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EVOLVING THERAPIES (R BUKOWSKI, SECTION EDITOR)

Endoglin for Targeted Cancer Treatment

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Abstract Endoglin is a homodimeric cell membrane glycoprotein receptor for transforming growth factor β and bone morphogenetic proteins. Endoglin is essential for angiogenesis, being densely expressed on proliferating endothelial cells and upregulated during hypoxia. Its expression is implicated in development of resistance to vascular endothelial growth factor (VEGF) inhibition. TRC105 is an antibody that binds endoglin and prevents endothelial cell activation. Targeting endoglin and the VEGF pathway concurrently improves treatment in vitro and appears to reverse resistance to bevacizumab in some refractory cancer patients. Randomized trials are under way to assess the clinical benefit of adding TRC105 therapy to bevacizumab therapy. Further trials are under way to assess the activity of TRC105 with small-molecule inhibitors of the VEGF pathway in renal cell carcinoma, hepatocellular carcinoma, and soft tissue sarcoma. Stratification of soft tissue sarcomas based on endoglin expression levels is proposed to identify patients most likely to benefit from TRC105 treatment. The development of a TRC105 antibody–drug conjugate is also described.

Keywords Endoglin · CD105 · TRC105 · Angiogenesis · Vascular endothelial growth factor resistance · Antibody–drug conjugate · Hypoxia · Bone morphogenic protein · Transforming growth factor β · Antibody · Radioimmunoconjugate · Renal cell cancer · Sarcoma · Glioblastoma · Prostate cancer · Hepatocellular cancer

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Introduction

Angiogenesis and Cancer

Angiogenesis is required for the survival and growth of solid cancers [1, 2]. It is generally accepted that solid cancers develop through two phases, an avascular phase and a vascular phase. During the initial avascular phase, tumors exist as small aggregates of malignant cells supported by simple diffusion of oxygen and nutrients. The progressive growth of solid cancers beyond clinically occult sizes requires the continuous formation of new blood vessels, a process known as angiogenesis. Tumor growth and metastasis require angiogenesis. Therefore, inhibition of tumor angiogenesis is a potentially effective strategy for the treatment of solid cancers.

Therapies directed against targets implicated in the development of tumor angiogenesis are attractive for many reasons. First, except for ovulation and wound healing, angiogenesis in adults is generally part of a pathologic process such as tumor growth or choroidal neovascularization. Second, treatments that interrupt tumor angiogenesis apply broadly to all solid cancers. Third, angiogenic targets are present either in the plasma or on the surface of endothelial cells, and therefore

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are readily accessible to antibody treatments, in contrast to targets expressed within tumors, which are more difficult for antibodies to access. Fourth, angiogenic targets on vascular endothelial cells are less prone to genetic mutation than targets expressed by genetically unstable cancer cells. As a result, development of resistance may be more predictable for agents that target endothelial cell functions than for those targeting cancer cells.

Indeed, agents that target pathways required for tumor angiogenesis have an important role in the treatment of cancer patients. The monoclonal antibody bevacizumab, which binds to the angiogenic cytokine vascular endothelial growth factor (VEGF), significantly prolongs overall survival for patients with advanced colorectal cancer and non-small-cell lung cancer when added to chemotherapy regimens [3, 4]. Bevacizumab is also effective therapy for malignant glioma, renal cell, and ovarian cancers [5–7]. Ziv-aflibercept is approved for the treatment of colorectal cancer, and orally available small-molecule VEGF receptor (VEGFR) tyrosine kinase inhibitors (TKI), including sunitinib, sorafenib, pazopanib, regorafenib, and axitinib, have been shown to prolong survival in patients with metastatic renal cell cancer, hepatocellular cancer, neuroendocrine cancers, sarcoma, and colorectal cancer [8–16].

Despite the clinical success of antiangiogenic agents that primarily target the VEGF pathway, patients uniformly develop resistance to this class of treatment or do not respond in the first place. The mechanisms by which antiangiogenic therapy potentiate the effects of cyotoxic drugs are as yet unclear, and much remains to be learned about how to combine the various available treatments optimally. Identification of subsets of patients more or less likely to respond to specific treatments continues to be an important goal. Generally, it is hypothesized that resistance to antiangiogenic agents occurs through the emergence of escape pathways rather than by acquisition of mutations to the VEGFR or its ligand [17, 18]. Endoglin is a leading angiogenic target implicated in VEGF resistance.

