

Angiopoietin-2: An Attractive Target for Improved Antiangiogenic Tumor Therapy

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Abstract

Anti-VEGF pathway therapies primarily target immature blood vessels in tumors. However, emerging approaches to combine with targeted therapies impacting the later stages of remodeling and vessel maturation are expected to improve clinical efficacy by expanding the target vessel population. The angiopoietin/Tie ligand/receptor system is a prototypic regulator of vessel remodeling and maturation. Angiopoietin-2 (Ang2) appears to be a particularly attractive therapeutic target. In fact, the experimental proof-of-concept showing improved efficacy when VEGF and Ang2-targeting therapies are combined has been solidly established in preclinical models, and several Ang2-targeting drugs are in clinical trials. However, rational development of these second-generation combination therapies is hampered by a limited understanding of the biological complexity that is generated from agonistic and antagonistic Ang/Tie signaling. This review discusses recent mechanistic advances in angiopoietin signaling, particularly in light of the recent study published on REGN910 and summarizes the status quo of Ang2-targeting therapies. In light of the clarified partial agonist function of Ang2, we propose that clarity on the expression profile of the angiopoietin ligands and Tie1 and Tie2 receptors in subsets of cancer vessels and cancer cells will provide clearer hypotheses for more focused rational clinical trials to exploit this seminal pathway and improve current antiangiogenic therapies. *Cancer Res*; 73(6); 1649–57. ©2013 AACR.

Introduction

In the last 9 years, antiangiogenic therapy has become part of standard antitumor treatment. However, the clinical efficacy of such therapies is limited, and it appears that the full therapeutic potential of antiangiogenic intervention has not been fully exploited. Moreover, the mechanistic goals of antiangiogenic intervention are presently less clear than ever. While concepts developed by Folkman in the 1970s suggested that antiangiogenic therapy was supposed to starve tumors to death by driving the tumor-associated vasculature into regression (1), we find that clinically established anti-VEGF/VEGF receptor therapies primarily promote stable disease by preferentially targeting the most immature blood vessels leaving behind normalized and resistant blood vessels (2). The enigmatic question around the importance of vascular regression,

tumor vessel normalization, or a spatiotemporal combination of both mechanisms for the efficacy to antiangiogenic intervention has increased to the forefront of basic and applied vascular biology research to guide future pharmaceutical development. One of the most challenging questions at the moment is: What drug combinations improve the clinical efficacy of targeted VEGF pathway therapies without causing toxicity by damaging the mature, resting organ vasculature outside tumors?

Recent Advances in the Mechanistic Understanding of Angiopoietin-2 Function

The vascular receptor tyrosine kinase Tie2 is physiologically activated by its agonistic ligand Ang1. Ang1- and Tie2-deficient mice die in midgestation around embryonic day 10.5 as a consequence of perturbed vessel remodeling and maturation (3). The phenotypic similarity between Ang1 ligand and Tie2 receptor-deficient mice has supported the concept that Ang1 is the single, nonredundant agonistic ligand of Tie2 (4, 5). Using similar logic, the antagonistic mode of action of the second angiopoietin ligand, angiopoietin-2 (Ang2), was inferred from genetic manipulation experiments in mice: Ang2-overexpressing transgenic mice essentially phenocopy the midgestational embryonic lethal phenotype of Ang1-deficient mice (6). In other words, too much Ang2 resembles too little Ang1. In addition, correlative expression of Ang2 at times of vessel regression in ovaries, tumor vascular cooption, and hyaloid vessel regression supported the concept that the dominant biologic role of Ang2 is destabilization of established blood vessels through the interruption of Tie2 signaling, as a prerequisite to sprouting angiogenesis in the presence of proangiogenic

