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Targeting the Tumor Microenvironment: From Understanding Pathways to Effective Clinical Trials

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Abstract

It is clear that tumor cells do not act alone but in close interaction with the extracellular matrix and with stromal cells in the tumor microenvironment (TME). As our understanding of tumor cell-stroma interactions increased over the last two decades, significant efforts have been made to develop agents that interfere with these interactions. Here, we discuss four different therapeutic strategies that target the TME, focusing on agents that are at the most advanced stage of preclinical or clinical development. We end this review by outlining some of the lessons we have learned so far from the development of TME-targeting agents.

Keywords

Tumor Microenvironment; Targeted Therapy; Preclinical and Clinical Trials

Introduction

During the second half of the 20th century, much of our focus in cancer biology and therapy has been on genetic and epigenetic alterations in malignant cells that are drivers in the malignant process (1). However, it is now quite clear that tumor cells do not act alone but in close interaction with the extracellular matrix (ECM) and with non-genetically altered stromal cells that constitute the tumor microenvironment (TME) (2) -- thus, tumors are in fact “malignant organs”. The interactions between tumor cells and the TME have a deep influence on cancer progression and contribute to almost all of the hallmarks of cancer (3).

The stromal cells that interact with tumor cells not only originate from neighboring tissues but can also be actively recruited from the bone marrow as endothelial progenitor cells, myeloid and lymphoid inflammatory cells, and mesenchymal cells. Once in the TME, they become “educated” as tumor-associated macrophages (TAM), tumor- (or cancer-) associated fibroblasts (TAF or CAF), or vascular and perivascular cells. Education of stromal cells is also a dynamic process where non-malignant cells progressively switch from a neutral or anti-tumorigenic role toward a pro-tumorigenic role. When these cells are targeted by

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therapies, it is therefore critical to take into consideration the specific stage at which they are in such an education process.

The mechanisms of communication between tumor cells and the TME are complex but fall into two main categories, contact-dependent mechanisms that involve cell-cell and cell-ECM adhesion molecules and contact-independent mechanisms in which soluble molecules such as growth factors, chemokines and cytokines, and soluble subcellular organelles including microvesicles and exosomes play an essential role. Ultimately, these interactions activate via juxtacrine and paracrine mechanisms, signaling pathways in malignant cells and in non-malignant stromal cells that can inhibit or favor tumor progression (4). As our understanding of tumor cell-stroma interactions has increased and pathways involved have been better characterized, significant efforts have been made over the last decade to identify, develop and test therapeutic agents interfering either with the recruitment of stromal cells into the TME, with tumor cell-stromal cell interaction or with specific pathways activated by the TME.

In this review, we will discuss four different therapeutic strategies that primarily target the TME, focusing on agents that are at the most advanced preclinical or clinical stages of development. Agents that specifically target the immune system and are used for immunotherapy are not discussed here since they have been the subject of recent comprehensive reviews (5, 6).

Strategy 1: Targeting the Tumor Vasculature

The vascularization of a tumor is an intricate process that is tightly regulated by pro- and anti-angiogenic factors produced by both malignant cells and non-malignant cells through autocrine and paracrine signaling pathways. The onset of angiogenesis, known as “angiogenic switch”, is induced when the pro-angiogenic factors are favored (7). Vascular endothelial growth factor (VEGF/VEGF-A) is the predominant pro-angiogenic factor involved in endothelial cell (EC) activation (8), but many other growth factors are also pro-angiogenic, such as fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and epidermal growth factor (EGF) (7). Different from the normal vasculature characterized by an organized formation of mature EC covered with pericytes, the tumor vasculature is typically abnormal with a deficit in pericytes and perivascular cells, and an increased permeability, resulting in a leaky vascular system (9). It is also now well recognized that bone marrow-derived endothelial progenitor cells contribute to the tumor vasculature through a process known as vasculogenesis (10).

Targeting VEGF signaling

Considering the important functions of VEGF signaling in tumor angiogenesis, growth, invasion and metastasis, several VEGF- and VEGFR- antagonists have been developed. The first effective agent, which is also the first one to provide clinical evidence supporting the validity of the concept of targeting the TME, is bevacizumab (Avastin). This recombinant humanized VEGF-neutralizing monoclonal antibody (mAb) was approved by the Food and Drug Administration (FDA) in 2004 for the treatment of metastatic colon cancer and subsequently several other metastatic cancers in combination with cytotoxic therapies. Other effective inhibitors of VEGF signaling include small molecule receptor tyrosine kinase inhibitors (e.g., sunitinib/Sutent, sorafenib/Nexavar, and pazopanib/Votrient) and receptor-specific antibodies (e.g., IMC-1121B/Ramucirumab, a mAb against VEGFR-2). Sunitinib, sorafenib, and pazopanib have already been approved by the FDA, in most cases for advanced renal cell carcinoma. This type of cancer is not only highly angiogenic but its tumor angiogenesis is predominantly driven by VEGF, which explains why four different VEGF-pathway targeting drugs have all been approved for this type of malignancy (11).

IMC-1121B/Ramacurimab has shown a favorable therapeutic index with early evidence of both stable disease and partial responses in a phase I trial of patients with advanced solid tumors (12) and is currently undergoing several phase III clinical trials in patients with breast and gastric cancer but its efficacy has not yet been demonstrated.