Endoglin and Angiogenesis

Endoglin (CD105) is a homodimeric cell membrane glycoprotein that was initially identified as a human leukemia-associated antigen [19] and was later found to be expressed densely on the surface of proliferating endothelial cells [20]. Endoglin is a transforming growth factor β (TGF-β) coreceptor that is required for angiogenesis [21], is densely expressed on proliferating vasculature [22, 23], and is upregulated following hypoxia induced by VEGF pathway inhibition. These properties render endoglin an attractive target for the antiangiogenic treatment of cancer [24], especially in combination with VEGF inhibitors.

Endoglin modulates signaling of multiple kinase receptor complexes of the TGF-β superfamily, including TGF-β receptors, activin-receptor-like kinases and activin receptors [22, 23]. Activation of TGF-β receptors in the absence of endoglin on quiescent endothelium results in SMAD 2/3 phosphorylation, which inhibits endothelial cell growth. However, the binding of bone morphogenic protein (BMP) to endoglin expressed on proliferating endothelium allows the phosphorylation of the activating SMADs 1, 5, and 8 (Fig. 1). The end result is release of the growth inhibitory effects of SMAD 2/3 activation on endothelium. Not surprisingly, inhibition of endoglin activation using anti-endoglin antibody acts synergistically with TGF-β to inhibit endothelial cell growth [24].

Endoglin expression is required for endothelial cell proliferation, and endoglin is upregulated in the setting of hypoxia through the induction of hypoxia-inducible factor (HIF) 1α [25, 26]. The expression of endoglin by endothelial cells is essential for the development of new vasculature, whether it be tumor vasculature or the choroidal neovascularization that is the pathologic determinant of age-related macular degeneration. Targeted inactivation (knockout) of endoglin results in defective vascular development, and mice lacking endoglin die in utero from the absence of angiogenesis at gestational day 10.5 [21]. Consistent with its requirement for angiogenesis in response to hypoxia, endoglin downregulation causes apoptosis of hypoxic endothelium [26].

Endoglin is critical for normal human blood vessel development [27]. Endoglin heterozygosity (i.e., haplotype insufficiency) causes a well-described syndrome of hereditary hemorrhagic telangiectasia type 1 (or Osler–Weber–Rendu syndrome). Osler–Weber–Rendu syndrome is a rare autosomal dominant genetic disorder characterized by localized angiodysplasia involving the nasal, buccal, and gastrointestinal mucosa and skin microvasculature [28]. Although the genotype is manifested in utero, the phenotype does not become apparent for many years following birth. Affected patients commonly present with epistaxis in the second decade of life. The phenotype of this disorder is limited to vascular effects, indicating the specific role of endoglin in vascular development [29].

Endoglin is highly overexpressed on the plasma membrane of proliferating endothelial cells in tumor vessels, including those of lung, breast, colorectal, gastric, liver, endometrial, renal cell, head and neck, and ovarian cancers (reviewed in [22]). In adult human tissues, endoglin expression is limited to proliferating endothelial cells, activated monocytes, and proerythroblasts, precursors of red blood cells [30].

Endoglin expression is a prognostic factor in solid tumor patients. High endoglin microvessel density (a marker of neovessels as opposed to mature vessels) is correlated with poor prognosis in clinical studies of breast cancer [31, 32], lung cancer [33], prostate cancer [34, 35], colorectal cancer