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stimulation or physiologic vascular regression in the absence of such stimuli (7–10). The molecular mechanism of this vessel destabilization phenotype has recently been bolstered to include the Ang2-induced internalization and degradation of endothelial cell surface integrins, which may eventually drive the cells into apoptosis (anoikis; ref. 11). The antagonistic mode of Ang2 function is also supported by the positional presentation of the angiopoietin ligands; whereas Ang1 is produced by periendothelial cells, including pericytes, to act in a paracrine manner, Ang2 is almost exclusively produced by endothelial cells and acts on endothelial cells in an autocrine manner, possibly even involving intracrine signaling mechanisms (12). In fact, Ang2 is not just produced by endothelial cells, but endothelial cells store it in Weibel–Palate bodies where it can be rapidly released in seconds to minutes to enable the responsiveness of endothelial cells to angiogenic, inflammatory, and other cytokines (13, 14). Tools to examine the impact of Ang2 on Tie2 phosphorylation are poor and have hampered extensive interrogation of this hypothesis; however, there is one study in which transgenic overexpression of Ang2 in endothelial cells was shown to be capable of reducing Tie2 phosphorylation *in vivo* (15). Ang2 production by endothelial cells is strongly regulated at the transcriptional level. In fact, almost any form of endothelial cell activation leads to upregulation of Ang2 mRNA. The mRNA induction of Ang2 in tumor endothelium has made Ang2 a very attractive circulating biomarker of angiogenic activation (e.g., ref. 16; summary in ref. 3). Thus, while the *in vivo* evidence for a predominately antagonistic mode of action of Ang2 is overwhelming, there is still only very limited examination of the molecular mechanisms of this phenotype.

As a challenge to the antagonist hypothesis, a number of cellular studies have provided compelling evidence that Ang2 may under certain conditions also be able to act agonistically on its receptor Tie2 (e.g., ref. 17). The typically higher concentrations of Ang2 required to observe this agonism have raised questions around the physiologic relevance of the *in vitro* data. However, there are a series of *in vivo* data suggesting that there are cellular contexts in which Ang2 may offer a proangiogenic function through agonism of the Tie2 receptor. One example is the situation in lymphatics, in which deletion of Ang2 led to lymphatic dysfunction with chylous ascites and edema. This lymphatic phenotype was corrected by replacing endogenous Ang2 with a cDNA for Ang1 (18). Thus, Ang2 seemed to substitute for Ang1-agonistic functions in lymphatics *in vivo*, which may be deprived of Ang1 signaling due to their low pericyte coverage. Similarly, in monocytes there is evidence of agonist activity by Ang2. A subpopulation of Tie2-expressing monocytes (TEM) are associated with tumor blood vessels and exhibit proangiogenic activity, which could be inhibited by Tie2 silencing or Ang2 neutralization (19, 20). *In vitro*, TEMs migrated toward Ang2, which induced Tie2 phosphorylation (21). Thus, Ang2 also functioned as an agonist of Tie2 in these cells.

To further complicate the question of Tie2 antagonism versus agonism, there is a series of reports that incorporate the orphan receptor Tie1 into the context of Ang2 function. Deletion of Tie1 in mice results in defects of vascular

integrity, localized hemorrhaging, and cardiac underdevelopment, indicating its essential role in vascular maintenance (22). Tie1 is highly expressed by arteries and their associated capillaries at branching points and is induced by disturbed flow, suggesting dynamic physiologic regulation of Tie1 (23). Multiple studies have addressed potential cross-talk between Tie1 and Tie2, as well as feedback regulatory loops that respond differently to Ang1 and Ang2 (24, 25). These data are still emerging but a take-home hypothesis generated by Seegar and colleagues suggests that in the presence of Tie1, Ang2 is unable to activate Tie2; however, loss of Tie1 reveals the agonist capabilities of Ang2 *in vitro* (26). These tantalizing data await a more physiologic context and confirmation *in vivo*.

