression; however, approaches for attenuating pro-inflammatory processes with single-track or combined single-track therapies repetitively show—as reviewed here—controversial data about the benefit of anti-inflammatory therapies. What are the reasons for the conflicting data? Recently published clinical data indicate that inflammation control in different metastatic tumor types requires differential therapeutic access, because pro-inflammatory processes are multifacetedly rationalized. Beyond the control of inflammation-related clinical symptoms (improved ECOG status) inflammation control is associated with attenuation of tumor growth. Tumor-specific rationalizations provide highly specific targets: Inflammation—as a ubiquitous tumor-associated normative notion—may be constituted in multiple ways, supported by quite different cell types of the tumor compartment. Dependent on the physical constitution of tumor-associated rationalization processes the interfaces to other rationalization processes, e.g., immune response or angiogenesis become displaced and form an obstacle for single-track approaches. Combined modularized, primarily multi-track therapies account for such displacements.

Introduction

Patients with metastatic tumors often suffer from severe symptoms, which are related to tumor-associated pro-inflammatory processes. Clinical symptoms do not necessarily correlate with tumor size or dissemination: There are patients with extensive tumor masses without relevant inflammation-related symptoms, but others with hardly detectable metastases suffer from intolerable symptoms and deteriorate within weeks.

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A clinically assessable symptom complex is associated with inflammatory processes caused by metastatic tumors, comprising cachexia, anorexia, fatigue, depression, neuropathic pain, anxiety, cognitive impairment, sleep disorder and delirium [1, 2].

Attenuation of inflammation-related symptoms is extremely important to improve palliative care in metastatic cancer. Despite the observation that inflammation associated clinical symptoms are highly diversified, the approaches to access the symptoms are uniform single-track or combined single-track therapies irrespective of the metastatic disease and the individual clinical presentation of symptoms.

Single-track approaches are commonly delineated from the observation that inflammation related symptoms are frequently associated with elevated levels of proinflammatory cytokines, particularly IL-1α, IL-1β, IL-6, TNFα, VEGF and others [3, 4]. Correspondingly, drugs have been introduced for inflammation control, which inhibit the activity of pro-inflammatory cytokines. To date, however, a convincing therapeutic breakthrough could not be achieved (Table 21.1 and 21.2).

Glucocorticoids

Glucocorticoids are very potent anti-inflammatory agents that inhibit the synthesis of many pro-inflammatory cytokines by transcriptional regulation. Therefore, they are widely used for symptom control in patients with metastatic cancer, particularly for the treatment of nausea, vomiting, depression, fatigue, anorexia and cachexia [32].

In 1985 a randomized, double-blind crossover trial in 40 terminally ill cancer patients compared oral methylprednisolone (daily dose of 32 mg) against placebo. The study end points were pain, psychiatric status, appetite, nutritional status and daily activity, and performance status [33]. Methylprednisolone led to increasing appetite and daily activity, and decreasing depression and analgesic consumption without serious toxicity and was preferred over placebo.

In 1999, a single arm observation trial enrolled fifty palliative home care patients. They received dexamethasone in doses ranging from 4–16 mg. Symptom intensity was assessed by the palliative home care team and graded on a scale of 0 (not at all) to 3 (severe). Dexamethasone was found to be effective in anorexia, weakness, headache, and nausea and vomiting [34].

A randomized controlled trial (51 patients) primarily assessed the antiemetic effects of dexamethasone 20 mg/day in patients with chronic nausea, refractory to metoclopramide (secondary endpoints: appetite, fatigue, and pain) [35]. The data revealed no difference between the dexamethasone and placebo groups: improvement in appetite and fatigue were observed on Day 3 and Day 8 in both groups as compared with baseline. Pain, vomiting, well-being, and quality of life remained unchanged in both groups at both time points.

The evaluation of corticosteroids (dexamethasone 8 mg orally) as adjuvant to opioid therapy for cancer pain (76 advanced cancer patients), indicated no differences in pain intensity, opioid consumption [36]. However, corticosteroids persistently

Table 21.1 Relation of pro-inflammatory markers and clinical syndromes (modified according to [1])

Cancer type	Inflammation-related changes/Disease traits
	Cachexia
Random	Skeletal muscle of cancer cachectic patients exhibited increased expression and activity of the TNF-a signaling pathway [5]
Lung cancer	Increased level of serum TNF-a in cachexia patients compared with those without cachexia [6]
Pancreatic cancer	Serum from cachectic patients exhibited an elevated IL-6 [7]
	Fatigue
Random	A significant correlation between IL-6 and fatigue in cancer patients [8]
Colorectal cancer	Significant correlation between IL-6 and fatigue in patients [3, 9]
Breast cancer	Exhibited significant increase in IL-6 following stimulation with LPS [10]
Random	A significant correlation between IL-6 and fatigue in cancer patients [11]
Lymphoma	Treatment of patients with humanized monoclonal antibodies against IL-6 caused a relief from fatigue [12]
Non-small cell lung cancer	Worsened the symptoms in the patients undergoing CXCRT [13]
Breast cancer	A significant increase in TNF-a following stimulation with LPS [10]
	Depression
Random	Significant correlation between plasma IL-6 and depression in cancer patients [14]
Ovarian cancer	Close association between plasma IL-6 and facets of depression [15]
Leukemia	Significant correlation between IL-6 gene expression and depression [16]
Pancreatic, esophageal, breast cancer	Exhibited higher concentrations of IL-6 compared with normal subjects and cancer patients without depression [17]
	Anxiety
Renal cell carcinoma, melanoma	Patients treated with a combination of IL-2 and INFa-2b had enhanced anxiety [18]
	Cognitive impairment
Leukemia, multiple myeloma	Patients exhibited higher level of IL-6 in association with poorer executive function [19]
	Sleep disorders
Non-small cell lung cancer	Patients undergoing CXCRT exhibited a strong correlation between serum IL-6 and severity of sleep disorder [13]
Random	Poor sleep in cancer patients was associated with elevated VEGF [20]

Table 21.2 Relation of pro-inflammatory markers and disease traits in animal models (modified according to [1])

Cytokine	Cancer type	Inflammation-related changes/Disease traits
		Cachexia
TNF-a	Hepatoma	Rats bearing AH-130 hepatoma cells showed enhanced protein degradation in gastrocnemius muscle, heart and liver that was diminished by administration of anti-TNF-a [21]
	Lung cancer	Wild-type mice implanted with LLC had enhanced loss of fat and muscle concomitant with activation of the ubiquitin–proteasome pathway that was absent in TNF-a receptor type I deficient mice [22]
	Colon cancer	Mice bearing colon-26 adenocarcinoma exhibited cachexia in association with a significant increase in TNF-a [23]
IL-6		
		Mice bearing colon-26 adenocarcinoma exhibited cachexia in association with a significant increase in IL-6 [23]
IL-1b	Squamous cell carcinoma	Wild-type mice had less lean body mass and fat mass and increased IL-1b compared with the TLR-non-functional mice [24]
		Anorexia
IL-1a	Sarcoma	Tumor-bearing rats exhibited negative correlation between CSF IL-1a and food intake [25]
		Intrahypothalamic microinjections of IL-1ra were associated with an improvement in food intake in tumor-bearing rats [26]
IL-1b	ProstateCancer	Rats bearing prostrate tumor cells developed anorexia that was associated with enhanced IL-1b in cerebellum, cortex and hypothalamus regions of the brain [27]
		Fatigue
IL-6	Mouse model	Exercise-induced IL-6 decreased the TNF-a levels in skeletal muscle of TNF-a transgenic mice and added to the beneficial effect of exercise against cancer treatment-related fatigue [28, 29]
IL-1b	Rats with tumors; Prostate cancer	Increase in IL-1b was correlated with spontaneous pain in female rats [30]
		Rats injected with prostrate cancer cells exhibited an increase in IL-1b and bone pain [31]
TNF-a	Rats with tumors	Increase in TNF-a was correlated with spontaneous pain in female rats [30]

decreased opioid-related gastrointestinal symptoms and improved the sense of well-being without relevant toxicity for these patients showing a limited survival of only 33–37 days.

Side effects of glucocorticoid therapies are not neglectable: High glucocorticoid doses and long treatment periods lead to severe complications in a relevant portion of patients: In a retrospective analysis of patients, who were previously irradiated for brain metastasis, 89 patients received dexamethasone in a dosage of at least 12 mg a day [37]. Overall, in 14 of the 89 patients (15.7 %) a complication of steroid therapy developed in form of peptic ulcer disease, steroid myopathy or diabetes mellitus (or a combination of these).

In a retrospective analysis of 88 patients with brain metastases treated with dexamethasone prior and during whole brain radiotherapy the most common side effects were increased appetite or weight gain in 46 %, proximal muscle weakness in 28 %, insomnia in 24 % and gastrointestinal symptoms in 20 % of the patients [38]. Many patients received high doses of steroids for considerable periods of time. Another frequent steroid side effect is the loss of bone mass, which causes osteoporosis and vertebral fractures mainly after long-term steroid treatment but sometimes also appearing after short courses of steroids [39].

