Chapter 19
Targeting Pro-Angiogenic TGF-β Signaling in the Tumor Microenvironment

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Abstract The recently developed targeted anti-angiogenic agents have been introduced into clinical practice over the course of the past decade. High hopes were placed on targeting the VEGF signaling pathway in endothelial cells following the preceding successful drug development in the preclinical setting. Indeed, the therapeutic efficacy observed in mouse models of cancer has in some cases been translated into clinical benefit for patients. Nevertheless, many anti-angiogenic therapies have failed to provide substantial improvement in survival in large phase III clinical trials. In the search for attractive and complementary angiogenic signaling pathways, the TGF-β family stands out as one of the most interesting. Our expanding knowledge on TGF-β signaling in the tumor vasculature has led to the development of specific inhibitors targeting TGF-β, ALK1, and endoglin. Many clinical trials exploring the concept of targeting pro-angiogenic TGF-β signaling are currently underway, and preliminary reports are encouraging. Here, we will discuss opportunities and challenges of targeting the TGF-β system for anti-angiogenic therapy of cancer.

Keywords Angiogenesis • BMP • Cancer • Targeted therapy • TGF-β

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A. Moustakas and K. Miyazawa (eds.), *TGF-β in Human Disease*,
DOI 10.1007/978-4-431-54409-8_19,

Abbreviations

ALK	Activin receptor-like kinase
BMP	Bone morphogenetic proteins
EC	Endothelial cell
HHT	Hereditary hemorrhagic telangiectasia
TGF-β	Transforming growth factor-β
VEGF	Vascular endothelial growth factor

19.1 Introduction

Based on several successful preclinical studies, the oncologic clinical panorama anticipated with hope the use of anti-angiogenic therapies to combat cancer. Nevertheless, the introduction of vasculature targeting drugs led to limited clinical benefit measured in months, with almost negligible effect on overall survival and mainly in combination with cytotoxic agents. Recent studies provide evidence for the development of resistance and acquisition of a more aggressive and invasive phenotype in tumors that have been treated with vascular endothelial growth factor (VEGF) anti-angiogenic approaches. This points to the need for additional examination of the biology of tumor-nurturing blood vessels, in order to accurately pinpoint promising and novel anti-angiogenic targets. Furthermore, it is reasonable to assume that curing cancer is a complex task that requires combined strategies aiming at different tumor cellular compartments, in addition to aiming at the ample spectrum of signaling networks that are commonly deregulated in malignant cells.

Often altered in neoplasms and involved in multiple cellular functions, the large transforming growth factor-β (TGF-β) family is, as a result, an obvious candidate for aiming such efforts. More specifically, because TGF-β signaling encompasses a number of vascular restricted receptors, including activin receptor-like kinase (ALK)1 and endoglin, they represent attractive anti-angiogenic therapeutic targets for cancer. For a thorough summary of endothelial cell signaling by components of the TGF-β family, please see Chap. 14.

19.2 TGF-β Signaling as Tumor Angiogenesis Targets

19.2.1 Clinical Relevance of ALK1 in Cancer

ALK1 expression in the early developing mouse embryo coincides with sites of vasculo- and angiogenesis (Roelen et al. 1997), with prevailing expression in developing arterial endothelium, while nearly absent from small capillaries (Seki et al. 2006). During early mouse development ALK1 is strongly expressed, whilst it tends

to be concealed in the adult quiescent vasculature. The expression of ALK1 is reversibly turned on during neo-angiogenesis events in wound healing or in tumors (Seki et al. 2006).

Information about pattern and intensity of ALK1 expression in human normal and cancerous tissues is unfortunately still very scarce. The public Human Protein Atlas program (Ponten et al. 2008) has characterized ALK1 immunostaining of both normal and neoplastic tissues. In this study, ALK1 exhibited frequent strong expression most notably in neuronal cells of the cerebral cortex, hippocampus, ventricle, and cerebellum, in the gall bladder, GI tract, and in tubular cells in the kidney, in line with the murine ALK1 expression profile (Panchenko et al. 1996; Wu et al. 1995). The same organization identified ALK1 prevailing expression in neoplasms in the colorectal tract, pancreas, stomach, and thyroid, as well in malignant lymphomas and melanomas. A preliminary descriptive study reported a weak but widespread pattern of expression in the vasculature of normal tissues, including positive staining in lymphatic tissues, lung, intestines, and pancreas. In a follow-up study by the same entity, ALK1 was found to be extensively present on tumor blood vessels, especially in lymphomas, and malignant tissues of the prostate, skin, thyroid, kidney, ovary, lung, pancreas, and liver (Hu-Lowe et al. 2011). Thorough studies analyzing the prognostic strength and diagnostic significance of ALK1 are highly warranted.

19.2.2 Inhibitory Drugs Targeting ALK1

Given the extensive literature describing paradoxical effects of signaling stemming from ALK1 in endothelial cells (ECs), the prediction for the net outcome for acute inhibition of ALK1 in the context of cancer is an intricate task. Furthermore, the complex ligand-receptor binding specificity and/or redundancy within the TGF-β family add multiple hurdles to the estimate of therapeutic efficacy, benefit and secondary effects for the various ALK1 inhibitors currently under development.