Ang2 has recently been shown to exert proangiogenic functions in a Tie2-independent manner. Angiogenic endothelial tip cells downregulate the Tie2 receptor and Ang2 signals in these cells through activated integrins to promote focal adhesion kinase (FAK) signaling and, subsequently, angiogenic sprouting (27). While not acting through an agonism of Tie2, this is nevertheless an unexpected proangiogenic function of Ang2 where neutralization of the ligand could be beneficial in a cancer context. It is notable that in terms of Ang2 functions, both its role in destabilizing a mature vasculature to facilitate cooption and neoangiogenesis and its role in promoting integrin stimulation of sprouting tip cells suggest that the consequences of Ang2 blockade will likely be contextual with different net outcomes on sprouting Tie2-negative tip cells and more mature Tie2-expressing stalk and phalanx endothelial cells (see Fig. 1). This is consistent with published reports using neutralizing molecules to Ang2 where tumor growth and retinal sprouting are blocked (e.g., ref. 28). These studies use antibodies that were developed to block Tie2 interactions and often to block the *in vitro* activation of Tie2 that can be observed in the absence of Ang1, suggesting that the antibodies or peptides block the Ang2–Tie2–interacting domain. It is unclear whether the observed Ang2 interactions with integrins use the same binding domains and whether this function is sensitive to the currently available blocking antibodies and peptides to Ang2 in therapeutic development described below.

In a recent issue of *Cancer Research*, Daly and colleagues reveal insights into the complexity of Ang/Tie signaling that provides a model for the contextuality of Ang2-antagonistic and -agonistic functions of Ang2 on Tie2 (Fig. 1). The authors show that a selective antibody to Ang2 (REGN910) can decrease tumor growth in a manner that is reversed by high-dose administration of an engineered form of Ang1 called Ang1-F₁-Fc-F₁ (which has an IC₅₀ of 9.7 nmol/L to Tie2 and is a forced tetramer), hinting that Tie2 signaling was attenuated (29, 30). This Ang1 construct has previously been shown to maximally activate Tie2 *in vitro* and *in vivo* and to block mustard oil–induced vascular leak. While this report falls short of directly showing the impact of REGN910 on activation of Tie2 by Ang2 *in vivo*, the tumor growth and resulting gene expression data presented, along with the above described cellular contexts in which Ang2 can have proangiogenic and