Beyond targeting VEGF signaling

Highlighting the need to develop more effective anti-angiogenic agents was the recent withdrawal of Avastin by the FDA from its previously approved indication for patients with metastatic Her-2 negative breast cancer, due to reports that Avastin had no favorable therapeutic efficacy and significant toxicity (bleeding, heart attack or heart failure, elevated blood pressure, and organ perforation) (13). This again emphasizes the fact that we should not assume VEGF-dependent angiogenesis to be present in all cancer types at the same level as in renal cell carcinoma. For example, the results of an analysis of a large number of primary breast cancer specimens from various types of disease progression that identified 6 different proangiogenic growth factors including VEGF and FGF, indicated that there was a greater level of pro-angiogenic growth factor redundancy in more advanced disease stage (14). This type of angiogenic pathway redundancy is now well accepted as the major resistance mechanism for VEGF-pathway targeting agents (15-17).

Thus, multiple anti-angiogenic agents targeting other proangiogenic signaling pathways are currently undergoing development.

Targeting FGF signaling

Emerging evidence suggests that the upregulation of FGF provides a mechanism of resistance to anti-VEGF therapy. The most advanced FGF/FGFR-targeting agent so far developed is BMS-582664 (brivanib), an oral dual inhibitor of VEGFR and FGFR tyrosine kinases, presently tested in multiple phase II/III clinical trials (18). In a recent phase II open-label study in patients with advanced hepatocellular carcinoma who had failed prior antiangiogenic treatment with sorafenib, brivanib has shown promising antitumor activity with a manageable safety profile (19).

Targeting PDGF signaling

PDGF and its receptors PDGFR have been detected in diverse human cancers, and PDGFR is upregulated in the tumor vasculature, particularly in pericytes, where it plays a central role in their proliferation, migration, and maturation (20). PDGF signaling also regulates angiogenesis by inducing VEGF expression and by recruiting pericytes to mature blood vessels (21). SU6668, a small molecule kinase inhibitor for PDGFR, VEGFR and FGFR, has currently completed phase I clinical trials in patients with advanced solid tumors. The results of this trial, however, have shown relatively low plasma levels of the free drug because of its high protein binding, discouraging further clinical development (22).

Targeting EGFR signaling

EGF receptor (EGFR) is a member of the HER growth factor receptor family, a group of homologous receptor tyrosine kinases known to modulate normal cell growth and differentiation (23). In solid tumors, VEGF and EGFR pathways are linked, thus promoting angiogenesis (24), and resistance to anti-EGFR therapy is accompanied by an increase in VEGF levels (25). Because tumor cells and tumor-associated EC both express EGFR, targeting EGFR has a dual function of inhibiting both tumor cells and EC. Several EGFR-neutralizing antibodies (e.g., cetuximab, panitumumab) and small molecule inhibitors (e.g., erlotinib, gefitinib) have been approved by the FDA and have been incorporated into the

standard care for different types of cancers, implying the significance of targeting both tumor cells and TME in cancer therapy.

Strategy 2: Targeting Cancer-Associated Inflammation

The presence of innate and adaptive immune cells in tumors was initially considered to be the sign of an effective attack of the immune system against cancer. It is now well recognized that immune cells can also promote cancer initiation, progression, and metastasis (26). Our understanding of this dual role of immune cells in cancer progression is centered on the concept of polarization. Immune cells can be polarized toward either a typically anti-tumorigenic T_H1-type or toward a T_H2-type that is pro-tumorigenic (27). The polarization of the immune system is characterized by changes in metabolic pathways and the production of cytokines by immune cells. TAM, which can be polarized toward a T_H2-type, have emerged as being among the most important players in a pro-tumorigenic inflammatory reaction. Their contribution to cancer angiogenesis, cancer metastasis, and drug resistance is now well recognized (28). As a result, strategies to inhibit their pro-tumorigenic activity have been actively pursued and two major strategies aimed at targeting TAM and targeting the pro-tumorigenic inflammatory pathways have been developed so far.

Inhibiting TAM

The recruitment of macrophages by tumors is driven by chemokines such as CCL2 (monocyte chemotactic protein-1 or MCP-1) and colony stimulating factor-1 (CSF-1) (29), and these factors also promote the polarization of macrophages toward a T_H2-type (also reported as M2). Targeting these chemokines has thus been actively tested in cancer therapy. CNTO 888, a neutralizing antibody to human CCL2, has proven to have anti-cancer activity in a preclinical model of prostate cancer (30). Bindarit, a small molecule inhibitor of CCL2, was recently shown to have anti-cancer activity in preclinical models of prostate and breast cancer, causing a significant decrease in the infiltration of TAM and myeloid-derived suppressor cells in tumors (31). Other studies have shown that anti-CSF-1 antibodies and antisense oligonucleotides suppress macrophage infiltration and the growth of xenografted mammary tumors in mice (32, 33). Tyrosine kinase inhibitors of CSF-1 receptor (also known as c-fms), Ki20227 and JNJ-28312141, suppress osteolysis in a bone metastasis mouse model (34) and inhibit angiogenesis and bone metastasis in preclinical models of solid tumors and acute myeloid leukemia, respectively (35). These favorable preclinical results support their further clinical development.

Inhibiting inflammatory pathways

Numerous signaling pathways become activated upon interaction between tumor cells and immune stromal cells. Inhibitors for several of these pathways have been developed.