Small molecules comprising broad inhibitory spectrum against bone morphogenetic protein (BMP) type I receptor kinases, including ALK1, have been generated. Smad-dependent and -independent signaling, induced by BMP can be blocked by compounds, such as dorsomorphin and its structural derivative LDN-193189 (Boergermann et al. 2010; Yu et al. 2008b). These drugs can be useful and potent in inhibiting BMP type I receptor signaling in a range of diseases and familial syndromes (Yu et al. 2008a); however, their effect on BMP-induced tumor angiogenesis remains to be determined. Nonselective inhibitors also raise the concern of off-target effects, as documented for dorsomorphin (Cannon et al. 2010; Vogt et al. 2011). Further development of small molecule compounds with a more specific inhibition profile aiming primarily at BMP type I receptors, and specifically towards ALK1, should be considered.

Antibodies or soluble extracellular receptor domain traps use may alternatively provide a tighter inhibition of specific ALK1 activity. ALK1 inhibitors are currently

under development for cancer treatment as anti-angiogenic drugs. Up to now, several biological inhibitors against ALK1 have been generated for in vivo use. Firstly, Pfizer is currently conducting phase I-trials of PF-3446962, a fully human monoclonal antibody against ALK1. Secondly, the use of an ALK1-Fc fusion protein (amino acids 23–119 of mouse ALK1) was reported by Genentech studies on hematogenous and lymphatic vessel development in the mouse (Niessen et al. 2010). Another ALK1 targeting agent is ACE-041 (mouse counterpart RAP-041, amino acids 22–117 of mouse ALK1), a human ALK1-Fc fusion protein, currently undergoing a phase II clinical trial, coordinated by Acceleron Pharma.

19.2.3 PF-3446692

Preclinical tumor studies using PF-3446962 were recently reported (Hu-Lowe et al. 2011). ALK1 blockade exhibited attenuation of VEGF-induced EC proliferation and tube formation in vitro. In addition, therapeutic treatment with the ALK1-neutralizing antibody delays tumor growth in vivo in MDA-MB-231 breast carcinoma and M24met/R melanoma mouse models.

The anti-hALK1 antibody from Pfizer has completed phase I clinical trials for patients with advanced solid tumors. Preliminary evidence from the trial indicates that the anti-hALK1 antibody reduced the amount of ALK1-positive circulating ECs, which were found to be present in increased levels in advanced stage cancer patients. The PF-3446962 antibody was well tolerated up to 10 mg/kg lacking concerning adverse events in all 44 patients participating in the phase I trial. The most common side effects included transient thrombocytopenia and asymptomatic elevation of pancreatic enzymes. Hence, preliminary observations evoke encouraging clinical activity where three partial responses were observed in patients who have previously received fruitless anti-angiogenic regimens.

The mechanism of action of this antibody has recently been described (van Meeteren et al. 2012). The anti-hALK1 antibody selectively recognizes human ALK1 and interferes with BMP9-induced signaling in ECs. Moreover, the anti-hALK1 antibody competitively obstructs BMP9 and TGF-β binding to ALK1 receptor and prevents BMP9-dependent recruitment of endoglin into the angiogenesis-mediating signaling complex, which may eventually hinder the BMP9/ALK1 proangiogenic effects.

19.2.4 ACE-041/RAP-041/dalantercept

The effects of RAP-041 were recently analyzed in the RIP1-Tag2 transgenic mouse model of pancreatic neuroendocrine tumorigenesis (Cunha et al. 2010). The RIP1-Tag2 tumors readily express ALK1 exclusively on ECs, mirroring the expression profile of common vascular markers during tumor progression.

RIP1-Tag2 mice treated with 2 weekly doses between 1 and 12 mg/kg RAP-041 results in a dose-dependent retardation of tumor growth and the highest dose effectively prevents further tumor expansion. Incidentally, Alk1 heterozygous mice under the RIP1-Tag2 context recapitulated the effects obtained by systemic inhibition by RAP-041. Specificity of the ALK1-targeting drug treatment was validated by decreased expression of ALK1 downstream target genes in tumors from mice treated with the RAP-041. Alternative studies with the same drug demonstrated that RAP-041 possesses growth-inhibitory response in orthotopic MCF-7 breast carcinomas (Mitchell et al. 2010).

Acceleron Pharma recently concluded a phase I trial for the human ALK1-Fc fusion protein ACE-041, which interestingly only binds and neutralizes BMP9 and BMP10, but not to any of the TGF-β isoforms (Cunha et al. 2010; Mitchell et al. 2010). Thirty seven patients with solid tumors or refractory multiple myeloma were recruited to assess safety and tolerability of ACE-041, as well as changes in tumor metabolism evaluated by ^{18}F deoxyglucose positron emission tomography. ACE-041 regimen included an administration every three weeks and was well tolerated at doses up to 1.6 mg/kg. Common collateral effects comprised peripheral edema, fatigue, nausea, headache, anorexia, and anemia. However, toxicities usually associated with VEGF inhibition, such as hypertension, proteinuria, GI tract perforation, and hemorrhaging, were not observed, possibly because ALK1 is predominately present in the actively cycling endothelium within the tumor milieu and preferentially localizes in arterial endothelium, whereas VEGFRs present a more global distribution (Seki et al. 2003).

In this clinical study, one patient with refractory head and neck squamous carcinoma exhibited a partial response (>30 weeks) and six other patients exhibited stable disease. Rapid reduction in tumor metabolic activity (>20%) was observed in ten patients, measured by FDG-PET scanning. Of note, many of the patients included in this trial had been previously inefficiently treated with other therapeutic regimens, including VEGF-targeting drugs.