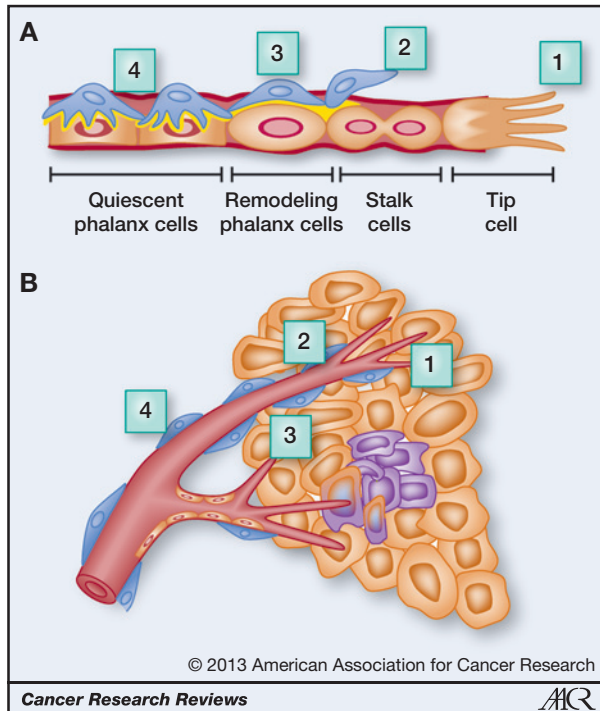


Figure 1. Endothelial cell phenotypes during tumor angiogenesis. A, endothelial cells during angiogenesis maintain distinct functions and acquire specific phenotypes during the individual steps of the angiogenic cascade. Sprouting tip cells (1) have an invasive filopodia-rich promigratory phenotype. They sense the gradient of angiogenic cytokines and migrate towards the angiogenic stimuli. Tip cells are followed by stalk cells (2), which proliferate and remodel to establish lumen-forming microvessels. Tip cell- and stalk cell-derived PDGFB is the best characterized pericyte-recruiting cytokine during angiogenesis. Pericyte recruitment leads to microvessel maturation. Loosely attached pericytes cover remodeling phalanx cells (3). Fully mature phalanx endothelial cells (4) are in intimate contact with pericytes, which control the quiescent endothelial cell phenotype. B, application of the tip cell/stalk cell/phalanx cell model of angiogenesis applied to the partial Ang2 agonist concept for cancer: The mature organ vasculature outside tumors (4) expresses Tie2, and the cells are surrounded by Ang1-producing pericytes. There is low-level Ang2 production, which is stored in quiescent phalanx endothelial cells, that can be induced and secreted upon activation. Release of Ang2 would likely result in a quenching of Ang1/Tie2 signal transduction indicative of an antagonistic mode of action. Conversely, the most immature tip cell vasculature (1) downregulates Tie2 and upregulates Ang2 creating a milieu in which Ang2 can signal agonistically through integrins. The exact positional information on Ang ligands and Tie receptors in stalk cells and in remodeling phalanx cells awaits further analysis, but it is most likely that Tie2- and Ang2-expressing endothelial cells in a low-Ang1 environment could be found in this zone (2, 3) where the partial agonist Ang2 results in a net activation for Tie2. The function and the positional expression of the Ang/Tie2 response, including the role of Tie1 in this context, similarly await further clarification.

possibly even Tie2-agonistic activity, provide a compelling case to revisit the simplistic view of the *Yin-Yang* of Ang1 and Ang2.

The data presented in the article by Daly and colleagues are compatible with a partial agonist model of Ang2 function. In line with classical pharmacologic concepts, the partial agonist competes with the strong agonist. In the presence of the strong agonist (in this case, Ang1), the partial

agonist Ang2 quenches the signal induced by the strong agonist, thereby exerting an inhibitory activity. In the absence of the strong agonist, the weak agonist may function as receptor-stimulating ligand. This model of Ang2 as a partial agonist can perhaps provide a unifying hypothesis to explain some of the contextuality of Ang/Tie biology. For example, the embryonic lethal phenotype of Ang1-deficient mice may be explained by the fact that Ang2 is only later expressed in endothelial cells during embryonic development. Conversely, the puzzling observation that Ang2 is only later expressed in endothelial cells during embryonic development. Conversely, the puzzling observation that Ang1 deletion in adult mice did not result in overt pathology unless the mice were pathologically challenged (31) may well be attributed by a compensatory agonistic function elicited by the partial antagonist Ang2, which would be a testable hypothesis. Clearly, the model proposed by Daly and colleagues provokes a long list of experiments on the contextuality of Ang/Tie function, which emerges at a time when better reagents for the tracing of receptor and ligand expression and for the conditional mutagenesis of the ligands and receptors becomes more readily available. An accelerated pace in the elucidation of the molecular mechanisms of Ang/Tie signaling in the tumor context holds great promise to enable the rational translation of Ang/Tie pathway therapeutics into clinical application.

Current Therapeutics Targeting the Angiopoietin and Tie2 Pathway

There are no selective Tie2 kinase inhibitors in clinical development; however, several approved agents show cellular activity against this pathway. Regorafenib is one of several small-molecule VEGFR2 inhibitors that also exhibit some activity against Tie2. Regorafenib has a 3 to 10 nmol/L cellular activity against VEGFR2, Ret, Kit, and Raf and modest (30–150 nmol/L) cellular activity against fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), and Tie2. Phase III trials showed a significant increase in progression-free survival (PFS) and overall survival in second-line metastatic colorectal cancer (CRC) leading to the accelerated approval of the drug (32). Cabozopanib, a related VEGFR TKIs that is also active against Ret, Tie2, and other kinases, is now approved in medullary thyroid cancer (33). Similarly, MGCD-265 is a Ron, Tie2, cMET, and a VEGFR1/2/3 inhibitor that is being currently tested in phase II trials in patients with a variety of advanced metastatic malignancies (34). In addition, there are several small-molecule inhibitors (ACTB-1003, CEP-11981, ARRY-614) in phase I trials. As it is unlikely that any of these molecules has dominant impact via Tie2 signaling, the remainder of this discussion will focus on the variety of more selective biologics currently under development.