Targeting IL-6/JAK/STAT3 signaling pathway

Because of its central role in cancer-associated inflammation and other diseases like rheumatoid arthritis and lupus, the IL-6/JAK/STAT3 signaling pathway has been the focus of much attention over the last several years (36). Strategies to inhibit this pathway have focused on 1) blocking IL-6/IL-6R interaction, 2) blocking JAK phosphorylation of STAT3, and 3) inhibiting STAT3 DNA binding activity.

An IL-6 neutralizing mAb (siltuximab/CNTO-328) is being tested in a number of phase I/II clinical trials. When given in combination with mitoxantrone and prednisone in patients with metastatic castration-resistant prostate cancer, siltuximab was found to be of no additional benefit (37). However, it has been shown to stabilize disease and induce partial responses in patients with platinum-resistant ovarian cancer (38) and metastatic renal cell carcinoma (39).

Further studies will be needed to demonstrate its clinical efficacy. Ruxolitinib (INCB018424), a JAK2 specific inhibitor, is currently being tested in a phase III clinical trial in patients with myelofibrosis (40) and in phase II clinical trials in adults and children with a variety of cancers. A STAT3 transcription factor decoy that inhibits STAT3 DNA binding was tested in a phase 0 (biological effect) clinical trial, in head and neck tumors, and shown to bind with high affinity to STAT3 protein, to reduce cellular viability, and to suppress STAT3-dependent gene expression in tumor cells (41).

Targeting NF-κB signaling pathway

Nuclear factor (NF)-κB is a family of transcriptional factors that play pivotal roles in both inflammation-induced tumorigenesis and anti-tumor immunity (42, 43). NF-κB signaling is also critical in bone metastasis, being activated in osteoclasts upon stimulation of the receptor activator of NF-κB (RANK) by its ligand RANKL produced by tumor cells and by osteoblasts (44). Activation of most forms of NF-κB depends on phosphorylation-induced ubiquitination of the inhibitor of κB (IκB) proteins, which is modulated by the IκB kinase (IKK) complex (45).

To date, most efforts in targeting NF-κB signaling have concentrated on the development of specific inhibitors for the IKKβ subunit(46). PS-1145, a small molecule inhibitor of IKKβ, was found to be effective in subtypes of diffuse large B-cell lymphomas where NF-κB is activated (47). Because IKKβ has many NF-κB independent functions, including the induction of genes involved in autophagy, the alteration of actin dynamics and the regulation of the phosphorylation of multiple proteins, its inhibition has resulted in undesired effects (45). Other approaches that target NF-κB include 1) the inhibition of proteasomes to disrupt the degradation of IκB, by agents like bortezomib (Velcade) used in multiple myeloma (48), and 2) the direct targeting of NF-κB-dependent gene expression with agents like curcumin and arsenic.

Targeting TNF-α signaling pathway

In animal models, TNF-α at high doses has a potent anticancer activity through stimulation of T cell-mediated immunity and the destruction of blood vessels (49). Conversely, at physiologic doses, TNF-α enhances tumor growth and metastasis by inducing the production of pro-inflammatory cytokines and chemokines (e.g., IL-6), pro-angiogenic factors (e.g., VEGF, FGF) and proteases (e.g., matrix metalloproteinases or MMPs) (49, 50). TNF inhibition could thus have a dual effect.

At least two TNF-α antagonists have been tested in patients with advanced cancers. The first antagonist, etanercept (Enbrel), a recombinant humanized soluble p75 TNF-α trapping receptor that binds to TNF-α, has been tested in several phase I/II clinical trials. Etanercept in combination with rituximab (a mAb against B cells) was well tolerated and produced durable remissions in chronic lymphocytic leukemia patients (51). When used as a single agent in patients with recurrent ovarian cancer, it resulted in disease stabilization and some partial responses (52). It has also shown safety and biological activity in metastatic breast cancer patients (53). The second TNF-α antagonist, infliximab (cA2, Remicade), a chimeric human-mouse mAb, has been demonstrated to produce stable disease or partial responses as a single agent in renal cell carcinoma (54). It did not provide additional therapeutic benefits when combined with sorafenib in patients with advanced renal cell carcinoma (55).

Targeting COX2 signaling pathway

COX2, an inducible enzyme responsible for the synthesis of prostanoids (prostaglandins, prostacyclin and thromboxane) from the precursor arachidonic acid, is another major player in inflammation. COX2 overexpression is observed in both cancer cells and stromal cells

during tumor progression and in response to anti-cancer therapies. Elevated levels of COX2 in tumors are associated with increased angiogenesis, tumor invasion, and tumor cell resistance to apoptosis (56, 57).

Celecoxib, a COX2 inhibitor, has shown efficacy in preventing colon cancer in patients with inflammatory bowel disease, but was not adopted clinically due to its unacceptable cardiac toxicity (58). The therapeutic administration of celecoxib has been tested in a number of phase II/III clinical trials for multiple cancers with mixed clinical results. Whereas it has improved the efficacy of conventional therapy in patients with locally advanced undifferentiated nasopharyngeal carcinoma (59), it had no effect in hormone-sensitive prostate cancer (60) or advanced non-small cell lung cancer (61). Its value as a therapeutic agent is uncertain at this point.