After such an encouraging phase I clinical trial, the ALK1 inhibiting agent ACE-041 is undertaking a phase II clinical study with an expanded cohort on head and neck squamous carcinoma patients (http://clinicaltrials.gov). Apart from its anticancer specification, ACE-041, is further being developed for testing in age-related macular disease treatment.

19.2.5 Possible Side Effects from ALK1 Inhibition

Anti-angiogenic therapies, mainly in the form of inhibitors of VEGF signaling, have been in routine clinical use for several years for various malignancies. Side effects from inhibiting angiogenesis are in general milder than those arising from conventional chemotherapeutic treatment and include bleeding, hypertension, fatigue, and nausea. Specifically, given the causal relationship between impaired ALK1 signaling and hereditary hemorrhagic telangiectasia (HHT)-related symptoms,

inhibition of ALK1 signaling in the vasculature may induce de novo arteriovenous malformations and hemorrhaging. In fact skin telangiectasis was observed in a patient treated with ACE-041, validating the on-target effect of the drug and indicating that the appearance of telangiectases may be useful as a surrogate marker for efficacy. Loss-of-function mutations in ALK1 linked with hereditary pulmonary arterial hypertension (Machado et al. 2009) highlight the risk for pulmonary circulation hemodynamics perturbations with ALK1 inhibitors. Interestingly, complete blockade of ALK1 signaling triggered by both BMP9 and BMP10 resulted in lung hemorrhaging (Ricard et al. 2012), an organ that should thus be primarily monitored. Furthermore, given the high expression levels of BMP9 in the liver, this organ should be also carefully monitored (Bidart et al. 2012). Finally, as ALK1 is reported to be expressed by, and conceivably important for lymphatic ECs, cells of the pituitary gland, hepatic stellate cells, chondrocytes, and pancreatic ductal cells, special care should be taken to record adverse events from the treatment of ALK1 inhibitors related to processes regulated by these particular tissues (Alexander et al. 1996; Finnson et al. 2008; Niessen et al. 2010; Ungefroren et al. 2007; Wiercinska et al. 2006).

19.3 Clinical Relevance of Endoglin in Cancer

Endoglin positive intratumoral microvascular density strongly correlates with poor prognosis in cancer, being associated with shorter survival and relapse-free survival rates (Bernabeu et al. 2009; Fonsatti et al. 2010). Moreover, subcutaneous tumor neovascularization and growth are impaired in endoglin heterozygous mice, reiterating the relevance of endoglin in tumor angiogenesis (Duwel et al. 2007). An enormous body of literature highlights the potential of endoglin as a tumor vasculature marker in preclinical and clinical studies (Bredow et al. 2000; Costello et al. 2004; Fonsatti et al. 2000; Fonsatti et al. 2010). In this respect, endoglin can be a more specific marker for new, immature tumor vessels, unlike other EC markers, which are expressed in both mature and immature vessels (Beresford et al. 2006).

High levels of endoglin expression have therefore been confirmed in several experimental models, such as breast, prostate, and colorectal cancer, for example (Akagi et al. 2002; Beresford et al. 2006; Wikstrom et al. 2002).

Endoglin expression has also been associated with predisposition of colorectal mucosa dysplastic tissues evolution into fully developed carcinomas (Akagi et al. 2002). Furthermore, in prostate cancer endoglin-positive microvessel density correlated with Gleason score, metastasis and tumor stage (Wikstrom et al. 2002). Paradoxically, the same study indicated that endoglin-positive vessels were more poorly covered by α-smooth muscle cells and correlated with survival. Another study in colorectal cancer patients reported that vessel density evaluated by endoglin staining significantly correlated with survival. Additionally, other reports correlate loss of endoglin with prostate cancer progression and aggressiveness

(Liu et al. 2002) and endoglin presence with decrease in prostate tumor cell motility (Craft et al. 2007). Our own work strengthens the vascular role of endoglin as protective against tumor cell metastatic seeding (Anderberg et al. 2013).

Endoglin exists in the body in two different forms: membrane-bound and circulating (Bernabeu et al. 2009). Levels of soluble endoglin have been reported in plasma of pregnant women suffering from preeclampsia (Venkatesha et al. 2006) and also in patients suffering from colorectal, breast, prostate, and leukemic cancers, correlating positively with metastatic disease (Fujita et al. 2009; Karam et al. 2008; Li et al. 2000; Takahashi et al. 2001b). However, the role of soluble endoglin in cancer is still poorly understood. Since soluble endoglin contains the binding site for different ligands of the TGF-β family, it may act as a scavenger of circulating ligands, preventing their binding to the functional receptors, hence interfering with vascular function and angiogenesis (Perez-Gomez et al. 2010). An intriguing question concerns the source of soluble endoglin detected in cancer patients. Since endoglin levels are higher in tumor vessels, soluble endoglin may conceivably derive from shedding from tumor ECs and, importantly, it may represent a surrogate marker of angiogenic activity (Fonsatti et al. 2003).

19.3.1 TRC105

The potential of endoglin-targeting monoclonal antibodies to be used as a therapeutic anti-angiogenic strategy in human cancer has received considerable support from preclinical studies.

Intravenous systemic administration of anti-endoglin monoclonal antibody TRC105 was shown to suppress angiogenesis, tumor growth, and metastasis without overt toxicity in mice (Seon et al. 1997; Tabata et al. 1999; Takahashi et al. 2001a). The combination of endoglin-targeting antibody with cyclophosphamide and doxorubicin was reported to exhibit synergistic antitumor efficacy in human skin/SCID mouse chimeras, including in metronomic regimens (Shiozaki et al. 2006; Takahashi et al. 2001a).