There are 2 types of biologics targeting the angiopoietins whose references can be found in Table 1. The first and most advanced are peptide based, using peptides with high affinity and differing selectivity to Ang1 and Ang2. These peptides are either linked to an Fc domain or to an irrelevant antibody. Trebananib (AMG-386) and PF04856884 (CVX-060) are the 2 peptide-based traps that are most advanced, in phase III and 2 trials, respectively.

Table 1. Therapeutics targeting Ang-Tie axis

	Company	Target	Highest phase of development	Clinical data	Preclinical data	
Small-molecule inhibitors						
	Regorafenib	Bayer AG	FGFR, Kit, Tie2, VEGFR1,2,3, RET, BRAF, CRAF, PDGFR and caspase-3/9 stimulator	Phase III	Positive phase III trials (significant increase in PFS) in second-line metastatic CRC and third-line gastrointestinal stromal tumors (46).	Showed inhibition of tumor growth in several tumor xenograft models including pancreatic, lung, colon carcinoma and regression in breast carcinoma model (47). In addition showed a significant increase in survival with fewer metastasis in syngeneic MC38 CRC liver metastasis model (48).
	Cabozantinib (XL-184/BMS-907351)	Exelixis	MET, VEGFR2, RET, Kit, FLT3, Tie2	Phase III	Positive phase III trials in medullary thyroid cancer and ongoing phase III trials in metastatic castration-resistant prostate cancer.	Reduced metastasis in RIP-TAG2 model of pancreatic neuroendocrine carcinoma compared with VEGF inhibitor alone (49).
	MGCD-265	MethylGene, Inc.	RON, Tie2, VEGFR1, 2, 3, cMET	Phase II	A parallel-arm, open-label trial in advanced metastatic malignancies and NSCLC began for assessing antitumor activity, PFS, and safety (50).	Showed tumor regression in MKN45 gastric cancer xenograft model in combination either with erlotinib or docetaxel. Showed antitumor activity and tumor growth regression in MET-driven human xenograft cells (gastric carcinoma) and non-MET-driven tumor cells (NSCLC and prostate) in combination with paclitaxel and docetaxel (34).
	Foretinib	Exelixis, Inc./GSK	MET, VEGFR1, 2, Tie2, RON, AXL, FLT3 and PDGFR-beta	Phase 2	A phase 1/2 trial in HCC and phase 2 trials in recurrent or metastatic squamous cell head and neck cancer, metastatic gastric cancer and hereditary or sporadic papillary RCC (NCT00725764, NCT00743067, NCT00920192).	Dose-dependently inhibited tumor growth in several models including glioblastoma, breast, CRC, and NSCLC (51).
	ACTB-1003	ACT Biotech/ Bayer AG	VEGFR2, Tie2, RSK, FGFR1 and S6K	Phase I	None.	Showed tumor growth inhibition in cell lines with FGFR genetic alterations—OPM2 human multiple myeloma and the murine leukemia Ba/F3-TEL-FGFR1 and in H460 (52).
	CEP-11981	Cephalon, Inc./Teva	Tie2, VEGFR1, 2,3, FGFR1	Phase I	Linear, dose-related increases in plasma pharmacokinetic exposure (mean C_{max} and AUC) with oral administration. Exposure-related increases in VEGFR2 inhibition <i>ex vivo</i> were shown in a human plasma-based cellular Bioassay (NCT00875264).	Dose-dependent tumor growth inhibition in U251 MG human glioblastoma xenograft and A375 human melanoma xenograft model (53).