Therapy recommendations According to the controversial data, there is no "existential impact" [40] for a general recommendation for starting corticosteroid treatment as symptom control in all patients with advanced metastatic cancer. The indication for initiating or maintaining a corticosteroid therapy in patients with metastatic cancer should be drawn on an individual basis considering the symptom burden, the expected life time and duration of the steroid therapy and the response to therapy on day 3–5. As corticosteroids have shown to increase appetite and improve a number of other symptoms only transiently without changing caloric intake or nutritional status, a brief course of corticosteroids may provide short-term symptomatic effects in patients with short expected survival [41].

Progestational Drugs/Megestrol Acetate

Megestrol acetate is a synthetic progesterone derivative, which is a potent agonist of the progesterone receptor causing suppression of the gonadotropin secretion and—via negative feedback—down-regulation of the hypothalamic-pituitary-gonadal axis resulting in a strong antiandrogenic and antiestrogenic effect. Additionally, megestrol acetate is a weak agonist of the glucocorticoid receptor and inhibits the secretion of multiple proinflammatory cytokines like IL-1α, IL-1β, IL-6, and TNF-α.

For patients with a longer expected survival time, megestrol acetate or other progestational drugs have been found to increase appetite, caloric intake, and nutritional status in a number of studies [41].

A randomized phase III clinical trial compared a progestational agent (megestrol acetate 800 mg/day p.o.), a corticosteroid (dexamethasone 0.75 mg qid), and an anabolic corticosteroid (fluoxymesterone 10 mg p.o. twice daily) over a longer

period with monthly observation: 475 assessable patients suffered from cancer anorexia/cachexia [42]. Megestrol acetate and dexamethasone improved the appetite and the non-fluid weight status to a similar degree, with dexamethasone causing more corticosteroid-type toxicity and a higher rate of drug discontinuation because of toxicity and/or patient refusal than megestrol acetate. Megestrol acetate, however, led to a higher rate of deep venous thrombosis. Fluoxymesterone was clearly inferior for the treatment of cancer anorexia/cachexia with significantly less appetite enhancement and an unfavorable toxicity profile.

A randomized three arm trial in 469 advanced cancer patients with loss of appetite or weight compared the efficacy of single agent megestrol acetate (oral megestrol acetate 800 mg/d liquid suspension plus placebo) or dronabinol (oral dronabinol 2.5 mg twice a day plus placebo) with the combination of both agents [43]. Significantly more megestrol acetate-treated patients reported improvement of appetite and weight gain compared with dronabinol-treated patients. Combination treatment resulted in no additional benefit in appetite or weight compared with megestrol acetate alone. Except of an increased incidence of impotence among men who received megestrol acetate, toxicity was comparable. The study revealed no correlation between changes in serum IL-6 and appetite, weight gain or global quality of life, providing no evidence that megestrol acetate down-regulates IL-6 in patients with cancer-associated anorexia and weight loss [44].

Megestrol acetate leads to a relevant number of severe side effects. In addition to thrombembolic disease in 5 % of megestrol acetate-treated patients [42], megestrol acetate treatment may also suppress the gonadal axis in male patients with cancer, resulting in symptomatic androgen deficiency and subsequently leading to a higher symptomatic burden due to adrenal insufficiency and hypogonadism [45].

Hypogonadism in cancer cachexia was investigated in a retrospective study of 98 consecutive male cancer patients [46]. The combination of low testosterone and elevated C-reactive protein (CRP) was associated with poorer prognosis. Elevated CRP levels were associated with increased symptom burden and decreased survival. Low testosterone was associated with decreased survival and correlated inversely with CRP levels, dyspnea, and insomnia.

No therapy recommendations According to the literature available in advanced cancer patients, recommendations cannot be provided for the administration of megestrol acetate.

Melatonin

Melatonin (*N*-acetyl-5-methoxytryptamine), a hormone produced by the pineal gland is involved in the regulation of the sleep-wake cycle. Recent laboratory investigations showed that melatonin (*N*-acetyl-5-methoxytryptamine) inhibits the DNA binding of activated NF-κB in androgen receptor positive prostate epithelial cells [47].

Further pre-clinical data have shown anti-inflammatory and anti-proliferative activity: In the human vascular smooth muscle (VSM) cell line CRL1999, stimulated

by lipopolysaccharide (LPS), treatment with melatonin significantly inhibited the production and expression of TNF-α and interleukin (IL)-1β, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase, prostaglandin E(2) (PGE2), and nitric oxide (NO). The suppression of these proinflammatory mediators by melatonin was caused by inhibition of MAPK, NF-κB, c/EBPβ, and p300 signaling [48]. In human MDA-MB-361 breast cancer cells melatonin significantly suppressed cell proliferation and induced apoptosis accompanied by the melatonin-mediated inhibition of COX-2, p300, and NF-κB signaling [49].

A meta-analysis including 21 clinical trials that randomized patients with solid tumors to chemotherapy treatment plus/minus melatonin showed improved response and reduced 1-year mortality for the melatonin group [50]. Melatonin also significantly reduced asthenia, leucopenia, nausea and vomiting, hypotension, and thrombocytopenia.

No therapy recommendations Although there is extensive literature about melatonin as an agent with potent anti-inflammatory, antioxidant, antiproliferative, immune-modulating, and hormone-modulating properties, it seems to have little or no effect on cachexia, anorexia or fatigue, suggesting that these cancer symptoms are not caused by "inflammation" in general, but by specific rationalizations of tumor-promoting inflammation or multiple rationalization processes for tumor-immanent normative notions, i.e., angiogenesis, immune response etc.

Melatonin for the treatment of cancer-related symptoms will be investigated by a randomized, placebo-controlled, double-blinded trial on the effect of melatonin on depression, anxiety, cognitive function and sleep disturbances in patients with breast cancer (MELODY trial) [51].

NSAIDs/COX-2 Inhibitors

In 1994 Lundholm et al impressively showed the link between inflammation-associated symptom control and improved survival. Anti-inflammatory treatment in patients with insidious or overt malnutrition due to various kinds of generalized solid tumor diseases not only improves the symptom burden but also results in a significantly prolonged survival [52–54]. One hundred thirty-five patients were randomized to receive placebo, prednisolone (10 mg twice daily), or indomethacin (50 mg twice daily) p.o. until death. Patients receiving anti-inflammatory treatment with indomethacin or prednisolone had a stable Karnowsky index, while the index declined in placebo-treated patients. Mean survival in the indomethacin group was prolonged to 510 ± 28 days compared to 250 ± 28 days in the placebo group. Additionally, patients in the indomethacin group suffered from less pain and consumed less additional analgetics compared to the other patient groups.

In a retrospective case control analysis of weight-losing cancer patients treated with long-term cyclo-oxygenase (COX) blockade (indomethacin), the initially elevated resting energy expenditure—as compared to undernourished non-cancer patients—was significantly reduced by indomethacin treatment, accompanied by

a decreased heart rate, increased systolic blood pressure, more preserved body fat and by a decrease in systemic inflammation (C-reactive protein, erythrocyte sedimentation rate) [55].

A prospective phase II trial studying the COX-2 inhibitor celecoxib (300 mg/day for 4 months; n = 24 patients) in advanced cancer patients showed a significant increase in mean body mass and improvement of grip strength, quality of life, performance status and a significant decrease of TNF-alpha. Patient compliance was good and no grade 3/4 toxicities occurred [56].

Therapy recommendations A systematic review of the literature on the use of non-steroidal anti-inflammatory drugs for the treatment of cancer cachexia—comprising four randomized controlled trials—revealed some positive effects on quality of life, performance status, inflammatory markers, weight gain and survival, but insufficient data for approving effectiveness of NSAIDs as single-track therapy for the treatment of cachexia in advanced cancer [57]. This is mainly due to a lack of uniformity of inclusion criteria across the studies [58]. However, as cachexia is a multifactorial syndrome, which may be differentially constituted in different histological tumor types; therapeutic approaches targeting a single factor need to be urgently supplemented by multi-track approaches.

Direct or Indirect Targeting IL-6 or TNF-α

Anti-Interleukin-6 Monoclonal Antibodies

Elevated IL-6 levels in serum are correlated with debilitating lung-cancer-related symptoms such as fatigue, thromboembolism, cachexia and anemia, resulting in a poor prognosis. Therefore, targeting IL-6 with a monoclonal antibody could be efficacious for the treatment of the pro- inflammatory microenvironment in lung cancer: In phase I and II trials the humanized monoclonal anti-IL-6 antibody ALD518 is well tolerated and improves NSCLC-related anemia and cachexia [59].