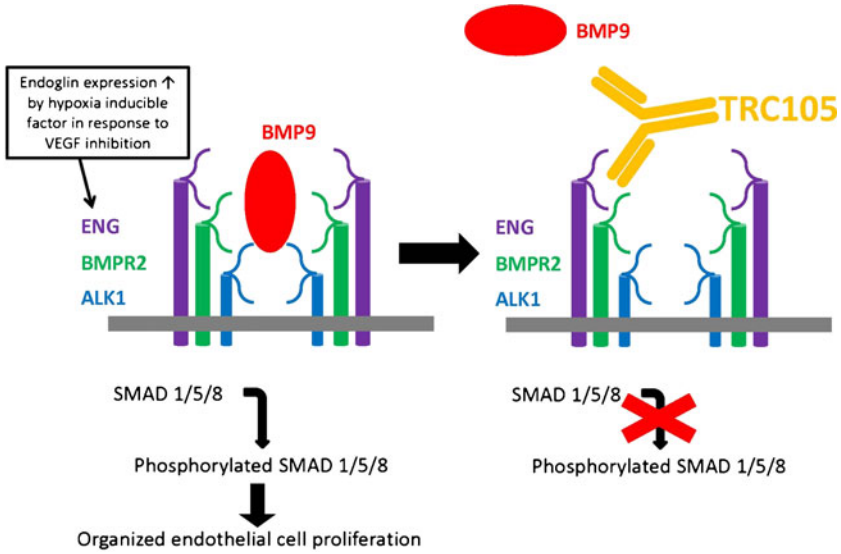

Fig. 1 Endoglin (*ENG*)-mediated signaling in proliferating endothelium and the effect of the anti-endoglin antibody TRC105. Bone morphogenic protein (*BMP*) binds to a receptor complex consisting of BMP receptor 2 (*BMPR2*), activin-receptor-like kinase 1 (*ALK1*), and endoglin ENG, thereby allowing phosphorylation of SMADs 1/5/8, which activates gene expression, allowing endothelial proliferation and maturation. ENG is expressed in response to hypoxia, including that caused by inhibition of vascular endothelial growth factor (*VEGF*). The monoclonal antibody TRC105 binds to ENG, competitively inhibiting BMP binding, thereby inhibiting angiogenesis. (Adapted from [69••])

[36, 37], ovarian cancer [38, 39], gastric cancer [40], endometrial cancer [41], astrocytic brain tumors [42], hepatocellular carcinoma [43], esophageal adenocarcinoma [44], head and neck cancer [45, 46], and renal cell cancer [47]. Plasma endoglin levels are prognostic in retrospective analyses. In one study, the mean plasma endoglin concentration in 76 patients with colorectal cancer was fourfold higher than the mean value in 40 healthy subjects without cancer [36]. In that study, a positive correlation was observed between the plasma endoglin concentration and the stage of disease. Further, endoglin microvessel density was an independent prognostic factor for survival.

Importantly, endoglin expression is upregulated in tumor endothelial cells in response to the hypoxia caused by inhibition of the VEGF pathway. Endoglin expression increased in human pancreatic cancer xenografts grown in mice treated with antibody that binds VEGF [48], and endoglin was one of only three genes of the 96 profiled that was significantly upregulated (the other two were placental growth factor and HIF-1α). As well, following treatment of human bladder cancers grown in mice with the antibody DC101, an antibody that binds VEGFR2 to prevent VEGF binding, endoglin-expressing vessels persisted at the tumor periphery and increased in density within the core tumor vasculature, allowing continued tumor growth [49].

Sennino et al. [50] determined that the endoglin ligand TGF-β was the most highly upregulated angiogenic factor (over 16-fold increased expression, whereas the expression of no other factor was more than fourfold elevated) in pancreatic cancers from RIP-Tag2 mice treated with antibody that binds VEGF. Tumors in RIP-Tag2 mice deficient in one copy of the endoglin gene exhibited a delayed onset of resistance to anti-VEGF agents, illustrating the therapeutic utility of combinatorial targeting of multiple angiogenic pathways for the treatment of cancer. Likewise, endoglin conditional knockout mice carrying subcutaneous lung tumors exhibited a dramatically reduced size of lung metastases following treatment with a VEGFR TKI [51]. Impressively, the tendency of agents targeting the VEGF or endoglin pathways separately to increase local invasion and distant metastasis [52] is reversed when therapies targeting the VEGF and endoglin pathways are used concurrently.

Further work indicates that stimulation of angiogenesis by VEGF and basic fibroblast growth factor (bFGF) requires TGF-β and BMP, as both ligands are required for VEGF- and bFGF-induced angiogenesis [53]. Cunha et al. [53] reported that the combined stimulation of endothelial cells with TGF-β and BMP-9 primes endothelial cells to the proangiogenic action of VEGF and bFGF, a synergy that was abrogated by inhibiting BMP binding to endoglin. In animal models, therapy targeting endoglin demonstrated antiangiogenic effects by inducing regression of established tumors as well as by preventing new tumor formation [54–57]. These effects have been noted with agents targeting other essential angiogenic pathways.