(Continued on the following page)

Table 1. Therapeutics targeting Ang-Tie axis (Cont'd)

	Company	Target	Highest phase of development	Clinical data	Preclinical data
ARRY-614	Array BioPharma, Inc.	p38, Tie2	Phase I	Drug was well tolerated and the MTD was not reached with QD dosing. Recently tested a new formulation with enhanced drug exposure and lower variability for future trials (54).	Antitumor activity shown in multiple myeloma in combination with lenalidomide. Complete regressions in BCR-Abl-driven model of chronic myeloid leukemia (55).
Large-molecule inhibitors					
Trebananib-peptide: Fc fusion-peptibody, AMG-386	Amgen	Ang1 and Ang2	Phase III	No improvement in PFS in combination with sorafenib in metastatic RCC (phase II; ref. 36). Evidence of antitumor activity and a dose-response effect in combination with paclitaxel in advanced recurrent epithelial ovarian or primary peritoneal cancer (37). No apparent prolongation of PFS in combination with paclitaxel in HER2-negative, metastatic, or locally recurrent breast cancer (56).	Significantly inhibited tumor growth of several tumor types, including Colo205 (CRC) and A431 (epidermoid carcinoma). A dose-dependent antiangiogenic effect in VEGF-mediated rat corneal angiogenesis assay. Had greater tumor growth suppression than inhibiting Ang1 or Ang2 alone (57).
CVX-060/PF04856884, recombinant humanized CovX body	CovX Pharmaceuticals (Pfizer)	Ang2	Phase II	A randomized, crossover, open-label phase II trial in metastatic RCC in combination with axitinib was initiated (NCT01441414).	As efficacious as bevacizumab in a colon cancer xenograft model and found to improve the response to irinotecan or docetaxel when administered together (35).
AMG-780, fully human anti-ang1/2 mAb	Amgen	Inhibits Tie2-Ang1/2 interaction	Phase I	Dose-escalation and expansion in patients with advanced solid malignancies (NCT01137552).	None.
MEDI-3617, fully human anti-Ang 2 mAb(3.19.3)	AstraZeneca	Ang2 (Ang1 weakly)	Phase I	Dose-escalation in patients with advanced solid malignancies. Modest toxicity was observed. No objective response. Five of 17 evaluated patients had stable disease at 12 wks (58).	Inhibited tumor growth in mice with human pancreatic and hepatocellular xenografts. Efficacy enhanced in combination with sorafenib and bevacizumab Efficacy also enhanced in human CRC models in combination with vandetanib, cediranib, or DC101 (59).
REGN-910, fully human anti-Ang2 mAb	Sanofi/Regeneron	Ang2	Phase I	Dose-escalation and expansion study in patients with advanced solid malignancies (60).	Significantly inhibited growth of several tumors types including PC3 (prostate), Colo205 (CRC), and A431 (epidermoid carcinoma). Reduced Colo205 tumor vascularity and tumor perfusion more than single agents, as per histologic and microultrasound analyses (61). Potentiates the effects of VEGF-Trap (Aflibercept; ref. 62).

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Table 1. Therapeutics targeting Ang-Tie axis (Cont'd)

	Company	Target	Highest phase of development	Clinical data	Preclinical data
TAvi6, a CrossMab tetravalent IgG-like bispecific antibody	Roche	Ang2 and VEGF-A	Discovery	None.	Effectively reduced angiogenesis, tumor growth, and metastasis in several subcutaneous and orthotopic <i>in vivo</i> models that are resistant to anti-VEGF treatment. Ang2-selective approach was found to have safety-related advantages over simultaneous Ang1 and Ang2 inhibition (63).
AT-006, fully human mab	Roche/ Affitech	Ang2	Discovery	None.	Inhibited tumor growth and neoangiogenesis (64).
Anti-Her2/anti-Ang2 mab, multivalent mab	Zyngenia	Her2 and Ang2	Discovery	None.	Remained intact and functional in mouse serum and can bind to Her2 and Ang2 simultaneously (65).
A-11, a peptide inhibitor of Ang2 and Tie2 interaction	Compugen	Ang2	Discovery	None.	Inhibited retinal neovascularization in a rat model of oxygen-induced retinopathy. Showed synergy with anti-VEGF antibody (66).