A total of 50 patients with advanced refractory cancer disease were included in TRC105 phase I clinical trial (Seon et al. 2010). Doses up to 1 mg/kg were administered every other week to assess efficacy, toxicity, and tolerability of TRC105. One patient with castrate-refractory prostate cancer remained in the study even after 3 years of TRC105 at 0.01 mg/kg with a complete response and bone scan normalization. In addition, an ovarian cancer patient presented with 6-month stable disease. Dose-limiting toxicities incorporate GI hemorrhage and anemia.

A phase I clinical trial has been initiated with breast metastatic cancer patients to determine maximum tolerated dose of TRC105 in combination with capecitabine, a DNA synthesis blocking agent, approved by FDA as adjuvant treatment for colon cancer, as well as, for metastatic breast and colorectal cancers (www.clinicaltrials.gov).

19.3.2 Endoglin-Fc

More recently, Acceleron Pharma characterized a soluble mouse and human endoglin extracellular domain fused to an immunoglobulin Fc domain (human endoglin amino acid sequence 26–359). This endoglin ligand trap binds specifically and with high affinity to BMP9 and BMP10 in vitro. This agent significantly impaired VEGF-induced chick chorioallantoic membrane assay in vivo. Finally, murine soluble endoglin extracellular domain acted as an anti-angiogenic factor decreasing blood vessel sprouting in VEGF/fibroblast growth factor-induced angiogenesis in in vivo angioreactors and tumor burden in the colon-26 mouse tumor model (Castonguay et al. 2011). Together these findings indicate an important role for soluble endoglin in the regulation of angiogenesis and evoke the prospective efficacy of endoglin-Fc as an anti-angiogenic therapeutic agent.

19.3.3 Possible Side Effects of Targeting Endoglin

Only two dose-limiting toxicities were reported in patients who received TRC105: one grade 4 gastric ulcer bleeding in a patient treated with 0.1 mg/kg after 4 days, which resolved spontaneously and one patient experienced grade 3 infusion reaction.

Based on a recent study, a careful follow-up of the patients treated with endoglin targeting antibodies should be in place, since the preclinical studies in mouse models, either endoglin heterozygous or endoglin-EC specifically ablated led to the emergence of worsened phenotypes with tumors acquiring a refractory behavior with increased metastatic seeding (Anderberg et al. 2013).

19.4 Clinical Relevance of TGF-β Signaling in Cancer

The role of TGF-β in cancer biology is complex and controversial, involving aspects of tumor suppression as well as tumor promotion. The ability of TGF-β to potently inhibit the proliferation of epithelial, endothelial, and hematopoietic cell lineages is central to the tumor-suppressive mechanism (Yingling et al. 2004). For example, the TGF-β receptors and their Smad signaling mediators are tumor suppressors that frequently become inactive in gastrointestinal, pancreatic, ovarian, and hepatocellular carcinomas, as well as in subsets of gliomas and lung adenocarcinomas (Bierie and Moses 2006a). As tumors evolve, they often become noncompliant to TGF-β-mediated growth inhibition and overexpress TGF-β, which in turn has a manifested autocrine impact on the biology of the malignant cells themselves. A tumor microenvironment that favors tumor growth and metastasis in a paracrine fashion is

therefore created. Interestingly, in breast carcinoma, glioblastoma, melanoma, and other cancer types, specific loss of TGF-β-mediated growth inhibitory responses often accumulate through alterations downstream of Smad, leaving the rest of the TGF-β pathway operational and available to co-opt in detriment of tumor progression (Massague and Gomis 2006).

19.4.1 Inhibitory Drugs Targeting TGF-β

Improvements in understanding how TGF-β impinges upon the tumor microenvironment have led to the development of TGF-β inhibitors for cancer treatment. Three categories of TGF-β inhibitors have been characterized: soluble antisense oligonucleotides, monoclonal antibodies, and small molecule inhibitors. At first sight, this pathway presents an attractive target for the development of cancer therapeutics that simultaneously attacks the tumor and its microenvironment. One could, thus, anticipate that TGF-β targeting drugs would have a very efficacious effect on malignant progression. Nonetheless, the ubiquitous and pleiotropic nature of TGF-β signaling and its dual role in tissue homeostasis and in tumorigenesis pose on this kind of inhibitory strategies a risk that cannot be underestimated in preclinical and clinical drug development programs. Multiple clinical trials targeting the TGF-β pathway are currently in progress (see Table 19.1).

19.4.2 TGF-β Antisense Compounds

The specificity of hybridization draws antisense oligonucleotides as targeted and functional therapeutic tools to selectively modulate the expression of a variety of genes involved in the pathogenesis of malignancies and other genetic diseases (Stahel and Zangemeister-Wittke 2003; Tamm et al. 2001; Tamm and Wagner 2006).