The side effects of COX2 inhibitors may result from the suppression of prostanoid production, including the protumorigenic PGE_2. A possible way to selectively block PGE_2 is to inhibit prostaglandin E synthase (PGES) (62). Selective inhibitors of this enzyme, which is overexpressed in cancer cells (63), may rationally be proposed in substitution for COX inhibitors. Another arachidonic acid-metabolizing enzyme, 5-lipoxygenase (5-LOX), also plays an important role in inflammation and inflammation-associated carcinogenesis and is over-expressed in many cancers. As a result, 5-LOX inhibitors have been developed. It was recently shown that the combination of zileuton (a specific 5-LOX inhibitor) and celecoxib prevented oral carcinogenesis through their joint inhibitory effects on arachidonic acid metabolism (64). Moreover, simultaneous treatment of premalignant and malignant human lung cell lines with a triple combination of one COX2 inhibitor (celecoxib) and 2 specific 5-LOX inhibitors, MK886 and REV5901, is more potent in suppressing growth and inducing cell death than a single or dual combination of these agents (65). Further clinical tests are however necessary to confirm the clinical effectiveness and safety of COX/LOX dual inhibitors.

Targeting TGF-β signaling pathway

TGF-β- has a dual function in cancer. It is tumor suppressive in pre-malignant cells and at the early stage of cancer development, but strongly pro-tumorigenic at later stages of cancer progression (66). Autocrine TGF-β signaling promotes epithelial-mesenchymal transformation (EMT) which increases cell invasion and metastasis. Paracrine TGF-β signaling stimulates angiogenesis and contributes to an immune-tolerant environment by suppressing T-lymphocytes and natural killer cells (67). TGF-β also has a central function in bone metastasis. It is released from the bone matrix during bone resorption by osteoclasts and contributes to a vicious circle that further fuels tumor growth and bone destruction (44, 68). Strategies to inhibit TGF-β activity include (69): 1) blocking the production of TGF-β with antisense molecules; 2) blocking the activity of TGF-β with neutralizing antibodies; 3) blocking the interaction between TGF-β and its receptors with soluble forms of TGF-β receptors; and 4) blocking TGF-β-mediated receptor signaling by small molecule tyrosine kinase inhibitors of TGF-βRII and TGF-βRIII. AP12009 (trabedersen), an 18-oligomer antisense phosphorothioate oligodeoxynucleotide that prevents the production of TGF-β2, is at the most advanced stage of development. On the basis of data demonstrating that TGF-β2 is over-expressed in more than 90% of high-grade gliomas, and that its levels are closely related to tumor progression, AP12009 was first tested in high grade glioma patients and showed an increase in 6 month median survival from 21.7 months to 39.1 months (70, 71). Furthermore, inhibition of TGF-β2 in tumor tissue leads to reversal of tumor-induced immune suppression as well as inhibition of tumor growth, invasion, and metastasis (72). In a phase II clinical trial for patients with brain tumors, the addition of AP12009 to conventional chemotherapy resulted in a significant increase in 14-month tumor control rate

in anaplastic astrocytoma patients and an increase in 2 and 3 year survival in a subgroup of high-grade glioma patients (73).

Strategy 3: Targeting the Communication between Tumor Cells and TME

Targeting Communication between Tumor Cells and ECM

Integrins are clustered at the cell surface in complexes known as focal adhesions, and play a central role in many normal physiological processes such as growth, anchorage-dependent differentiation, adhesion, motility, apoptosis and angiogenesis (74, 75). They are expressed by EC where they control angiogenesis and by tumor cells where they potentiate tumor metastasis by facilitating migration, survival and invasion. Integrin inhibition can thus affect both tumor cells and EC.

Cilengitide (EMD 121974), a cyclic RGD pentapeptide, was the first potent integrin inhibitor for both $\alpha_v\beta_3$ and $\alpha_v\beta_5$ that reached clinical testing (76). In a phase II trial for recurrent glioblastoma multiforme patients (77), cilengitide monotherapy was found to be well tolerated but exhibited modest antitumor activity. Intetumumab, (CNTO 95), a monoclonal antibody against the human α_v integrin, has shown a favorable safety profile and a trend towards improved overall survival in a phase II clinical trial of metastatic melanoma patients (78). In contrast, in another phase II trial of Stage IV melanoma patients, etaracizumab (MEDI-522), a mAb against the human $\alpha_v\beta_3$ integrin, did not provide any therapeutic benefit (79). Whether these inhibitors should be used alone or in combination with other therapeutic agents remains to be determined.

Targeting Communication between Tumor Cells and the Bone Microenvironment

The bone is among the most common metastatic sites in many different types of cancer, and the mechanisms involved in bone metastasis have been well characterized (80). Tumor cells secrete parathyroid hormone related peptide (PTHrP), which promotes the expression of RANKL at the surface of osteoblasts. By binding to its receptor RANK present on osteoclast precursor cells, RANKL promotes their differentiation into mature osteoclasts and their activation. RANKL is inhibited by a natural inhibitor, osteoprotegerin (OPG). Osteoclasts, PTHrP, and RANKL have thus been considered as therapeutic targets.