Renal Cell Carcinoma

Advanced renal cell carcinoma (RCC) is an ideal setting to test novel antiangiogenic combination regimens. Renal cell cancer with clear cell histologic features (70 % of renal cell cancers)

has aberrant von Hippel–Lindau protein expression that results in upregulation of HIF-α. HIF-α upregulates the expression of endoglin and VEGF, making RCC a highly vascular tumor [58]. Renal cancer stem cells isolated from nephrectomy specimens also express endoglin, allowing targeting of the tumor vasculature and stem cells in this tumor type [59].

Angiogenesis inhibition has been shown to be useful with multiple agents across multiple lines of therapy in the treatment of RCC. These features also allow efficient biomarker evaluations. The approval of axitinib in the second-line setting, with a relatively short progression-free survival of 4 months in a VEGFR TKI refractory setting, presents an opportunity to combine this VEGFR TKI with antibody to endoglin in an effort to prevent escape from VEGF inhibition.

Soft Tissue Sarcoma

The primary target for therapy directed at endoglin in most epithelial tumors is the proliferating endothelial cell, which expresses endoglin at very high density. Sarcomas, however, are candidates for direct tumor targeting, as endoglin is a mesenchymal stem cell marker that is expressed on many sarcoma subtypes [60, 61]. In one report, high surface expression of endoglin as evidenced by whole-cell flow cytometry was seen in seven of eight sarcoma cell lines [62]. Moreover, the level of endoglin expression correlated with proliferative capacity, and the addition of neutralizing anti-endoglin antibodies reversed the increase in proliferation. Endoglin-mediated SMAD phosphorylation was associated with plasticity and progression of aggressive sarcoma cell lines in vitro and in vivo [63].

Endoglin expression in human sarcoma tumor tissue has been reported by several groups. Gromova et al. [64] demonstrated endoglin on 26 of 49 human gastrointestinal stromal tumors, and higher expression correlated with more aggressive tumors and high-risk disease. They concluded that endoglin deserves consideration as a target for treatment of gastrointestinal stromal tumors. Endoglin expression on Ewing sarcoma has been reported, and high expression correlates with poor survival. Moreover, endoglin knockdown reversed the increased tumor cell plasticity, invasiveness, and anchorage-independent growth associated with endoglin expression [63]. Other endoglin-expressing sarcomas include angiosarcoma, osteosarcoma, leiomyosarcoma, malignant fibrous histiocytoma, Kaposi sarcoma, Wilms tumor, and chondrosarcoma [65–68].

In addition to endothelial cells and sarcoma cells, endoglin is found on the surface of benign stromal cells present in sarcomatous tumors. Morozov et al. [67] identified endoglin-positive sarcoma-derived benign mesenchymal stromal cells in primary sarcoma cultures. These cells express pericyte markers and cooperate with endothelial cells in vascular tube formation assays. In coculture experiments, endoglin-positive sarcoma-derived benign mesenchymal stromal cells as well as normal endoglin-positive human pericytes markedly stimulated the growth of sarcoma cell lines. By targeting endoglin-positive benign stromal cells, antibody to endoglin may further inhibit sarcoma progression.

TRC105

TRC105 is a novel human chimeric IgG1 that binds the endoglin orphan domain with high avidity (5 pM), and competitively inhibits BMP binding to prevent SMAD 1/5/8 phosphorylation [69••]. Anti-endoglin antibodies that bind outside the receptor orphan domain do not affect SMAD-mediated signal transduction and are inactive. Although TRC105 potently inhibits angiogenesis in vitro in response to VEGF and bFGF, inhibition of angiogenesis is not seen with anti-endoglin antibodies that fail to bind the orphan domain and competitively inhibit BMP binding. Notably, the ability of TRC105 to inhibit angiogenesis, which requires the interaction of endothelium with supporting stromal cells, was far more impressive than its ability to inhibit human endothelial proliferation in vitro, emphasizing the importance of assays that mimic the interplay between endothelial and perivascular cells during angiogenesis.