With regard to survival, another anti-interleukin-6 chimeric monoclonal antibody siltuximab (CNTO 328) was investigated in a randomized phase II trial in patients with metastatic castration-resistant prostate cancer receiving mitoxantrone/prednisone with or without siltuximab after a docetaxel-based chemotherapy [60]. While siltuximab plus mitoxantrone/prednisone was well tolerated and induced a more intensive decrease of serum C-reactive protein levels, enrolment had to be prematurely terminated due to a much shorter progression free survival in the combination treatment arm.

Siltuximab combined with docetaxel in an open-label, dose-escalation, multicenter, phase I study in patients with metastatic, progressive castration-resistant prostate cancer showed preliminary efficacy with respect to PSA response, with a confirmed ≥ 50 % PSA decline in 23 of 37 patients [61]. However, the combination caused a significant burden of grade ≥ 3 adverse events. Symptom control (cachexia, fatigue, etc.) was not the purpose of this trial.

A third humanized monoclonal antibody targeting the human interleukin-6 (IL-6) receptor initially introduced for the treatment of rheumatoid arthritis was investigated in multi-centric Castleman disease (28 patients enrolled). This disease is characterized by systemic lymphadenopathy and constitutional inflammatory symptoms. The clinical presentation is caused by dysregulated overproduction of interleukin-6 [12]. Antibody treatment consistently resulted in diminished fatigue and control of chronic inflammatory symptoms as well as alleviated lymphadenopathy in all inflammatory parameters. Additionally a significant increase in hemoglobin, albumin, and total cholesterol levels, high-density lipoprotein cholesterol values, and body mass index could be observed with only mild to moderate adverse events.

Blocking TNF-Alpha

Tumor necrosis factor-alpha (TNF-alpha) presumably contributes to the development of anorexia/cachexia and fatigue in advanced cancer patients. The anti- tumor necrosis factor alpha antibody infliximab was investigated in a pilot study enrolling seventeen eligible outpatients with fatigue [62]. The majority of these patients had modest improvements in serum C-reactive protein, erythrocyte sedimentation rate, or leptin concentrations. Subjective functional improvement was found in 9 of 14 patients concerning fatigue severity scale, in 3 of 15 patients concerning Karnofsky performance status, and in 7 of 15 patients concerning total hospital anxiety and depression scale. However, infliximab treatment was accompanied by five serious adverse events including two serious infections, which were possibly related to treatment.

Other approaches targeting TNF-alpha were performed with etanercep, a dimeric fusion protein consisting of the extracellular ligand-binding portion of the human 75-kilodalton TNF receptor linked to the Fc portion of human immunoglobulin [Ig] G1. In a placebo-controlled double blind trial of etanercept for improving the cancer anorexia/weight loss syndrome, 63 patients with an incurable malignancy, loss of weight and/or appetite, and/or a far reduced daily intake were randomly assigned to receive etanercept (25 mg s.c. twice weekly) versus placebo [63]. Both groups showed only minimal weight gain of less than 10 % of their baseline weight, negligible appetite improvement and comparable median survival.

With the aim of disease stabilization, in another non-randomized, open-labeled phase II study [64] (16 patients with progressive metastatic breast cancer and refractory to conventional therapy) patients were treated with etanercept 25 mg s.c. twice weekly (median treatment duration 8.1 weeks). Immune-reactive elevation of TNF and consistently decreased interleukin-6 and CCL2 levels were found compared with base-line values (serial blood samples), but only one patient had a short period of disease stabilization outbalanced by frequent and relevant side effects (injection site reactions in 6 patients, fatigue in 5 patients, loss of appetite in 2 patients, and nausea, headache, and dizziness in 1 patient each).

For achieving disease stabilization a phase IB trial [65] was conducted in 30 patients with recurrent ovarian cancer. This study investigated the therapeutic

efficacy of etanercept at a dose of 25 mg twice weekly versus three times weekly until disease progression. In contrast to a previous survey, six patients achieved prolonged disease stabilization (two patients with etanercept application twice weekly, four patients with three times weekly). Again a significant elevation of immune-reactive TNF and significant decreases of interleukin-6 and CCL2 levels were found in all patients (pretreatment compared with end of treatment). Injection-site reactions and fatigue were frequent side effects.

These contradictory results on etanercept treatment may be explained by differences in the constitution of pro-inflammatory processes in breast or ovarian cancer; or the clinical symptoms are caused by multiple rationalizations for tumor-promoting normative notions, i.e., angiogenesis, immune response etc.

Thalidomide

Thalidomide was developed as sedative drug with additional antiemetic properties and is meanwhile widely used in cancer treatment due to its anti-angiogenetic, anti-inflammatory, immune-modulatory and pro-apoptotic effects. Thalidomide suppresses TNF-alpha mediated NF-kappa B activation [66] and inhibits the production of interleukin 6 [67], among other mechanisms of action.

Preliminary results from an open study in patients with metastatic cancer with decreased appetite and weight loss of more than 5 % and without antineoplastic therapy [68] suggested, that thalidomide 100 mg at night for at least 10 days could be a well tolerated and effective medication for cancer-related anorexia, improving not only anorexia, cachexia, appetite, and "overall sensation of well-being", but also resulted in improved nausea.

A recent randomized, double-blind study investigated the effects of thalidomide 100 mg versus placebo for 14 days in advanced cancer patients with fatigue, anorexia, weight loss of more than 5 % and with either anxiety, or depression or sleep disturbances [69]. The data revealed that significant cytokine reduction was achieved in both, the thalidomide as well as the placebo group, with no differences in symptom control between the groups.

Nutritional Supplements

In different rat or mouse models several nutritional agents (curcumin, genistein, resveratrol, epigallocatechin gallate and lycopene) have shown efficacy by improving symptoms like fatigue, anorexia, neuropathic pain, and cognitive deficits, thereby, decreasing inflammatory markers such as IL-1beta, IL-6 and TNF-alpha [1].

Also in humans nutritional agents seem to have positive effects on inflammatory markers as well as on cancer-related symptoms.

In a study (22 patients) with advanced lung cancer and Systemic Immune-Metabolic Syndrome (SIMS)—including systemic syndromes due to cytokine

release, such as paraneoplastic hemopathies, hypercalcemia, coagulopathies, fatigue, weakness, cachexia, chronic nausea, anorexia, and early satiety—treatment with eicosapentaenoic and docosahexaenoic n-3 fatty acids from fish oil, 2 g thrice daily, for six weeks and additional oral food supplementation resulted in significantly more appetite, less fatigue, and lower C-reactive protein values than the respective baselines values [70]. Patients, additionally receiving celecoxib 200 mg twice daily, showed significantly lower C-reactive protein levels, higher muscle strength and body weight.

However, a large randomized study with 421 patients investigating the efficacy of eicosapentaenoic acid, an omega-3 fatty acid, concluded, that eicosapentaenoic acid supplementation at a much lower dose, only 1.09 g twice daily, either alone or in combination with megestrol acetate (MA), does not improve weight or appetite compared to megestrol acetate alone [71]. Survival and global quality of life were not significantly different among the groups. Also toxicity was comparable, except of increased impotence in MA-treated patients.

In a prospective, multicentre, placebo-controlled, randomized and double-blinded trial in 72 patients suffering from advanced pancreatic cancer and cachexia [72] oral L-carnitine supplementation (4 g daily) for 12 weeks improved nutritional status (body cell mass, body fat) and quality-of-life parameters compared to the placebo group and caused a significant increase of the body-mass-index, but non-significant trends towards an increased overall survival and towards a reduced time of hospitalization.

In a randomized study (309 unselected weight-losing patients with malignant disease) all patients received a COX inhibitor (indomethacin, 50 mg twice daily) and erythropoietin (15–40,000 units per week) [55]. The efficacy of specialized, nutrition-focused patient care (oral nutritional support and home total parenteral nutrition) was compared to the outcome of control patients without the added nutritional support. All patients were treated and followed until death. Nutritional support resulted in a significantly prolonged survival, improved energy balance, increased body fat, and a greater maximum exercise capacity in these patients with progressive cachexia secondary to malignant disease.

Cannabinoids

A large randomized study in 496 advanced cancer patients investigated the efficacy of dronabinol (delta-9-tetrahydrocannabinol, THC), megestrol acetate or the combination of both agents for the treatment of cancer associated anorexia. Megestrol acetate treatment caused significant appetite improvement and weight gain compared with dronabinol-treated patients, whereas the combination caused no additional effect [43].

Also another large randomized study [73] comparing the effects of cannabis extract, delta-9-tetrahydrocannabinol, and placebo on appetite and quality of life in patients with cancer-related anorexia-cachexia syndrome showed no significant differences between the three arms for appetite, quality of life, or cannabinoid-related toxicity.

Combination Treatments

As reported above the combination of eicosapentaenoic and docosahexaenoic n-3 fatty acids from fish oil, 2 g thrice daily, for six weeks with celecoxib 200 mg twice daily was more effective for the treatment of cancer related symptoms like fatigue, cachexia, anorexia etc. than the single agents [70].