Targeting osteoclasts with bisphosphonates has been among the first successful strategies targeting the TME in cancer bone metastasis. Zoledronic acid, a nitrogen-binding bisphosphonate, is FDA approved for the treatment of patients with multiple myeloma and documented bone metastases from solid tumors, in conjunction with standard antineoplastic therapy. Despite having a limited effect on overall survival, this agent significantly decreases the number of skeletal morbidities in these patients (81). A humanized mAb targeting PTHrP, CAL, has completed a phase I/II study for breast cancer patients with bone metastasis but its clinical efficacy has not yet been reported. Denosumab, a fully humanized mAb that specifically targets RANKL and was originally approved by the FDA in postmenopausal women with risk of osteoporosis, is now approved for patients with cancer bone metastasis and for the prevention of skeletal related events in patients with solid tumors (82). In 2011, the FDA also granted approval for denosumab to increase bone mass in patients who are at high risk of fracture from receiving androgen deprivation therapy for non-metastatic prostate cancer or in patients receiving adjuvant aromatase inhibitor therapy for breast cancer.

Strategy 4: Targeting Hypoxia in the TME

A major metabolic feature of the TME is that it is hypoxic. Tumor cells adapt to this hypoxic environment using mechanisms that promote genomic instability and deregulate

DNA repair systems and the expression of proto-oncogenes (83). Altogether these mechanisms promote therapeutic resistance. There is also recent evidence that hypoxia through the expression of hypoxia-inducible factor-1α (HIF-1α) and -2α (HIF-2α) regulates the polarization of macrophages (84).

Bioreductive Prodrugs

A first therapeutic strategy that takes advantage of the unique hypoxic environment of tumors consists of the use of bioreductive prodrugs, which are activated by enzymatic reduction (bioreduction) into toxic products in hypoxic tissues (85). Among those bioreductive prodrugs was Tirapazamine (TPZ) that, despite promising results from preclinical studies and early-phase clinical trials, was abandoned as it failed to demonstrate any benefit in combination with chemotherapy or radiation therapy in several phase III trials in patients with non-small cell lung and head and neck cancer (86). Other bioreductive prodrugs currently being investigated in phase I/II clinical trials include TH-302 and AQ4N, which have both shown minimal toxicity in a phase I trial in patients with advanced malignancies (87, 88). In contrast, others like PR-104 have shown severe hematological toxicities that may limit their use in the clinic (89). The future of these agents is thus uncertain and further preclinical and clinical testing is required.

Targeting HIF-1α Signaling

Multiple agents developed as HIF inhibitors are currently being evaluated in clinical trials. Among the most advanced ones are EZN-2968, a HIF-1α antisense mRNA, and PX-478, a small molecule inhibitor of HIF derived from melphalan by oxidation of the nitrogen mustard moiety (90). Both of these agents have completed phase I clinical testing but no data on toxicity and efficacy have been reported yet.

Targeting Unfolded Protein Response (UPR) Signaling

Severe hypoxia leads to increased levels of unfolded proteins in the endoplasmic reticulum (ER), leading to the induction of UPR. UPR suppresses protein synthesis, stimulates protein degradation in the ER, and activates apoptosis or autophagy to resolve ER stress (91). One therapeutic strategy to target UPR signaling seeks to exacerbate ER stress in order to overwhelm the UPR on the assumption that it is near capacity in hypoxic cells (91).

Agents having such action include inhibitors of 26S proteasome (such as bortezomib, nelfinavir) and inhibitors of HSP90 (such as IPI-504, 17-AAG). Bortezomib has been approved by the FDA for multiple myeloma and mantle cell lymphoma and nelfinavir is still under investigation in phase I trials. When tested in a phase II trial, IPI-504 (retaspimycin), used as a single agent, had a minimal effect on tumor burden and an unacceptable toxicity in patients with castration-resistant prostate cancer (92). In contrast, 17-AAG (tanespimycin) had significant anticancer efficacy when combined with trastuzumab in patients with HER2-positive metastatic breast cancer in a phase II trial (93), clearly warranting further clinical investigation.

What Have We Learned so Far?

With more than a decade of experience using drugs that more specifically target the TME, it is appropriate to ask the question of “what we have learned?” as we continue to move forward. Among the many lessons learned, we want to highlight three in particular in this review.

Lesson 1: Targeting the TME can be toxic

As clinical studies using agents targeting the TME were initiated, it was somewhat assumed that these agents would be much less toxic than cytotoxic agents used in chemotherapy. It is now clear that this is not the case as many initially promising studies on agents targeting the TME had to be discontinued because of unacceptable and often unanticipated toxicity. The reasons are multiple and complex.

The first and most obvious reason is that many agents targeting the TME alter the homeostatic balance in normal organs and tissues, as was well illustrated in the case of small molecule inhibitors of MMPs. Cell and ECM proteins in tissues are not static but are subject to a constant and dynamic turnover that requires a delicate balance between growth and death, synthesis and degradation, and activation and inhibition of specific proteolytic processes or pathways. By disturbing the balance between MMP activation and inhibition, inhibitors of MMPs were found to increase collagen deposition in tissues and to cause musculoskeletal pain and inflammation that although reversible, necessitated stopping their use in one third of the patients (94). Similarly, the use of Avastin in clinical trials has been associated with significant toxicities, in particular hypertension, proteinuria, thromboembolism and congestive heart failure, caused in part by a decrease in NO production associated with vasoconstriction and a lack of vascular integrity causing proteinuria, hemorrhage and thrombosis. As previously discussed, these side effects have resulted in the FDA's decision to remove Avastin from previous approval in breast cancer (95). However, this is not always the case. One would have anticipated that inhibition of proteasomes by drugs like Bortezomib would be associated with major toxicities, considering the central role the proteasome complex is playing in degradating ubiquinated proteins. Somewhat surprisingly this drug which has been tested in more than 200 clinical trials, is relatively well tolerated and has controllable toxicity, primarily neuropathy and thrombocytopenia (96).