Trabedersen (AP-12009), developed by Antisense Pharma GmbH, is a synthetic antisense oligodeoxynucleotide designed to block the production and tumorigenic effects of TGF-β2. Trabedersen is indicated for the treatment of malignant brain tumors and other TGF-β2 overexpressing solid tumors, such as those of the skin, pancreas, and colon. Preclinical studies provided evidence that trabedersen reduced the secretion of TGF-β2 in cultured tumor cells and exhibited antitumor activity ex vivo. Chronic intracerebral or systemic administration of trabedersen does not cause life-threatening collateral effects in animals, confirmed in early clinical trials in advanced cancer patients. In the initial phase I and II open-label dose-escalation study the compound was able to significantly prolong the median time to relapse compared with the published relapse times for temozolomide chemotherapy (Bierie and Moses 2006b).

Table 19.1 Summary of TGF-β family targeting clinical trials implicated in cancer

Drug target	Malignant condition	Intervention	Study type	Status	Evaluations
TGF-β2	Advanced metastatic carcinoma	Trabedersen with GM-CSF gene modified autologous tumor cell vaccine	Phase I	Completed	Primary outcome: – Safety Secondary outcomes: – Time to progression – Effect on immune stimulation
TGF-β2	Pancreatic neoplasms Melanoma Colorectal neoplasms	Trabebersen	Phase I	Completed	Primary outcome: – Maximum tolerated dose – Dose-limiting toxicity Secondary outcomes: – Safety and tolerability – Plasma pharmacokinetics – Method to assess the urine Pharmacokinetic profile – Effect on TGF-β2 plasma levels – Antitumor activity:effect on tumor size and tumor markers
TGF-β2	Glioblastoma Anaplastic astrocytoma	Trabedersen	Phase IIb	Completed	Primary outcome: – Overall response rate evaluated by brain MRI scans Secondary outcomes: – Overall survival – Response rates – Progression-free survival – Time to progression – Time to response – Survival and variation of tumor size on brain MRI – Change in quality of life – Safety and tolerability

TGF-β2	Anaplastic astrocytoma Glioblastoma	Trabedersen	Phase III	Completed	Primary outcome: – Survival rate Secondary outcomes: – Progression rate – Time to death – Overall response rate – Tumor control rate – Duration of response – Time to progression – Survival rate – Quality of life
TGF-β	Advanced malignant melanoma or renal cell carcinoma	GC1008	Phase I	Completed	Primary outcome: – Maximum tolerated dose – Dose-limiting toxicity – Safety Secondary outcomes: – Pharmacokinetic and dynamic
shFurin	Colon carcinoma with liver metastasis	FANG vaccine	Phase II	Recruiting	Primary outcome: – Overall survival Secondary outcome: – Immune response
shFurin	Advanced melanoma	FANG vaccine	Phase II	Recruiting	Primary outcome: – Immune response Secondary outcome: – Survival

(continued)

Table 19.1 (continued)

Drug target	Malignant condition	Intervention	Study type	Status	Evaluations
TGF-β1, 2, 3	Metastatic breast cancer	GC1008 + Radiation	Safety	Recruiting	Primary outcome: – Adverse events Secondary outcome: – Local response rate by PET/CT
TGF-β RI	Malignant glioma	LY2157299 with Temozolomide-based Radiochemotherapy	Phase Ib/2a	Recruiting	Primary outcome: Phase 1b – Safety and tolerability – dose Primary outcome: Phase 2a – Confirm tolerability – Pharmacodynamics
TGF-β 1, 2, 3	Relapsed malignant pleural mesothelioma	GC1008	Phase II	Ongoing	Primary outcome: – 3-month PFS rate Secondary outcomes: – Progression-free survival – Immune response parameters

ALK1	Advanced solid tumors	PF-03446962	Phase I	Recruiting	Primary outcomes: – Maximum tolerated dose Secondary outcomes: – Pharmacokinetics – Evaluation of immunogenicity – Evidence of antitumor activity – Effect on vascular function – Circulating ECs, circulating endothelial progenitors, and soluble proteins related to ALK1 signaling – Safety
ALK1	Squamous cell carcinoma of the head and neck, renal cell carcinoma, endometrial cancer Glioblastoma, renal cell carcinoma, breast carcinoma, others	ACE-041/dalantercept	Phase II	Recruiting	Primary outcome: – Response rate Secondary outcome: – Adverse events – Serum concentration – Progression-free survival – Overall survival – Time progression – Duration of response – Disease control rate – Expression of ALK1 in tumor tissue and blood
Endoglin		TRC105	Phase I/II	Recruiting	Primary outcome: – Progression-free survival Secondary outcome: – Response rate

In a phase IIb trial, improved survival was observed in refractory high-grade gliomas patients who were administered trabedersen intratumorally, by convection-enhanced delivery (Bierie and Moses 2006b; Vallieres 2009; Yingling et al. 2004). One hundred and forty-five patients with histopathology of recurrent/refractory glioblastoma multiforme or anaplastic astrocytoma were randomly assigned to receive trabedersen or standard chemotherapy (Bogdahn et al. 2011). One patient achieved a complete response in all tumor sites after a single cycle of trabedersen, and several patients achieved tumor reductions of more than 80 % (Bogdahn et al. 2011; Yingling et al. 2004). In addition, disease stabilized in seven out of 24 patients, and two patients were in complete remission after treatment. However, this observation requires validation by an ongoing large-scale phase III randomized controlled trial (Vallieres 2009). Meanwhile, continued research on trabedersen should help determine the roles of TGF-β2 in cancer. As a result of these studies, trabedersen received orphan drug status in the European Union in 2002.