In a phase II study, 39 advanced cancer patients with cancer-related anorexia/cachexia and oxidative stress received an "integrated" treatment for four months consisting of diet with high polyphenols content (400 mg), antioxidant treatment (300 mg/d alpha-lipoic acid + 2.7 g/d carbocysteine lysine salt + 400 mg/d vitamin E + 30,000 IU/d vitamin A + 500 mg/d vitamin C), and pharmaconutritional support enriched with 2 cans per day (n-3)-PUFA (eicosapentaenoic acid and docosahexaenoic acid), 500 mg/d medroxyprogesterone acetate, and 200 mg/d selective cyclooxygenase-2 inhibitor celecoxib [74]. The results showed a significantly increased body weight, lean body mass and appetite compared to baseline and an important decrease of proinflammatory cytokines interleukin-6 and tumor necrosis factor-alpha in 22 of the 39 patients. Additionally, a marked improvement was found in the quality of life and fatigue.

In a consecutive phase III, randomized study 332 patients with cancer-related anorexia/cachexia syndrome were randomly assigned to one of five treatment arms (single-track versus multi-track approaches): arm 1, medroxyprogesterone (500 mg/day) or megestrol acetate (320 mg/day); arm 2, oral supplementation with eicosapentaenoic acid; arm 3, L-carnitine (4 g/day); arm 4, thalidomide (200 mg/day); and arm 5, a combination of the above for a treatment duration of 4 months [75, 76]. The primarily multi-track approach, including all selected agents, was the most effective treatment and significantly improved cancer cachexia-lean body mass, resting energy expenditure and fatigue as well as the secondary endpoints appetite, IL-6, Glasgow Prognostic Score, and ECOG performance status.

In a randomized phase III study in 104 advanced-stage gynecological cancer patients the multi-track approach with megestrol acetate (MA) plus L-carnitine, celecoxib, and antioxidants for four months was more effective than megestrol acetate alone regarding to lean body mass, resting energy expenditure, fatigue, and global quality of life [77]. The combination treatment significantly decreased the inflammation and oxidative stress parameters interleukin 6, tumor necrosis factor α, C-reactive protein, and reactive oxygen species.

Conclusions

Consistent with the general controversial study results, pharmacological interventions are considered as experimental in the guidelines for cancer-related fatigue, cachexia, anorexia etc. The NCCN guidelines for cancer-related fatigue [78, for Version1.2011 see NCCN.org] recommend antidepressants for depression or erythropoietin for anemia and claim further research on the use of corticosteroids

and psycho-stimulants as potential treatment modalities in managing cancer-related fatigue.

The European Palliative Care Research Collaborative (EPCRC) guidelines on cancer cachexia give only a strong positive recommendation for steroids to stimulate appetite and to improve quality of life, but restricted to short treatment periods, up to two weeks [79]. Weak positive recommendations are given for megestrol acetate, for pro-kinetics and for multimodal therapies. Non-steroidal anti-inflammatory drugs alone, cannabinoids, and thalidomide or cytokine antagonists are not recommended in patients with refractory cachexia.

In 2008 a structured literature review was performed to develop evidence-based recommendations for cancer fatigue, anorexia, dyspnea and depression by a multidisciplinary expert panel [80]. Because of lack of evidence, a clear guideline for clinical situations, pharmacological interventions for cancer fatigue or anorexia (e.g., appetite stimulants) could not be elaborated by the experts.

Despite almost two decades of applied research regarding inflammation-related symptom control in advanced cancer, there is still not enough evidence for guideline recommendations. Research about anti-inflammatory treatment in metastatic cancer still seems to be in its infancy.

On the one hand laboratory data provide myriads of hints that indeed pro-inflammatory processes are related to multifold clinically evaluable disease traits associated with metastatic cancer; that pro-inflammatory processes are even drivers of tumor progression; on the other hand approaches for attenuating pro-inflammatory processes repetitively show controversial data about the benefit of anti-inflammatory therapy approaches. What are the reasons for the conflicting data?

Recently published clinical data show that inflammation control in different metastatic tumor types requires differential therapeutic access, as pro-inflammatory processes are multifacetedly rationalized. Beyond control of inflammation-related clinical symptoms (improved ECOG status) inflammation control was associated in these trials with attenuation of tumor growth [81, 82].

Rationalizations provide highly specific targets for 'top-down' strategies: Inflammation—as ubiquitous tumor-associated normative notion—may be constituted in multiple ways, supported by quite different cell types of the tumor stroma compartment [81–86]. Cells of the tumor compartment have redundant resources available to sustain pro-inflammatory acting rationalizations [87].

The following consequences emerge from these data: An evolution-adjusted tumor pathophysiology is necessary that systematizes the constitution of respective rationalization processes dependent on the histological tumor type (Fig. 21.1). Based on these data, rationalization-directed combined modularized therapies can be designed. 'Top-down' approaches should be preferred, as inflammation cannot be exclusively tethered at the classical pro-inflammatory cytokines or transcription factors, i.e., II-6, II-1, TNFalpha, NFkappaB, STAT3 etc. (Chap. 17).

Recent investigations in ovarian cancer by Balkwill et.al could show, how three key cytokine/chemokine mediators of cancer-related inflammation, TNF, CXCL12,

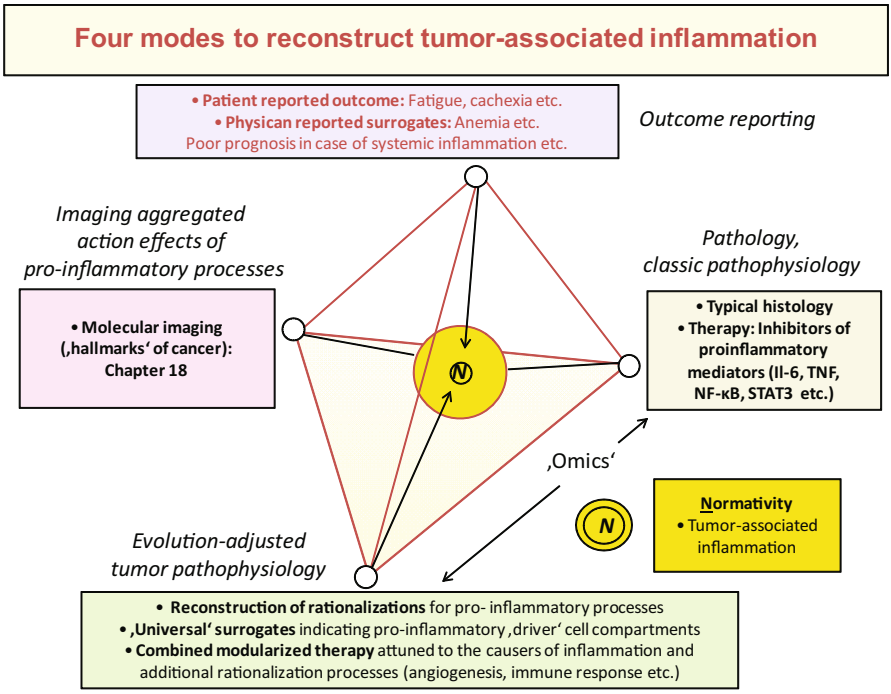

Fig. 21.1 Tumor-associated inflammation may be depicted by reductionist, holistically reconstructive, outcome-oriented, and imaging approaches. Respective results are available for cross-validation

and interleukin 6, are specifically involved in an autocrine cytokine network in human ovarian cancer [83]: It is not undefined "general" inflammation, which causes the symptoms. Understanding the crosstalk and the involved pathways for different tumor entities will probably enable specific "differential" treatment strategies with tailored combination therapies for the different tumors.

Dependent on the physical constitution of tumor-associated rationalization processes the interfaces to other rationalization processes, e.g., immune response, angiogenesis become displaced. Combined modularized therapies account for such displacements.

Thus we postulate that inflammation control should be primarily designed as a multi-track approach, and combined with the redirection and modulation of closely related tumor-promoting normative notions, such as immune-modulation or anti-angiogenetic approaches according to data systematically provided by an evolution-adjusted tumor pathophysiology (Chap. 2, 22). Then, inflammation control can be simultaneously directed towards tumor control and attenuation of related disease traits.