A second reason is that agents targeting the TME often target cells or pathways that are not always the enemy of cancer cells. This is again well illustrated in the case of MMP inhibitors, which were found to inhibit not only MMPs used by tumor cells for invasion but also MMPs involved in the production of anti-angiogenic peptides by cleavage of precursor proteins (94). Similarly, targeting NF-κB or TGF-β can have significant side effects as these pathways, depending on the state of tumor progression, can either promote or inhibit tumor growth. As agents targeting inflammation trail behind anti-angiogenic therapies in clinical development, it will be important to remember the lessons learned from the testing of MMP and angiogenesis inhibitors and to consider the role of these agents within the dynamic context of an immune system that can be pro- as well as anti-tumorigenic.

Lesson 2: Targeting the TME does not prevent resistance

Because agents targeting the TME are affecting non-malignant cells, it was initially assumed that--in contrast to their malignant counterparts—they would not be subjected to genomic instability, and that the acquisition of resistance would not be a significant problem. This, however, has not been the case as we have seen with Avastin. Based on a systems biology approach, it could in fact be predicted that the redundancy of angiogenic signals might limit the efficacy of anti-angiogenic monotherapies (97). In support of this idea, it has been recently shown that tumors become refractory or even evade the inhibition of a single pro-angiogenic pathway like VEGF by upregulating compensatory angiogenic factors or other pathways that are favorable to tumor cells (16, 98). Another important consideration is that drugs that target the TME often target pathways activated not only in non-malignant cells but also in malignant cells which are prone to become intrinsically drug-resistant or to activate by-pass mechanisms.

Lesson 3: Targeting the TME requires knowing the optimal biological dose (OBD)

Somewhat unfortunately, agents targeting the TME have been tested in clinical trials often at their maximal tolerated dose (MTD), which has been the strategy for chemotherapy trials where it is often assumed that more is better. However, this may not be the case when targeting the TME. One of the objectives in targeting the TME is in fact to re-establish a disrupted homeostasis in a “malignant organ” rather than to flip the balance in an entirely opposite direction by “over-hitting” the target at MTD. The complex relationship between the ECM and tumor cells illustrates well this latter point.

Determining the OBD of TME-targeting agents may thus be more important than finding their MTD. This requires: 1) that the drug hits the target, 2) that the target is altered by the drug, 3) that the tumor is altered by hitting the target, and 4) that giving a higher dose fails to improve outcomes further (99). It will therefore be critical to develop “companion” biomarkers that can serve as indicators of the effect of agents targeting the TME. Sensitive functional imaging techniques and molecular markers such as cytokines and chemokines profiles and immunometrics and immunoscore (6) are likely to play an important role in our evaluation of clinical trials targeting the TME.

Conclusions

It has now been almost 10 years since the FDA's approval of Avastin as the first drug specifically targeting the TME in cancer. Since this milestone, we have witnessed a dramatic increase in the pre-clinical development and clinical testing of agents that target the TME. Several of them are already part of the standard treatment in patients with specific cancers, while others are still at the early stages of clinical testing. So far, strategies targeting the tumor vasculature appear to be the most successful, as demonstrated by the large number of agents approved by the FDA. Strategies inhibiting the pro-tumorigenic inflammatory response of the TME are rapidly being developed with agents either targeting TAM and their recruitment or pro-tumorigenic inflammatory pathways activated in tumor cells and stromal cells. Inhibition of tumor cell-stroma interactions is another strategy where new agents are also rapidly emerging.

As more agents targeting the TME are proposed for clinical trials, it is important to remember that there are several questions in regard to their activity and place in our therapeutic arsenal against cancer that remain to be answered. One, for example, is whether these agents are most effective when used alone or in combination with chemotherapy, radiation therapy, or other molecularly targeted therapy. Recent data suggest that the TME is an important contributor to therapeutic resistance (100). Thus combining agents targeting the TME with chemotherapeutic agents to prevent the emergence of minimal residual disease from drug-resistant tumor cells may be critical. If those TME-targeting agents are used in combination with myeloablative agents, it will be important to determine whether some agents such as those inhibiting the recruitment of bone marrow-derived cells should be specifically used between courses of intensive and myelosuppressive therapy to prevent the release of precursor cells into the blood circulation (101). Another question is whether some agents would be more effective at early stages on cancer progression and others at later stages (i.e., TGF-β inhibitors), considering the dynamic changes in the pro- or anti-tumorigenic functions of the TME during cancer progression. As discussed above, the question of the development of resistance to agents targeting the TME remains an important consideration.

As we are rapidly entering the era of precision cancer treatment and are using a genomic and biomarker-integrated approach to determine the best front line therapies for cancer patients, it will be equally important to develop reliable biomarkers that indicate the type of TME

present in a specific tumor. For example, precise molecular or cellular information on the nature of immune cells present in a specific tumor and on their polarization could be as critical as the identification of the driver(s) mutation(s) that should be targeted. The development of such TME biomarkers and molecular signatures should be an exciting and valuable research direction in the TME over the next several years.