Overexpression of TGF-β2 in pancreatic malignancies is suggested to be a pivotal factor for malignant progression by inducing proliferation, immunosuppression, angiogenesis, and metastasis. Antitumor activity was reported for trabedersen in human pancreatic cancer cells and in an orthotopic xenograft mouse model of metastatic pancreatic cancer (Schlingensiepen et al. 2010), significantly reducing tumor growth, lymph node metastasis, and angiogenesis. According to these promising results, trabedersen appears attractive for the treatment of pancreatic adenocarcinoma but warrants further clinical insight.

19.4.3 TGF-β Antibodies

GC1008, the only TGF-β antibody in clinical trials, is a human IgG4 monoclonal antibody capable of neutralizing all TGF-β isoforms. Cohorts of patients with advanced malignant melanoma or renal cell carcinoma, who had failed at least one prior therapy were treated to assess effectiveness with GC1008 at doses from 0.1 to 15 mg/kg. Twenty-two patients were included (21 malignant melanoma, one renal cell carcinoma) and treated in this trial. No dose-limiting toxicities were observed and the highest dose of GC1008 was determined to be safe. Adverse events included skin rash, fatigue, headache, epistaxis, gingival bleeding, and gastrointestinal symptoms. So far, five patients reached stable disease and received extended treatment. Out of these five responders, three patients presented with metastasis shrinkage in the liver and other sites. One melanoma patient achieved a partial response with a lesion reduction of >75 %. All in all, GC1008 is well tolerated and neutralization of all TGF-β isoforms seems to hold promise in the treatment of one of the most aggressive cancer types, for which a phase II protocol is being expanded.

19.4.4 Small-Molecule Kinase Inhibitors Targeting TGF-β Receptors

There are several well-characterized small-molecule kinase inhibitors identified to target the TGF-β pathway, including A-80-01, LY364947, LY580276, LY566578, SB-505124, SD-093, SD-208, and SB-431542 (Bierie and Moses 2006a). The above ALK5 small-molecule inhibitors can, in general, also target ALK4 and ALK7 receptor, so results obtained through their application might not directly correlate with TGF-β/ALK5-specific signaling and dissecting specific effects stemming from each of the three receptors may require substantial validation (DaCosta Byfield et al. 2004; Inman et al. 2002; Peng et al. 2005). The inhibition effect on ALK4 and ALK7, in addition to the TGF-β-specific ALK5 effect on tumorigenesis, is yet to be determined. In particular ALK4 could be important, as its upregulation in the MMTV-Neu mouse model has been shown to correlate with activated Smad2 and loss of ALK5 expression (Landis et al. 2005). Furthermore, in the absence of ALK5, ALK4 mediates Smad2 phosphorylation and consequently αSMA expression during mouse yolk sac vasculogenesis (Carvalho et al. 2007).

Many of the TGF-β inhibitors were tested on breast and lung cancer xenograft tumor-bearing mice demonstrating significant tumor growth delay, providing supporting evidence for further development of these kinase inhibitors for clinical investigation (Lahn et al. 2005).

Treatment of syngeneic R3T or 4T1 tumor-bearing mice with orally supplied SD-208 inhibited primary tumor growth, angiogenesis, and number and size of metastasis. In contrast, SD-208 failed to inhibit R3T tumor growth or metastasis in athymic nude mice, suggesting that the antitumor effect is predominantly affecting immune responses (Ge et al. 2006).

SD-208 also inhibited kinase activity in SMA-560 gliomas in syngeneic mice, exhibiting a 25 % survival advantage in treated animals. No effect was detected on microvascular density. However, histological analysis of SD-208 treated tumors revealed an increase of infiltrated NK cells, CD8 T cell, and CD11b positive macrophages and neutrophils into responding tumors despite the negligible effects on overall tumor burden (Uhl et al. 2004).

Consistent with these observations, TGF-β signaling inhibition with SD-208 in the Panc-1 orthotopic pancreatic cancer model showed significant reduction of primary tumor weight and decreased incidence of metastasis. Histological evaluation revealed that SD-208 treatment reduced proliferation and induced apoptosis and vessel density in the tumor microenvironment. Additionally, an immune system contribution was observed with a greater B-cell infiltration in SD-208-treated tumors (Gaspar et al. 2007; Medicherla et al. 2007). Therapeutic benefit may be primarily driven by the host immune response against the tumor.

SD-208 also rendered beneficial effects on a model of melanoma bone metastasis (Mohammad et al. 2011). In this study 1205Lu melanoma cells were inoculated into the left cardiac ventricle of nude mice and metastasis formation and dissemination was monitored with and without administration of SD-208 prior to tumor cell

injection. SD-208 treatment prevented the development of osteolytic bone metastases compared with vehicle treated mice. Moreover, in mice where bone metastases did form, the size of the osteolytic lesions was significantly reduced after 4 weeks treatment.

All in all, these results evoke the therapeutic potential of TGF-β inhibitory agents by primarily interfering with TGF-β-mediated immune suppression and, thus, generate a more potent immune response, a result anticipated taking into consideration the phenotype obtained by complete genetic ablation of TGF-β1 in mice.

19.4.5 Possible Side Effects of TGF-β Targeting Agents

All the studies with genetically deficient mice demonstrate that TGF-β signaling pathway is essential during development. Nevertheless, the potential deleterious effects of TGF-β inhibition in adult mice still remain to be determined.