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Part IX
Novel Clinical and Pharmaceutical Technology for Bioengineering Tumor Response

Chapter 22
Combined Modularized Tumor Therapy—Tumor Biology—and Prognostic Factors: Bioengineering Tumor Response

Albrecht Reichle

Abstract Two rather different understandings of tumor pathophysiology exist; one is based on a reductionist or an observer's point of view ('bottom-up' approach) and the other on an evolution-adjusted or a participator's perspective ('top-down' approach). Important clinical requirements can be addressed by both 'top-down' and 'bottom-up' strategies. The first requirement is the incorporation of clinical knowledge derived from the application of different therapeutic methodologies; 'top-down' strategies, for instance, provide therapeutic access to communication-associated pathologies. The second requirement is overcoming limitations of 'bottom-up' strategies by focusing on the systematization of a tumor systems' normativity. Such strategies involve the heterogeneity of the communicative expression of tumor-promoting pathways, the tumor-specific and stage-specific accessibility and distribution of targets among cellular compartments, the heterogeneity of chromosomal or molecular-genetic aberrations in stroma and tumor cells, the presence of basic mechanisms implementing robustness, and repair mechanisms. Information derived from biomodulatory therapy approaches may be translated into an alternative tumor classification on the basis of evolution-adjusted tumor pathophysiology. This translation and the generation of a novel response and prognostic markers will hopefully further the future development of novel combined modularized therapeutic strategies for overcoming the problems due to the cytogenetically and molecular-genetically defined heterogeneity of tumor and stroma cells.

Introduction

Despite significant improvements in the histological, molecular-biological, and molecular-genetic specification of diagnoses, surgical procedures, radiotherapy, and advancements in supportive care, the majority of deaths from cancer are eventually caused by continuous metastatic growth that is resistant to available therapies.

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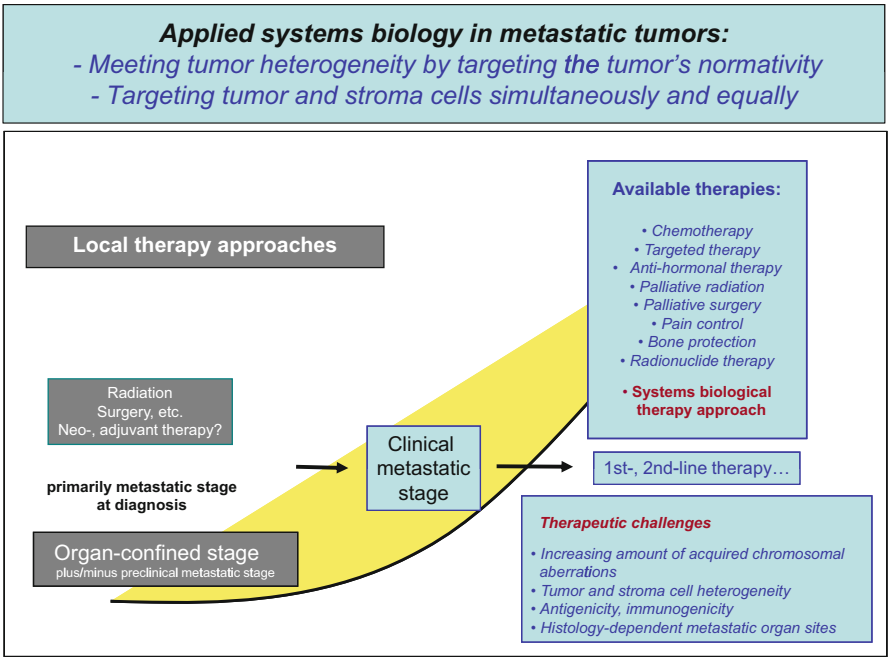

Fig. 22.1 A major obstacle for the eradication of metastases, particularly with targeted therapies, remains the biologic heterogeneity of tumor and neighboring stroma cells. A systemic therapy approach aims at targeting a tumor's normativity. Inducing continuous complete remission or long-time disease stabilization at a low tumor burden indicates that tumor heterogeneity may have been overcome by combined modularized therapy approaches

More and more evidence arises that cancer dissemination is already present at the time of diagnosis of localized tumors. During tumor evolution, metastases can be located in typical distant organ sites, depending on the tumor histology, and in different regions within a single organ [1].

A major obstacle for the eradication of metastases, particularly with targeted therapies, remains the biologic heterogeneity of tumor and neighboring stroma cells (Fig. 22.1).

At the time of diagnosis, primary tumors comprise a broad diversity of cellular tumor and stroma cell compartments, which are characterized by their heterogeneity in molecular-genetic and chromosomal aberrations, tumor-promoting communication lines, cell surface features, antigenicity, immunogenicity, and their sensitivity to therapies [2, 3].

Biologic heterogeneity is also found in metastatic tumor sites. However, tumor-immanent rationalizations for immune response, such as the composition and organization of the innate and adaptive immune-microenvironment in metastases

and respective primary tumors, are all the same, as shown by a comparison of primary tumors and corresponding lung metastases in renal clear cell carcinoma and colo-rectal cancer [4].

Tumor-immanent normative notions, i.e., inflammation, angiogenesis, etc., as well as structures, functions, and decision maxims are not only differently organized in various histological tumor types but even within the same histological tumor type [5]. Distinct rationalizations of tumor-immanent normative notions—here the immune response to tumor cells—achieve prognostic importance.

However, the fact that primary tumor and metastases may share tumor-immanent rationalizations is of pivotal therapeutic interest: Despite the frequently shown genetic heterogeneity between metastases and primary tumors, rationalizations of tumor-immanent normative notions may be maintained among primary tumor sites and metastases.

The findings by Fridman et al. [5] could explain the successful application of combined modularized tumor therapies that aim at redirecting tumor-immanent normative notions by implementing non-normative boundary conditions into the evolutionarily constrained systems world of a tumor.

The present paper describes how targeting tumor-associated normative notions for tumor control necessitates a novel understanding of tumor pathophysiology, therapy, and corresponding prognostic parameters. All three benchmarks are communicatively linked by a formal-pragmatic communication theory. The communicative interaction of tumor systems participators on the basis of modularity, subjectivity, intersubjectivity, and normativity may launch a continuous adaptive process by facilitating the routine implementation of tumor pathophysiology (evolution-adjusted tumor pathophysiology) for therapeutic purposes. Combined modularized therapies and corresponding prognostic parameters indicate the evolvability of tumor systems. This concept aims at including 'all' patients in therapy approaches by modulating and redirecting tumor-immanent normative notions with biomodulatory therapies (adaptive bioengineering). Designing 'top-down' therapies does not primarily comply with acquired genomic constellations in the tumor cells, but with tumor-specific rationalization processes.

'Top-down' Approaches, Inclusion Strategies, Versus 'Bottom-up' Approaches, Exclusion Strategies

'Top'-down therapy approaches focus on redirecting and modulating the communicative expression of tumor-promoting communication lines, i.e., pathways, receptors, etc. Thereby, the communicative 'background' of tumor-promoting communication lines is modified for attenuating tumor growth (Fig. 22.2).

'Bottom-up' strategies exclusively use single-track or combined single-track approaches, thereby including drugs with mono-activity.

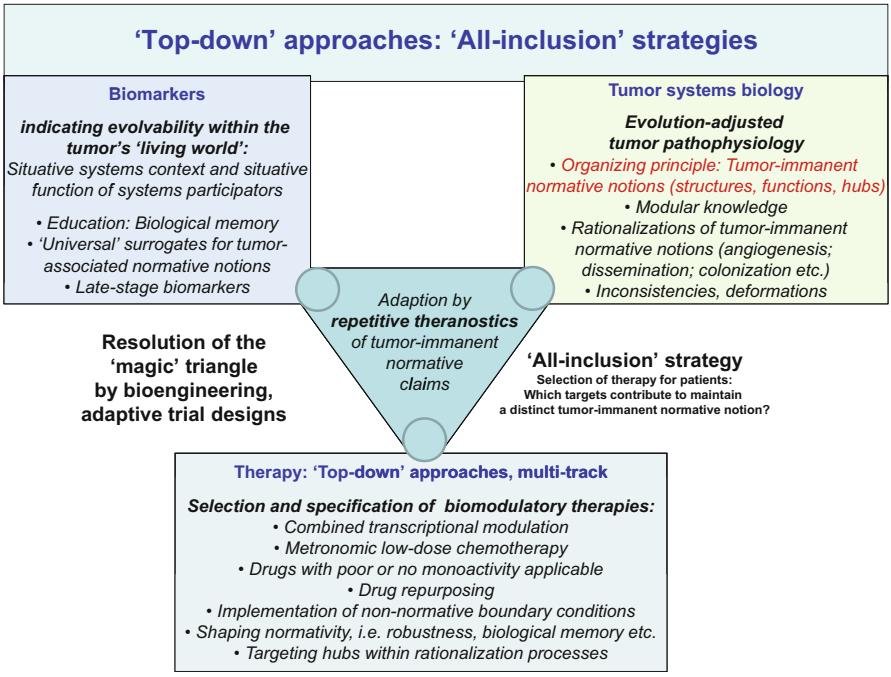

Fig. 22.2 As 'top-down' approaches target a tumor's normativity, treatment strategies try to include 'all' possible rationalizations of normative systems structures, functions, and decision maxims ('all-inclusive'). The implementation of non-normative boundary conditions into a tumor's systems world necessitates a novel understanding of tumor pathophysiology and the generation of novel predictors

Both treatment strategies have in common that their success is inevitably mediated by redirecting tumor-immanent normative notions, structures, functions, and decision maxims (hubs). 'Bottom-up' strategies use a 'surgical' intervention or combined 'surgical' interventions by directly targeting potentially tumor-promoting pathways, regardless of their evolutionarily confined situative validity and denotation due to acquired chromosomal aberrations (Fig. 22.3). 'Top-down' strategies, on the other hand, alter a tumor's normativity by 'indirect' modulation and redirection of its modularity, subjectivity, and intersubjectivity, thereby altering the communicative expression of tumor-promoting communication lines.