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Table 1

Agents targeting the tumor microenvironment

Category	Pathway	Molecular Target	Agent	Mechanism of Action	Indication	Clinical Status
Tumor Vasculature	VEGF signaling	VEGF	Bevacizumab (Avastin)	humanized monoclonal Ab against VEGF	metastatic colorectal cancer, metastatic renal cell carcinoma, non-small cell lung cancer, glioblastoma	FDA approved
		VEGFR	IMC-1121B (Ramucirumab)	VEGFR-2 neutralizing Ab	HER-2 negative breast cancer, non-small cell lung cancer, hepatocellular carcinoma, gastric adenocarcinoma	Phase III
		VEGFR	Sunitinib (Sutent)	TKI of VEGFR1-3, PDGFR, c-Kit	advanced renal cell carcinoma, pancreatic neuroendocrine tumors, gastrointestinal stromal tumors	FDA approved
		VEGFR	Sorafenib (Nexavat)	TKI of VEGFR2, PDGFRβ, Raf	advanced renal cell carcinoma, unresectable hepatocellular carcinoma	FDA approved
		VEGFR	Pazopanib (Votrient)	TKI of VEGFR1-3, PDGFR, c-Kit	advanced renal cell carcinoma, soft tissue sarcoma	FDA approved
	FGF signaling	FGFR	BMS-582664 (Brivanib)	FGFR and VEGFR	hepatocellular carcinoma, colorectal cancer	Phase II/III
	PDGF signaling	PDGF	SU6668	PDGFR, VEGFR, and FGFR,	not specific	Phase I
	EGFR signaling	EGFR	Cetuximab (Erbitux)	monoclonal Ab against EGFR	metastatic colorectal cancer, head and neck squamous carcinoma	FDA approved
		EGFR	Panitumumab	monoclonal Ab against EGFR	metastatic colorectal cancer	FDA approved
		EGFR	Erlotinib	TKI of EGFR	non-small cell lung cancer and pancreatic cancer	FDA approved
		EGFR	Gefitinib	TKI of EGFR	non-small cell lung cancer	FDA approved
Inflammation	Macrophage recruitment	CCL2	CNTO888	neutralizing antibody to human CCL2	prostate cancer model	Preclinical
		CCL2	Bindarit	a small molecule inhibitor of CCL2	prostate and breast cancer model	Preclinical
		CSF-1	mouse CSF-1 antisense oligonucleotides, CSF-1 or CSF-1 receptor small interfering RNAs	CSF-1 antibodies and antisense oligonucleotides	breast cancer model	Preclinical
		CSF-1 receptor	Ki20227	TKI of CSF-1 receptor	bone metastasis mouse model	Preclinical
		CSF-1 receptor	JNJ-28312141	TKI of CSF-1 receptor	lung adenocarcinoma, breast cancer, acute myeloid leukemia models	Preclinical
	IL-6/JAK/STAT3 signaling	IL-6	CNTO-328 (Siltuximab)	monoclonal neutralizing Ab against IL-6	prostate cancer, multiple myeloma, metastatic renal cell carcinoma, ovarian cancer	Phase I/II
		IL-6R	Tocilizumab	an IL-6R blocking antibody	N/A	Preclinical
		JAK1/2	AZD1480	small molecule inhibitor of Jak1/2	solid tumors	Phase I

Category	Pathway	Molecular Target	Agent	Mechanism of Action	Indication	Clinical Status
		JAK1/2	Ruxolitinib (INCB018424)	small molecule inhibitor of Jak1/2	advanced breast cancer, advanced hematologic malignancies, metastatic pancreatic adenocarcinoma	Phase II
		STAT3	STAT3 decoy	STAT3 DNA competitor	head and neck cancer	Phase 0
	NF-κB signaling	IKK	PS-1145	small molecule inhibitor of IKK kinases	B-cell lymphoma cells	Preclinical
		26S proteasome	Bortezomib (Velcade)	inhibitor of 26S proteasome disrupting Iκb degradation	multiple myeloma, mantle cell lymphoma	FDA approved
		NF-κB	Curcumin	phytochemical extract from turmeric that suppress NF-kB activation and NF-kB-dependent gene expression	colon cancer	Phase I/II
		NF-κB	Arsenic	NF-kB inhibitor	acute promyelocytic leukemia	Phase I/II
	TNF-α signaling	TNF-α	Etanercept (Enbrel)	recombinant inhibitory TNF-α receptors	recurrent ovarian cancer, metastatic breast cancer, chronic lymphocytic leukemia	Phase I/II
		TNF-α	Infliximab (cA2, Remicade)	neutralizing Ab against TNF-α	renal cell carcinoma	Phase II
	COX2 signaling	COX2	Celecoxib	COX2 inhibitor	non-small cell lung cancer, nasopharyngeal carcinoma, hormone sensitive prostate cancer	Phase II/III
	TGFβ signaling	TGFβ	AP12009 (trabedersen)	antisense phosphorothioate oligodeoxynuclotide for TGFβ2	anaplastic astrocytoma, glioblastoma	Phase II/III
		TGFβ	soluble TGFβ receptor II/III	TGFβ ligand traps	metastatic pancreatic cancer model, colon cancer model	Preclinical
		TGFβ	soluble TGFβ receptor II fusion protein	TGFβ ligand traps	pancreatic cancer and melanoma model	Preclinical
		TGFβR	LY2109761	small molecule inhibitor for TGFβ-receptor kinase	metastatic pancreatic and colorectal cancer model	Preclinical
		TGFβR	SB-431542	small molecule inhibitor for TGFβ-receptor kinase	N/A	Preclinical
		interaction between TGFβ ligands and receptors	2G7	neutralizing Ab minimizing the interaction between ligands and receptors	breast cancer model	Preclinical
		interaction between TGFβ ligands and receptors	1D11	neutralizing Ab minimizing the interaction between ligands and receptors	breast cancer model	Preclinical
Interactions between tumor cells and their microenvironment	Integrin signaling	$\alpha_v\beta_3$ and $\alpha_v\beta_5$	Cilengitide	RGD peptide as an inhibitor of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins	glioblastoma, prostate cancer	Phase II
		$\alpha_v\beta_3$	Etaracizumab (Medi-522)	monoclonal Ab against $\alpha_v\beta_3$ integrin	prostate cancer, colorectal cancer, melanoma	Phase I/II
		α_v	Intetumumab (CNTO 95)	monoclonal Ab against α_v integrin	prostate cancer, melanoma	Phase I/II
	Osteoclast survival signaling	Farnesyl pyrophosphate synthase	Zoledronic acid (Zoledronate, Zometa, Reclast, Aclasta)	Nitrogen-binding bisphosphonate	Multiple myeloma, bone metastases from solid tumors, hypercalcemia caused by malignant tumors	FDA approved