First-in-Human Study of TRC105

Fifty patients with advanced and refractory solid tumors were treated with escalating doses of TRC105 in a first-in-human trial [70••]. The on-target effects of TRC105 administration included hypoproliferative anemia, infusion reactions, and telangiectasia that were sometimes associated with mild superficial mucosal bleeding (epistaxis and gingival bleeding). The dose-limiting toxicity of single-agent TRC105 was hypoproliferative anemia associated with drug accumulation at 15 mg/kg weekly. The anemia was believed to result from TRC105-mediated suppression of proerythroblasts, the only cells in the bone marrow known to express substantial levels of endoglin [30]. The anemia could be easily monitored, and was easily reversed and treated without adverse sequelae.

Mucocutaneous telangiectasia was dose-dependent and developed early in the course of therapy at the maximum tolerated dose of 10 mg/kg given weekly by intravenous infusion. Telangiectasia is a notable clinical feature of patients with Osler–Weber–Rendu syndrome, a genetically inherited disease characterized by mutation of one copy of the endoglin gene [28]. This condition of endoglin haplotype insufficiency results in the development of mucocutaneous telangiectasia that causes epistaxis and gingival bleeding. Telangiectasia has

also been reported with other developmental therapeutics that target the endoglin signaling axis, including antibody to activin-receptor-like kinase 1 (an endoglin coreceptor), which is currently in early phase 2 testing, and a fusion protein to BMP (an endoglin ligand), which is also currently in early phase 2 testing [71, 72]. The development of telangiectasia, therefore, appears to represent a class effect for therapies targeting the endoglin pathway, and serves as confirmation of on-target effects. However, TRC105 did not cause pulmonary edema or thrombocytopenia characteristic of other therapeutics targeting the endoglin pathway [71, 72].

Infusion reactions were expected during dose escalation in the absence of premedication since TRC105 is an IgG1 antibody that engages antibody-dependent cellular cytotoxicity at low concentrations in vitro on binding endoglin on proliferating endothelium. These events generally occurred with the initial TRC105 dose and were treated with premedication. Infusion reactions were not observed in patients dosed at the recommended phase 2 dose of 10 mg/kg weekly, as these patients uniformly maintained measurable TRC105 serum levels at the time of redosing, so de novo binding of endoglin did not occur.

Classic toxicities associated with VEGF inhibition, including hypertension, proteinuria and thrombosis, were very rare. One patient with recurrent anal cancer developed proteinuria considered possibly related to TRC105, but proteinuria was also noted prior to TRC105 dosing. Transient hypertension (156/112 mmHg) without QT changes occurred in a single patient one day following infusion of 15 mg/kg, and was controlled by a single dose of oral antihypertensive medication. There were no arterial or venous thromboembolic events, nor were there gastrointestinal or other perforations in the phase 1 patients

TRC105 exposure increased with increasing dose, and continuous serum concentrations that saturate endoglin receptors were maintained at 10 mg/kg weekly (the maximum tolerated dose) and 15 mg/kg every 2 weeks, allowing there to be broad potential in the design of combination therapies. In the completed phase 1 study, antibodies to TRC105 were not detected in patients treated with TRC105 manufactured from Chinese hamster ovary cells. Stable disease or better was achieved in 21 of 45 evaluable patients (47 %), including an ongoing complete prostate-specific antigen (PSA) response with bone scan normalization in a prostate cancer patient at more than 5 years and a radiographic response in a heavily pretreated uterine carcinosarcoma patient who was progression free for 18 months. In sum, TRC105 was well tolerated at 10 mg/kg weekly and 15 mg/kg every 2 weeks with a safety profile that was distinct from that of VEGF inhibitors. Evidence of single-agent clinical activity was seen in a refractory cancer patient population.

Studies of angiogenic biomarkers indicated downregulation of VEGF and platelet-derived growth factor isoforms following TRC105 treatment, and elevation of the levels of VEGF isoforms at the time of progression [73]. In addition, there was a dose-dependent increase in the level of soluble endoglin measurable in plasma. Soluble endoglin has antiangiogenic effects, by serving as a sink for endoglin ligands, including BMP [74]. Thus, increases in soluble endoglin concentration resulting from TRC105 treatment may represent an additional mechanism of angiogenesis inhibition.