Therapy

'Top-down' therapy approaches are primarily combined modularized multi-track therapies, because they only try to alter the functionality of tumor promoters but do not aim at 'knocking-down' the tumor-promoting pathways themselves with 'bottom-up' strategies, i.e., single-tack or combined single-track approaches. Single-track

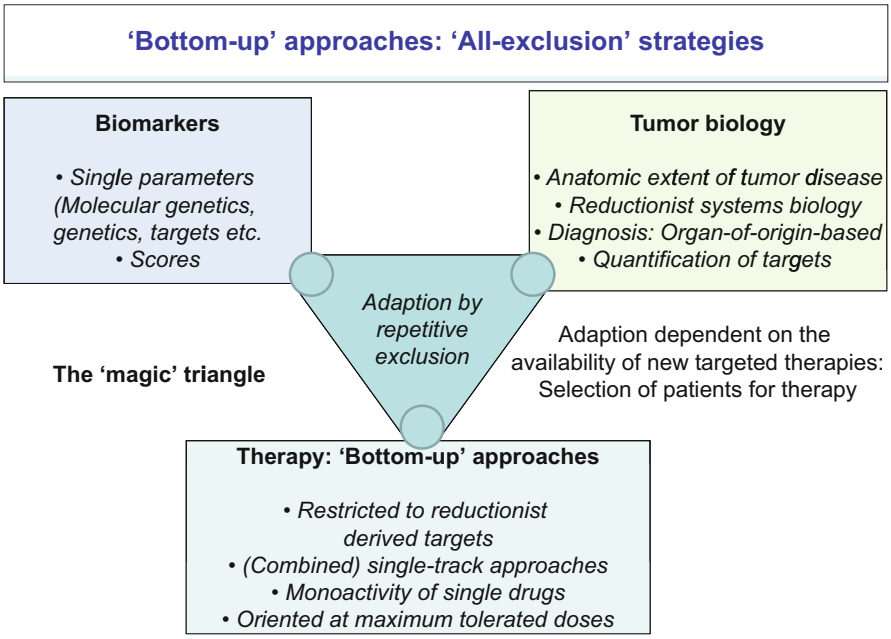

Fig. 22.3 'Bottom-up' strategies describe the traditional understanding of tumor therapy, i.e., of tumor pathophysiology associated with the generation of corresponding predictors. 'Bottom-up' strategies assume unchanged validity and denotation of tumor-promoting communication lines—regardless of the communicative context—given by ever changing chromosomal or molecular-genetic aberrations

approaches are targeted therapies that are administered at maximal tolerable doses to enhance their activity profile.

In contrast, the biomodulatory activity of a single drug is the focus of a multi-track approach, and the single drug in such a 'cocktail' must not be mono-active in any metastatic tumor disease, as shown in many phase II trials [6]. 'Top-down' approaches may include drugs that evolve tumor systems by inducing stable therapy-relevant epigenetic changes (combined transcriptional modulation). Biomodulatory therapies are directed to the same extent at stroma and tumor cells. Therefore, non-oncogene-addicted and commonly ubiquitously accessible targets may be used as therapeutic structures.

'Top-down' approaches try to implement non-normative boundary conditions into a tumor systems' world. Consecutively, the tumor is forced to change its normativity via communication-based rules. Modular knowledge of systems participators may be therapeutically redeemed: Novel arising communicative presuppositions alter the validity and denotation of tumor-promoting systems participators.

The acquisition of context-dependent communicative expression has been frequently described as 'moonlighting' [7]. 'Moonlighting' is no illegal, random, or

predominantly analogously working procedure, but a redeemable and therefore digitalized process that is well integrated into the 'metabolism' of evolution.

Another aspect of 'moonlighting' is the fact that the functionality of drugs may be completely repurposed within multi-track therapies, as shown in many phase II trials on metastatic tumors. Particularly, the activity profile of drugs targeting ubiquitously accessible structures depends on the distribution of the targets within the cellular compartments of a tumor environment.

In renal clear cell carcinoma and castration-refractory prostate cancer, successfully combined transcriptional modulation with metronomic low-dose chemotherapy serves as a pivotal example of drug repurposing: Completely novel mechanisms, namely a biological memory, may be activated for achieving continuous disease stabilization.

As normativity is a tumor-inherent biologic feature, a comparatively restricted tool of rationalizations is provided for establishing distinct tumor-associated normative notions compared to the myriads of seemingly unrestricted patterns of non-random chromosomal aberrations, which represent the genetic prerequisite for tumor-immanent normativity. From that point of view, we must postulate hubs within rationalization processes that allow the contingent evolution of normativity by bundling rather diverse communication lines via hubs or nodes within an overall evolutionarily restricted tool of rationalizations available for constituting normativity.

Here, an interesting link could arise for novel designed 'bottom-up' therapies, because the postulated hubs within rationalization processes could serve as structures for reductionistically derived targeted therapies.

Tumor Biology

Evolution-adjusted tumor pathophysiology is a clinically based discipline that aims at systematically establishing the constitution of tumor-immanent normativity by comparatively describing its physical rationalizations regardless of its tumor histology. Therefore, normativity constitutes a novel principle that comparatively organizes and systematizes tumor-immanent normative notions, i.e., normative structures, functions, and decision maxims, by describing corresponding evolutionarily constrained rationalization processes. This way, tumor pathophysiology may be reopened for describing and summarizing the tumor-related organization of communication lines that constitute distinct communicative expression. Tumor biology undergoes a turnaround from reductionist descriptions of pathophysiology and pathology to evolution-adjusted pathophysiological systematizations. For the first time, we have an instrument for conceiving communication-based pathologies, which develop between the claims of systems participators and those of the evolutionarily developing systems world. Thereby, the systems world subjects systems participators to situative validities and denotations. The enhancement of communication-driven pathologies (Achilles' heels) by combined modularized therapies could be the reason of a rapid response but also of a delayed complete response in metastatic cancer (chapter 2).

A reductionist comprehension of tumor biology is based on the detection and quantification of diagnostic parameters (categorization) and potential therapeutic targets. Tumor diagnosis is primarily organ-of-origin-based and necessitates describing the extent of a tumor disease. The summarizing categorization of the tumor is 'all-exclusive': Tumors with a missing biomarker profile or insufficient expression are excluded.

Therapy Adaption According to Evolutionarily Developing Systems

Tumors with negatively tested diagnostic parameters or therapeutic targets are subjected to repetitive exclusion procedures to find an adequate gateway for specific therapeutic interventions. Therefore, the adaptability of therapeutic procedures strongly depends on the availability of novel targeted therapies, and these therapies necessarily operate in more and more pathophysiological niches.

In contrast, continuous therapeutic adaption to individual normativity of tumor systems (novel adaptive trial designs) may be realized by repetitive theranostics of tumor-immanent normative claims and their rationalization. The communicative character of combined modularized therapeutic approaches opens up novel repeated diagnostic steps and provides access to prognostic markers, which may represent 'universal' surrogates indicating the functional status of a tumor's normativity (cellular secretome analytics in serum, molecular imaging of pragmatically selected 'hallmarks' of cancer).

Biomarkers: Response Parameters, Prognostic Factors

Both approaches for personalizing tumor therapy, i.e., the therapeutic adaption of combined modularized therapies by repetitive theranostics of tumor-immanent normative claims and adaption to available reductionistically derived targeted therapies by repetitive exclusion technique, necessitate the uncovering of methodology-specific parameters indicating response and prognosis.

Tumor response to combined modularized therapies and prognosis may depend on the evolvability of a tumor's 'living world' and the evolutionarily confined situative and systems-mediated function of systems participators. As shown, changes in C-reactive protein (CRP) levels in serum are indicators for tumor response and overall survival. Specificity and sensitivity of serum CRP changes during combined modularized therapy for predicting response depend on the metastatic tumor type. Changes in CRP levels in serum may be induced by rather different modular therapy elements. This observation indicates diversified rationalizations for tumor-associated pro-inflammatory processes [8, 9].

CRP emerges as a 'universal' surrogate for the efficacious modulation of a tumor-associated disease trait. Anti-inflammatory response could be specified by cellular secretome analytics in the serum or by molecular imaging techniques.