Category	Pathway	Molecular Target	Agent	Mechanism of Action	Indication	Clinical Status
	RANK/RANKL signaling	RANKL	Denosumab (Prolia, Xgeva)	monoclonal Ab against RANKL	bone metastases from solid tumors, patients at high risk of fracture with non-metastatic prostate cancer on androgen-deprivation therapy or adjuvant aromatase inhibitor therapy for breast cancer	FDA approved
	PTHrP signaling	PTHrP	CAL	monoclonal Ab against PTHrP	bone metastatic breast cancer	Phase I/II
Hypoxia	Hypoxic environment	Hypoxia	Tirapazamine (SR4233)	bio reductive prodrug of an aromatic N-oxide generating a DNA-reactive free radical	cervix cancer, advanced head and neck squamous carcinoma	Phase III(closed)
		Hypoxia	TH-302	bio reductive prodrug of a 2-nitroimidazole-based nitrogen mustard	multiple myeloma, advanced renal cell carcinoma, soft tissue sarcoma, pancreatic adenocarcinoma, non-small cell lung cancer	Phase I/II/III
		Hypoxia	PR-104	bio reductive prodrug of a 3,5-dinitrobenzamide nitrogen mustard	leukemia, solid tumors	Phase I/II
		Hypoxia	AQ4N	bioreductive prodrug of an aliphatic N-oxide generating a DNA intercalator	Glioblastoma, Non-Hodgkin's Lymphoma; Leukemia	Phase I/II
	HIF-1α signaling	HIF-1α	EZN-2968	HIF-1α antisense mRNA	advanced solid tumors, lymphoma	Phase I
		HIF-1α	PX-478	small molecule inhibitor of HIF-1α	advanced solid tumors, lymphoma	Phase I
	UPR signaling	26S proteasome	Bortezomib (Velcade)	inhibitor of 26S proteasome that cause ER stress	multiple myeloma, mantle cell lymphoma	FDA approved
		26S proteasome	Nelfinavir	inhibitor of 26S proteasome that causes ER stress, impairs HIF-1α and VEGF expression	colorectal cancer	Phase I/II
		HSP90	IPI-504 (Retaspimycin)	small molecule inhibitor of HSP90 that is in part responsible for protein folding	castration-resistant prostate cancer, gastrointestinal stromal tumors, breast cancer, lung cancer	Phase I/II
		HSP90	17-AAG (Tanespimycin)	small molecule inhibitor of HSP90 that is in part responsible for protein folding	metastatic breast cancer	Phase II/III

Abbreviations: VEGF, vascular endothelial growth factor; Ab, antibody; VEGFR, vascular endothelial growth factor receptor; FDA, Food and Drug Administration; HER2, human epidermal growth factor receptor 2; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; PDGF, platelet-derived growth factor; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; IL-6, interleukin-6; IL-6R, interleukin-6 receptor; JAK, Janus kinase; STAT3, signal transducer and activator of transcription 3; NF-κB, nuclear factor-kappaB; IKK, inhibitor of kappaB kinase complex; TNFα, tumor necrosis factor α; COX2, prostaglandin-endoperoxide synthase 2; TGFβ, transforming growth factor β; TGFβR, transforming growth factor β receptor; CCL-2, chemokine (C-C motif) ligand 2; CSF-1, colony stimulating factor-1; RGD, Arginine-Glycine-Aspartic Acid; RANK, receptor activator of nuclear factor kappa-B ligand; RANKL, receptor activator of nuclear factor kappa-B ligand; PTHrP, parathyroid hormone-related protein; HIF-1α, hypoxia-inducible factor-1α; UPR, unfolded protein response; ER, endoplasmic reticulum; HSP, heat shock protein. N/A, not available.