Single-Agent Phase 2 Studies of TRC105

TRC105 was studied as a single agent in refractory epithelial cancer patient populations that have traditionally been resistant to angiogenesis inhibition. Given the profound response in metastatic prostate cancer in the first-in-human trials, a dose-escalation trial at the National Cancer Institute treated 21 castrate-resistant prostate cancer patients, in most of whom chemotherapy had failed and there were soft tissue and skeletal metastases, and demonstrated reductions in the levels of PSA in six patients (20–59 %) and progression-free survival beyond 6 months in three patients [75]. Additional single-agent trials in refractory bladder, hepatocellular, and ovarian cancer have completed enrollment.

Studies of TRC105 with Bevacizumab and VEGFR TKIs

TRC105 was studied in a dose-escalation study with bevacizumab [76••]. Promising signs of activity were observed that are consistent with the biologic role of endoglin in promoting resistance to VEGF inhibition, and emphasize the importance of development of TRC105 with VEGF inhibitor therapy.

Administration of TRC105 in combination with bevacizumab was accompanied by migraine headaches following the first day of dosing both drugs together and prompted revision of the TRC105 dosing schedule. Delaying the first TRC105 dose by 1 week and administering the first TRC105 dose over 2 days resulted in both drugs being well tolerated at their recommended phase 2 doses of 10 mg/kg each. Other adverse events characteristic of each individual drug were not increased in frequency or severity when the two drugs were administered together. The concurrent administration of bevacizumab and TRC105 did not potentiate the known toxicities of bevacizumab of hypertension, hemorrhage (including tumor-associated hemorrhage or hemoptysis), and proteinuria. In fact, hypertension and proteinuria were unusual in patients treated with both agents. Reversible posterior leukoencephalopathy syndrome, congestive heart failure, fistulae, hypersensitivity reactions, gastrointestinal perforation, impaired wound healing, and arterial thromboembolic events were not observed.

Evidence of endoglin receptor modulation (telangiectasia with resulting low-grade epistaxis and gingival bleeding) was

seen routinely. The TRC105 pharmacokinetic parameters were similar to those observed in the TRC105 single-agent phase 1 trial. Laboratory evidence of immunogenicity was rare (below 10 %) and was not associated with clinical events.

Most impressive was the activity of the combination of TRC105 and bevacizumab observed in VEGF-inhibitor-refractory patients, including antitumor activity recorded in patients with metastatic colorectal and ovarian cancer previously treated with antiangiogenic therapy. Approximately 25 % of patients who were refractory to bevacizumab or VEGF TKI treatment experienced reductions in overall tumor volume (10-25 %) when TRC105 was administered in combination with bevacizumab, and remained without tumor progression for periods longer than experienced with prior anti-VEGF therapy [76••]. CT scans demonstrated favorable changes in tumor morphology (i.e., decreased tumor density) [77, 78].

These encouraging safety and activity data prompted the initiation of two randomized trials comparing bevacizumab versus bevacizumab and TRC105 in bevacizumab-naïve patients, one in RCC and one in glioblastoma ((NCT01727089 and NCT01648348, respectively; Table 1). TRC105 also combined safely with capecitabine in a dose-escalation trial, and trials of the combination of TRC105 with bevacizumab and chemotherapy are the logical next step in TRC105 development. In addition, three studies have been initiated combining TRC105 with VEGFR TKIs: with sorafenib in hepatocellular carcinoma, with axitinib in RCC, and with pazopanib in soft tissue sarcoma (Table 1).

Future Development

Biomarker Stratification

An assessment of TRC105 expression directly on sarcoma cells will be incorporated into the phase 1b dose-escalation study with pazopanib. Endoglin expression on malignant cells will be correlated with clinical response to determine an enrichment strategy for sarcoma subtypes of patients enrolled in a phase 2 trial. Melanoma and leukemia also represent indications where stratification of patients based on endoglin expression on malignant tissue could be used. Enrichment of epithelial tumor patients may be possible on the basis of the CT characteristics of metastatic deposits. An exploratory analysis, applying novel quantitative textural analysis measures of standard spiral CT scans, indicates markers of tumor heterogeneity and hypoxia at baseline correlate with individual lesion responses and are worthy of prospective evaluation as predictive imaging biomarkers [78]. Soluble biomarker expression is also being assessed in ongoing TRC105 trials in an effort to identify a responsive profile. Notably, marked elevations of TGF-β and VEGF-A levels at baseline were observed in a patient with castrate-resistant prostate cancer with an ongoing long-term complete PSA response following TRC105 monotherapy [73]. These biomarkers are part of a panel of more than 30 soluble markers that will be evaluated in ongoing TRC105 trials.