TGF-β systemic inhibition affects the entire tumor microenvironment, from the malignant epithelium to all stromal players. Given the importance of TGF-β in normal tissue homeostasis, broad inhibition is predicted to affect a wide array of normal cell functions. One concern with TGF-β targeting therapies is the potential for detrimental side effects, despite that long-term treatments with TGF-β inhibitors do not seem to significantly alter animal morbidity. In particular, because of the immune-mediated disease and lethality associated with the genetic ablation or inhibition of TGF-β signaling in mice, it was unclear if inhibiting this pathway to treat cancer would be compatible with patient survival when delivered for a sustained period.

In contrast to predictions of severe toxicity stemming from TGF-β signaling inhibition, it has been shown that lifetime exposure to systemic soluble TGF-β1, TGF-β3, or pan-TGF-β neutralizing antibody 1D11 in mouse models does not result in significant adverse effects (Ruzek et al. 2003; Yang et al. 2002; Yingling et al. 2004). The soluble TGF-βRII antagonist SR2F was expressed in mice to determine its impact on tumor growth and long-term effect. No severe toxicities were observed over the lifespan of these SR2F-overexpressing mice (Yang et al. 2002); however, lifelong inhibition of TGF-β at biological levels is sufficient to block experimentally induced or implanted mammary tumors and metastatic dissemination (Lahn et al. 2005; Yang et al. 2002).

Expansion of the hypertrophic and proliferation zones of the physes in the femur and tibia was observed in rats treated with TGF-βRI inhibitors (Lahn et al. 2005). Chondrocytes and chondroid matrix were also increased. The degree of hypertrophy was dose- and time-dependent and only affected young animals, presumably due to active physeal growth. The evident role of TGF-β in remodeling of bone claims for monitoring of bone metabolism in clinical studies with TGF-β inhibitors (Lahn et al. 2005).

The effect on the immune system is more complicated to estimate. In animals deficient for TGF-βRII expression on CD4+ T cells, antigen-activated T-cell

activity is enhanced and tumor growth inhibited (Chen and Wahl 2002; Lahn et al. 2005). However, increasing the activity of antigen-activated T cells by blocking TGF-β can result in autoimmune reactions.

The effect on the tumor vasculature is usually not a common readout for TGF-β inhibition therapeutic benefit. However, multiple are the preclinical reports providing evidence that blocking TGF-β signaling has a direct and indirect effect on tumor angiogenesis. Caution should be taken in VEGF-dependent tumors as type-I TGF-β receptor kinase inhibitors (SB-431542 and LY-2157299) and VEGF ligand availability were shown to synergistically promote blood-vessel formation via integrin 5 (Liu et al. 2009). Similar results were reported on SB-431542 facilitating proliferation and sheet formation of ESC-derived ECs (Watabe et al. 2003). VEGF is typically abundant in hypoxic tumors; hence, this may pose a risk for using TGF-β signaling inhibitors that may potentiate VEGF/VEGFR signaling and undesirably improve the angiogenic response.

19.5 Potential for Combinatorial Therapeutic Studies

The still scarce preclinical and clinical data currently available advocate for an anti-angiogenic and growth inhibitory effect of attenuated ALK1 signaling in cancer, hence sustaining the clinical development of drugs blocking ALK1. Furthermore, while ALK1 targeting monotherapies have been incredibly successful both in preclinical and clinical settings, so far, it is reasonable to anticipate that a combined targeted therapy can hold an agonistic effect fighting cancer disease. The multitude of cancers and heterogeneity within each malignancy combined with case-to-case special demands highly requests for incorporation of two or three different targeted drugs that have independently been beneficial. Future studies should therefore embark on such endeavurs.

Interestingly, VEGF levels are elevated in the aorta, lungs, liver, and intestine of ALK1-deficient mice (Shao et al. 2009), suggesting that double targeting VEGFR and ALK1 signaling pathways may not only be the route to a more efficacious treatment plan but may also circumvent the risk of refractoriness to anti-angiogenic drugs. Of note, bevacizumab was recently reported to attenuate VEGF-induced angiogenesis in ALK1 deletion-induced vascular malformations in the adult mouse brain (Walker et al. 2012).

In a human/mouse chimera tumor model, targeting human ALK1 decreased tumor vessel density and improved antitumor efficacy when combined with bevacizumab (Hu-Lowe et al. 2011). This study raised thus the question whether ALK1 signaling may be part of a set of adaptive mechanisms in tumors refractory to VEGF inhibition (Hu-Lowe et al. 2011). Moreover, ACE-041 used in combination with sunitinib impaired tumor growth in two xenograft models of VEGF-inhibitor resistant renal cell carcinoma, A498 and 786-O. These two models represent surrogates of renal cell carcinoma tumors that typically transiently shrink upon VEGF inhibition but quickly restore angiogenesis and resume tumorigenic program, despite continuation of treatment.

None of the ALK1 targeting studies have so far analyzed the potential adaptive effects of ALK1 targeting drugs in prolonged regimens. In order for ALK1 inhibitors to prevail and make a stand as opposed to VEGF targeting drugs, such analysis is mandatory.

Given the recent studies suggesting a regulatory crosstalk loop amongst BMP9/ALK1 and Notch signaling coordinating tip versus stalk cell specification, one may immediately anticipate possible therapeutic benefit arising from a combinatorial targeting of both pathways in tumor biology.