Education of tumor and stroma cells to maintain the attenuation of tumor growth after therapy discontinuation (biological memory) seems to be a novel field for establishing long-term tumor control. Novel late-stage parameters indicating progression-free survival could be detected in metastatic melanoma [10].

Novel biomarkers have to be generated for indicating the attenuation of metastatic tumor growth: Systematic investigations during tumor progression after combined modularized therapies have indicated metastatic progression at the original tumor sites but no additional metastases [6] in the majority of cases (67%), chapter 2.

Reductionist investigations aim at uncovering specific single parameters, patterns, or scores to confirm and specify diagnoses, to look for potential therapeutic targets, or to determine prognosis [11, 12]. Completely different reductionist therapies may share identical prognostic parameters, such as intensive chemotherapy and allogeneic transplantation in acute myelocytic leukemia (major prognostic parameters are cytogenetics or molecular-genetic) [13], or vascular endothelial growth factor antagonists in renal clear cell carcinoma (RCCC), and earlier standard therapies in RCCC (Heng score). The common denominator seems to be that all approaches operate on reductionistically derived targets [14].

Discussion

By now, clinical oncology has all necessary prerequisites for progressing towards personalized tumor therapies and, in particular, towards differently designed palliative care approaches (Fig. 22.4): Two rather different understandings of tumor pathophysiology exist; one is based on a reductionist or an observer's point of view, the other on an evolution-adjusted participator's perspective. Both basic starting points virtually dissect a tumor's 'living world' and provide qualitatively rather different target patterns for accessing a tumor's systems biology, once a tumor's normativity serves as a target via combined modularized therapies. The two divergent approaches have in common that they disturb the established growth-promoting normativity of tumor systems for attenuating tumor growth.

In the past few years, an ever growing number of reductionistically derived therapies have been developed, accompanied by technological progress that enhances our understanding of how metastases develop and how they might be accessible by therapy.

Important clinical needs can be addressed at this point by 'top-down' and 'bottom-up' strategies.

The first is the incorporation of clinical knowledge derived from the different application of therapeutic methodologies. 'Top-down' strategies in addition to 'bottom-up' strategies increase the variety of instruments for bioengineering tumors in a purposive-rational manner. At that stage, communication-associated pathologies

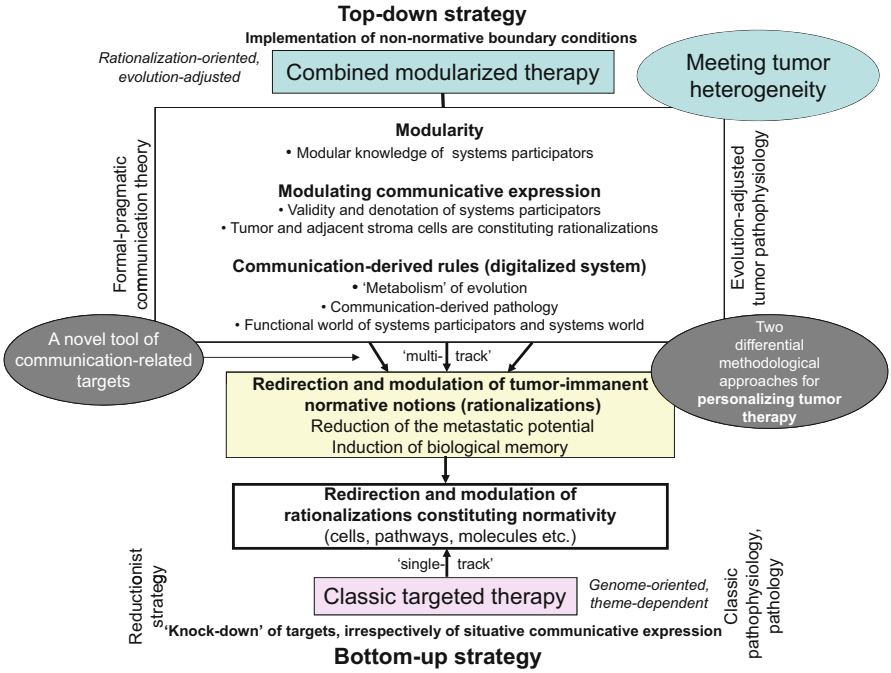

Fig. 22.4 Both 'top-down' and bottom-up strategies provide different approaches for personalizing tumor therapy and specifying palliative care. The strategies necessitate a specific presentation of tumor pathophysiology, either as classic pathophysiology and pathology or as evolution-adjusted tumor pathophysiology, and require the evaluation of strategy-derived prognostic markers. The strategies have in common that tumor response is mediated by redirecting and modulating situative and evolutionarily confined rationalizations that constitute a tumor's normativity

become therapeutically accessible. Bioengineering tumor response means to exploit bottom-up, top-down, as well as synthetic biolological therapeutic approaches.

Secondly, limitations of 'bottom-up' strategies are the heterogeneity of the communicative expression of tumor-promoting pathways, accessibility, and distribution of targets among cellular compartments, the heterogeneity of chromosomal or molecular-genetic aberrations in stroma and tumor cells, as well as basic mechanisms implementing robustness, and repair mechanisms. Thus, these limitations prompt to change the point of view from that of a systems observer to that of a systems participator and put the pathosphysiologic and therapeutic focus on the systematization of the comparative normativity of tumor systems (Table 22.1).

Finally, the novel point of view distracts from reductionist pathophysiologic and pathologic considerations, which dissect metastases into reductionistically relevant cellular and molecular components that drive the process of organ-specific colonization. The 'declination' of a tumor's normativity in its physical constituents, i.e.,

Table 22.1 'Bottom-up' and 'top-down' strategies are complementary and have their typical methodological limitations. Limitations by methodology may be overcome by repeated 'all-exclusion' strategies within the 'bottom-up' approach and an adaptive 'all-inclusion' strategy within the 'top-down' approach that specifically targets the rationalizations of a tumors' normativity

Limitations of exclusion and inclusion strategies

Strategy	Limitations
Exclusion strategy	• *Heterogeneity of the communicative expression of tumor-promoting pathways* • *Accessibility and distribution of targets among cellular compartments* • *Tumor heterogeneity:* – *Evolutionary confined broad diversity of chromosomal or molecular-genetic aberrations* • *Robustness, repair mechanisms (resistance)* • *Target and targeted therapy not available*
Inclusion strategy	• *Degree of evolvability (evolutionary constraints)* • *Biomodulatory accessibility of communicative expression by redirection and modulation of communicative presuppositions* • *Physical organization of rationalization processes* • *Heterogeneity of rationalization processes in a tumor* • *Robustness (resistance)*

rationalization processes, becomes the novel starting point for comparatively selecting and systematizing tumor-immanent normative structures, functions, and decision maxims and their corresponding rationalizations.

The 'all-inclusion' strategy favored by 'top-down' strategies has its limitations in the evolutionarily given degree of evolvability, the biomodulatory accessibility of the communicative expression by redirection and modulation of communicative presuppositions, the physical organization of rationalization processes, a tumor's robustness, but also in the development of resistance mechanisms (Table 22.1).

The translation of information derived from biomodulatory therapy approaches into an alternative communication-based classification of tumors, i.e., into evolution-adjusted tumor pathophysiology, results in novel biomodulatory therapy designs. Further communication relevant targets can be uncovered by the comparative reconstruction of tumor-associated normative claims. After this diversification of the technical prerequisites for assessing communication relevant tumor-promoting processes we have the freedom of choice for the selective or combined targeting of tumor-immanent normative notions. This way palliative care becomes patient-orientated: Targeting rationalizations of tumor-immanent normative notions and the

generation of novel response and prognostic biomarkers will hopefully further the future development of novel combined modularized therapeutic strategies for overcoming the problems due to the cytogenetically and molecular-genetically defined heterogeneity of tumor and stroma cells.

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Part X
Objectifying the Systems Objects' Subjectivity in Biological Systems: A Novel Reification of the Scientific Picture About the 'Objective' World

Chapter 23
The Subjectivity of Systems Objects as a Scientific Object

Albrecht Reichle

Abstract The subjectivity of systems participators emerges as ubiquitously occurring attainment of biological systems and facilitates multifaceted rationalizations for constituting the characteristic normativity of biological systems. The 'living world' of biological systems creates the background for establishing subjectivity of socialized—that means reciprocally communicating—systems participators and allows the redirection of systems normativity by combined modularized approaches: (1) Biomodulatory interventions exemplarily show that the available background knowledge of biological systems participators and practices of the related communication-derived rules of the systems 'living world' may now be conveyed for scientific proof, namely to the accustomed object side of natural sciences. (2) Evolution-adjusted pathophysiology may now practically use the architecture of the 'living world'. (3) Evolution-adjusted pathophysiology has access to the 'living world' of biological systems by providing the knowledge for evolving and redirecting the normativity of these systems. (4) Because of the assumed evolutionarily linked subjectivity of systems objects on the background of the systems 'living world' and the modular knowledge of systems participators, evolution fuses a dichotomy by simultaneously emerging in a directed and an undirected manner. In response, the modular knowledge of systems participators may be redeemed endogenously by 'natural' tumor evolution and 'artificially' (synthetic biology, gene transfection, etc.) by therapeutic approaches, i.e. combined modularized (top-down) or single track (bottom-up) interventions. The alternative to the introduction of a 'living world' of biological systems would be the assumption of some deeply grounded biological perspectives: Then, biological systems would disintegrate into the particularism of suggested relevant cuttings of the 'living world' in the sense of neopragmatism. Darwinian 'selection' as a possible principle to explain evolution history would be one of these perspectives.