Imaging of Tumor Vasculature with TRC105

The expression of endoglin on proliferating endothelial cells rather than quiescent endothelial cells allows the use of a TRC105 radioconjugate to image proliferating vasculature of tumors. Preclinical data indicate that TRC105 conjugated through 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid to radioactive zirconium (^{89}Zr) binds specifically to the proliferating vasculature of syngeneic tumor xenografts growing in mice, and is easily imaged by PET [79]. Further work indicates that imaging of tumor vasculature can be accomplished using TRC105 conjugated to near-infrared fluorescent dye and nanoparticles. TRC105-directed tumor imaging may be useful for staging purposes and could be used to select

Table 1 Ongoing clinical trials of the anti-endoglin antibody TRC105

Design	Type of study (no. of patients)	Sponsor	Indication	End point
Bevacizumab + TRC105	Randomized phase 2b (86)	NCI (CTEP)	Renal cell carcinoma	PFS
	Randomized phase 2b (88)	NCI (CTEP)	Glioblastoma	PFS
	Phase 2a (28)	TRACON	Glioblastoma	OS
	Phase 1b (38)	TRACON	Advanced solid tumors	ORR
Sorafenib + TRC105	Phase 1b (18)	NCI	Hepatocellular carcinoma	PFS
Capecitabine + TRC105	Phase 1b (19)	TRACON Pharmaceuticals	HER2-negative breast cancer	ORR
Axitinib + TRC105	Phase 1b (18)	TRACON Pharmaceuticals	Renal cell carcinoma	ORR
Pazopanib + TRC105	Phase 1b (18)	TRACON Pharmaceuticals	Sarcoma	ORR

CTEP Cancer Therapy Evaluation Program, *NCI* National Cancer Institute, *ORR* objective response rate, *OS* overall survival, *PFS* progression-free survival

patients most likely to respond to angiogenesis inhibition by TRC105, a TRC105 radioimmunoconjugate, or a VEGF inhibitor.

TRC105 Antibody–Drug Conjugate

Endoglin is internalized following binding by TRC105, and this presents an opportunity for the development of a TRC105 antibody–drug conjugate. Preclinical studies using endoglin-targeted antibody–drug conjugates indicate significant activity in mice at doses that are well tolerated. For example, antibody to mouse endoglin conjugated to the ribosomal toxin nigrin B causes complete regression of established tumor xenografts in mice despite the lack of activity of the naked antibody in the same model [80]. A TRC105 antibody–drug conjugate could be developed as a single agent in refractory cancer patients to complement the development of TRC105 with VEGF inhibitor therapies.

Conclusion

Endoglin is an essential angiogenic target that is distinct from the VEGF family and is implicated as a mechanism of VEGF resistance. Endoglin is upregulated following the hypoxia induced by VEGF inhibition and serves to promote continued endothelial proliferation. Targeting endoglin and the VEGF pathway concurrently appears to improve antiangiogenic treatment in vitro and reverses resistance to bevacizumab in some refractory cancer patients. Randomized trials are under way to assess the clinical benefit of adding the anti-endoglin antibody TRC105 to bevacizumab treatment. Further trials are under way to assess the activity of TRC105 with VEGFR TKIs in RCC, hepatocellular carcinoma, and soft tissue sarcoma. Stratification of soft tissue sarcoma by endoglin expression directly on malignant cells may identify those patients most likely to benefit from TRC105 treatment. The development of a TRC105 antibody–drug conjugate is also contemplated.

Compliance with Ethics Guidelines

Conflict of Interest Lee S. Rosen received funding from Tracon Pharma for a clinical trial.

Michael S. Gordon, Francisco Robert, and Daniela E. Matei declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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