Neutralization of Dll4-Notch signaling in tumors results in excessive, nonproductive angiogenesis with subsequent inhibitory effects on tumor growth, due to poor perfusion-induced hypoxia (Noguera-Troise et al. 2006; Ridgway et al. 2006). Furthermore, Dll4 has been reported to mediate tumor resistance to bevacizumab in vivo as a compensatory mechanism to VEGF neutralization (Li et al. 2011). Pharmacological targeting of Dll4/Notch signaling in preclinical tumor models has been achieved by several different inhibitory strategies. Specific targeting with anti-Dll4 antibodies does not induce overt toxicity and Dll4 has, thus, emerged as an attractive target for anti-angiogenic cancer therapy (Kuhnert et al. 2011). As Dll4 inhibitors are entering clinical trials for the treatment of solid malignancies, this may pose a novel combinatorial therapeutic opportunity in breast, colon, and renal cancer, where Dll4 is selectively expressed by the endothelium of malignant tissues (Jubb et al. 2006; Jubb et al. 2009; Patel et al. 2005) and where patients may profit from combinatorial actions.

The phase I clinical trial on TRC105 evokes the combinatorial use of drugs impinging their inhibitory effect on both endoglin and VEGFRs, which have shown to generate an ameliorated therapeutic benefit both in the primary tumor burden but also on metastatic dissemination. In fact, a clinical trial analyzing the effect of TRC105 in combination with standard-dose bevacizumab in advanced solid tumors for which bevacizumab is indicated has been launched (www.clinicaltrials.gov). Interestingly, a study reported on a patient with HHT1 who had a substantiated response to bevacizumab (Bose et al. 2009). In this study, the epistaxis episodes became sparser and shorter. In different studies, a patient with HHT1 who received bevacizumab for malignant mesothelioma had a dramatic reduction in gastrointestinal bleeding from arteriovenous malformations (Flieger et al. 2006). Also, a patient with severe hepatic HHT who received six courses of bevacizumab no longer required liver transplantation and was well 6 months after completing the treatment (Mitchell et al. 2008). These studies strongly suggest a strong collaborative action of double targeting simultaneously endoglin and VEGF signaling to reacquire endothelial homeostasis. Furthermore, studies in our laboratory provide evidence for an agonistic effect by using endoglin and VEGFR targeting strategies reducing primary tumor burden and metastatic dissemination (Anderberg et al. 2013).

TGF-β has been reported to promote migration, invasion, and survival in breast cancer cells overexpressing the HER2 oncogene and to accelerate the metastasis of neu-induced mammary tumors in mice (Muraoka-Cook et al. 2005; Muraoka et al. 2003; Siegel et al. 2003). A clearer understanding of the molecular mechanisms underlying the crosstalk between TGF-β and HER2 has started to emerge.

In recent studies the synergistic effect of TGF-β and HER2 on tumor progression has been shown to likely be a combined result of two distinct features: loss of TGF-β tumor suppressive effect and gain of pro-survival and -migratory function through HER2-dependent mechanisms (Chow et al. 2011). In HER2-overexpressing breast cancer, this crosstalk results in increased cancer cell proliferation, survival and invasion, accelerated metastasis, and resistance to chemotherapy and HER2-targeted therapy (Chow et al. 2011). The transformed cellular context stemming from HER2 amplification disrupts the tumor suppressive role of TGF-β and promotes its oncogenic role. In turn, TGF-β potentiates oncogenic HER2 signaling by eliciting shedding of the ERBB ligands and clustering of HER2 with integrins (Wang et al. 2009). Blockade of TGF-β-HER2 crosstalk may suppress breast cancer progression and metastasis and enhance the efficiency of conventional therapies in patients with HER2-overexpressing breast cancer, which afflicts 25–30 % of all breast cancer patients. Moreover, targeting both TGF-β and EGFR/HER2 signaling can represent a stronger action towards the tumor neoendothelium as both have been shown to, among other functions, impair tumor angiogenesis (Izumi et al. 2002). More specifically, Herceptin, a HER2 neutralizing antibody, upregulates TGF-β target genes, such as PAI-1 and Thrombospondin-1 in vivo, rendering a decrease in vessel diameter and in tumor burden (Izumi et al. 2002).

Combinatorial studies on TGF-β inhibition with VEGFR impairment have not yet been established; however, some studies suggest that targeting both pathways may bypass the tumor adaptive actions by means of exploring alternative pathways to maintain tumor growth (Liu et al. 2009).

19.6 Perspective

The pleiotropy and intricacy of TGF-β family signaling conveys effects virtually in all cell types in the body. In cancerous disease, it is well established that TGF-β holds a bipolar role in carcinogenesis, acting as tumor suppressor during the initial stages of tumor development, whilst promoting tumor growth and metastatic spread in advanced stages (Ikushima and Miyazono 2010). As a consequence, the potential of using to our advantage the knowledge on TGF-β biology is still not fully embraced. The development and use of inhibitors of TGF-β family activity for the treatment of cancer may conduct to disparate effects depending on the stage of the disease. Furthermore, in ECs, the overall result of signaling from TGF-β family receptors is manifold and determined by a plethora of factors: ligand specificity or redundancy, engaged type I, type II receptors, and co-receptors (Cunha and Pietras 2011). In spite of the challenges of the elaborated signaling network outcomes from the TGF-β receptors in the various cell types of a tumor, the dividends for modulating the TGF-β network in therapeutic regimens may be rewarding. The current preclinical data and preliminary clinical results readily support the feasibility of using ALK1 and endoglin inhibitors as angiogenesis counteracting agents and TGF-β inhibitors affecting all the tumor cellular compartments.

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