The subjectivity of systems participators emerges as ubiquitously occurring attainment of biological systems and facilitates multifaceted rationalizations for

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constituting the normativity of biological systems. The subjectivity of systems participators is the basis for acquiring evolutionary, either endogenously or interventionally redeemable, modular knowledge of systems participators and leads to the concept of a 'living world' of biological systems. The 'living world' creates the prerequisite for situatively redirecting and modulating the normativity of biological systems on the background of robust maintenance of systems integrity. Robustness is fed by the systems living world [1].

The possibility to exogenously induce evolutionary processes with consecutive digitalization on an epigenetic or chromosomal level exemplarily shows that the communicative background, constituted by the living world, is steadily present for further developing the normativity of biological systems [2–4]. Without the proof by exogenous interventions, which aim at implementing non-normative boundary conditions (combined modularized and single track approaches, synthetic biology, gene transfection, targeting of hubs within rationalization processes, etc.) to redirect systems normativity, the realization of developmental possibilities provided by the 'living world' could not be addressed and communication-derived rules would not be accessible to scientific proof (chap. 12, 13).

Combined modularized interventional approaches exemplarily show that the performatively available background knowledge of biological systems participators and the practices of the related communication-derived rules of the systems 'living world' may be now conveyed for scientific proof, namely to the accustomed object side of natural sciences [5, 6].

All biological systems are marked by a tension-filled interaction between reasons 'fixed' to biological 'traditions' and realized in institutionalized structures, functions, and decision maxims, and reasons connected to 'mobile' communication, which may be realized in the framework of the systems living world, for example, by implementing non-normative boundary conditions, etc. Evolution dogmatizes even corrupt rationalizations, for example, in tumors, particularly for constituting systems normativity as well as the functional and structural benchmarks for generated reasons. Thereby, biological systems evolve novel developmental constraints and specifically accessible developmental possibilities, such as a biological memory (chap. 19).

This way, a 'living world' of biological systems epitomizes reasons for endogenously or interventionally redeemable activity profiles. The symbolization of reasons within biological systems takes place in form of systems participators, which may be considered as systems artifacts. The subjectivity of artifacts is realized by the physical and chemical constitution of the artifacts and the functionally important communicative background. Subjectivity is expressed in distinct systems-relevant validity and denotation of the artifact. Epitomized reasons are constitutive for a tumor's living world and represent the context for the specific communicative access by biomodulatory interventional approaches.

In a reconstructive way, epitomized reasons are scientifically accessible and represent prerequisites for designing interventional approaches directed at diversifying the normativity of biological systems [5, chap. 17].

Reasons may be generated by developing the 'grammar' of communication, that means by modularly differentiating the communicative expression of pathways and

tumor cell compartments, etc. via situatively evolving communicative contexts: Systems participators adopt and use communicative symbols to mediate communicative expression, and systems participators in addition to the relevant communicative context reciprocally accept perspectives and acquire intentional attitudes [7].

Can scientific fragments compiled in a reductionist manner be composed for constituting evolutionarily developing biological systems, which adhere to the 'metabolism' of evolution, if we proceed from the perception of the 'living world' of biological systems?

1. The 'living world' of biological systems is a communication-theoretical term that encompasses a fund of communication-relevant rules, which are able to stably constitute the normativity of cell systems, tissues, organs, and living beings. The robustness of biological systems is founded by the systems living world. The 'living world' is no virtual space, but it can also be experimentally exploited by biomodulatory interventions, gene transfection etc. [2–5].
2. The living world is an evolution-theoretically and experimentally (biomodulation) accessible, inevitable horizon of experience of socialized biologic systems. The living world becomes operationally available by systematically studying the modular knowledge of systems participators and its specific redeemability within an evolutionarily confined systems context.
3. The existence of a living world can be objectified by implementing evolution-theoretical knowledge in biological systems with the aim of evolving systems by redirecting and modulating their normativity by the establishment of respective rationalization processes.
4. Systems participators are socialized in their relations and practices as intervening subjects. Their subjectivity is determined by the functional world of the systems participators and simultaneously by the requirements of the respective systems living world. Therefore, the communicative expression of a systems participator is a situative, evolutionarily confined phenomenon that is determined by the communication-derived rules present in the living world.
5. The living world of biological systems comprises all endogenously or interventionally redeemable communicative systems conditions (e.g. by implementing non-normative boundary conditions, etc.) for evolving systems normativity. Evolution is delimited by a distinct evolutionary background present in the living world and requires systems stage-dependent informative boundary conditions for establishing the subjectivity of systems participators by simultaneously maintaining systems integrity (robustness) or by redirecting communicative processes with the aim of disintegrating the normativity of biological systems for growth control, as shown in tumor systems.
6. Thus, disintegrating the normativity of biological systems by communication-derived approaches is postulated to be an independent mechanism to achieve systems 'death' similar to the broad variety of apoptosis and other 'death' pathways [8].
7. Because of the assumption of the evolutionarily linked subjectivity of systems objects on the background of the systems living world and the modular knowledge

of systems participators [1], evolution fuses the epistemic dualism of necessity and openness of a biological development by simultaneously emerging in a directional and an undirectional manner. Determinism in the common descent of living beings would not be in accordance with a formal-pragmatic communication theory underlying the presented evolution theory (chap. 12).
8. The formal-pragmatic communication theory supports experimental findings that knowledge of developmental conditions of biological systems allows to recourse on 'earlier' systems stages [9, 10].

Evolution-adjusted tumor-pathophysiology may now practically use the architecture of the living world. Descriptions of evolution-adjusted pathophysiology have access to the biological living world for evolving systems. For the time being, an empty space remains that has to be filled by the accessible knowledge about the 'metabolism' of evolution described by an evolution theory [1, 7].

With the analysis of background knowledge of systems participators and the reasons constituted by the 'living world', reductionist knowledge loses its exclusive orienting function that automatically promises practical information about what is 'right'.

To what extent does the epistemic function of the 'living world' draw boundaries to the 'metabolism' of evolution and to interventions with biomodulatory approaches?

1. The 'living world' steadily promotes the viewing of systems participators from an evolutionary side. Therefore, the 'world of objects' is critically scrutinized by objectifying the subjectivity of systems objects. The communicative context to which systems objects are subjected determines the evolutionarily confined communicative expression and, finally, the normativity of biological systems.
2. Consequent evaluation and reconstruction of the communicative expression of systems participators leads to a stepwise relief of the 'objective', reductionistically acquired knowledge from the projections of the living world [11–14, chap. 8, 18].
3. The result is a novel reification of the scientific picture about the 'objective' world.
4. Modularly redeemable validities and denotations of systems participators achieved by the implementation of non-normative boundary conditions (biomodulation, gene transfection, synthetic biology, etc.) show that the living world cannot be betrayed [2, 5, 9, 10]. This epistemic dualism contradicts the general desideratum for monistic scientific descriptions based on reductionistically derived investigations [15].
5. The living world of biological systems draws on evolutionarily constrained boundaries—as indicated by the diverse rationalizations for tumor-immanent normative notions—and provides specific access to combined modularized interventions [5, 15].

Evolution-generating communicative practices can be comprehended as something 'in the world', which does not fictively or arbitrarily emerge, because rules constituted or communication-technically 'allowed' by the living world may be uncovered by communicative interaction with socialized biological systems.

The reciprocal specific interaction between experimental or interventional practices implementing non-normative boundary conditions and the biological system itself finally contributes to uncovering communication-related rules supporting the 'metabolism' of evolution via the systems living world as 'vis a tergo'.

The failure of reductionistically derived interventional approaches based on the insufficient recognition of the performative subjectivity of systems objects shows that the characteristic communicative expression is generated by the interaction between the communicative context and the respective systems participator (chap. 11).

The alternative to the introduction of a living world would be the assumption of some deeply grounded biological perspectives [16, 17]: Then, biological systems would disintegrate into the particularism of suggested relevant cuttings of the 'living world' in the sense of neopragmatism [18, chap. 12]. Darwinian 'selection' as a possible principle to explain evolution history would be one of these perspectives [19].

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Index

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