Abbreviations: NSCLC, non-small cell lung cancer; qd, once daily.

Trebananib is a peptide-Fc fusion protein (peptibody) that blocks the interaction of both Ang1 and Ang2 to Tie2 with an affinity of 0.67 nmol/L by BiAcore (35). Several phase II trials were conducted with trebananib in which some hints of clinical efficacy and experience with pharmacokinetics (PK)/pharmacodynamics (PD) has led to further development in phase III, particularly in ovarian cancer. Phase II data showed an improved response rate but no difference in PFS when trebananib was combined with sorafenib in patients with metastatic clear cell renal carcinoma (RCC; ref. 36). However, an encouraging dose-response effect was observed when trebananib was used in combination with paclitaxel in patients with advanced recurrent epithelial ovarian or primary peritoneal cancer (37). PK/PD analysis of the phase II data suggest that maximum benefit in PFS may not have been reached with a trebananib dose of 10 mg/kg once every week and predict that a dose of 15 mg/kg once every week may provide greater exposure and may yield further improvements in PFS (38). The 3 phase III trials now ongoing at 15 mg/kg once-a-week dose of trebananib combine either paclitaxel (NCT01204749/TRINOVA-1), pegylated liposomal doxorubicin (NCT01281254/TRINOVA-2), or a combination with paclitaxel and carboplatin (NCT01493505/TRINOVA-3). TRINOVA-1 and -2 are in recurrent platinum-sensitive or -resistant epithelial ovari-

an, primary peritoneal, or fallopian tube cancers, whereas TRINOVA-3 is in first-line FIGO Stage III-IV epithelial ovarian, primary peritoneal, or fallopian tube cancers (39).

PF04856884 (CVX-060) is a CovX-body specific to Ang2. CovX-bodies are an antibody platform that are generated by linking an irrelevant (aldolase) IgG to a target-binding peptide. CVX-060 has a binding affinity of 4.9 nmol/L to Ang2, similar to published affinity for Ang2 to Tie2. The peptide is linked using a branched azetidinone (AZD). The antibody scaffold imparts IgG-like half-life, and distribution and the peptide pharmacophores are responsible for functional activities (6, 40). CVX-060 inhibited tumor growth, tumor microvessel density, and intratumor proangiogenic Tie2/CD11b-positive cells (TEMs) in Colo205 xenograft tumors. Even greater efficacy (>80%) was observed when combined with sunitinib, sorafenib, bevacizumab, irinotecan, or docetaxel in this model (35). Significant mean decreases in tumor blood flow assessed by K^{trans} (DCE-MRI) and increase in serum Ang2 levels were observed in a phase I study of CVX-060. The maximum tolerated dose (MTD) was not defined and the most common adverse event was fatigue. PK/PD analysis has recommended 15 mg/kg once weekly dosing (41). It is unclear whether increased serum Ang2 levels in this study are due to peptibody binding and accumulation of stabilized complexes in the circulation as opposed to a pharmacodynamics induction of *de novo* Ang2

expression. A phase II study is ongoing in combination with axitinib in patients with previously treated metastatic RCC (NCT01441414; ref. 39). In general, peptides tend to have lower affinities for Ang2 binding than antibodies, and understanding how competitive they are as neutralizing agents clinically awaits further development of the antibodies described below.

The second type of biologic therapeutics are antibodies. Two selective anti-Ang2 antibodies are in development, MEDI-3617 (AstraZeneca) and REGN-910 (Sanofi). Both MEDI-3617 and REGN-910 have ongoing phase I dose-escalation studies as single agents or in combination with other chemotherapy in patients with advanced solid tumors (NCT01248949 and NCT01271972). MEDI-3617 reported drug-related adverse events (% of patients) including fatigue (24%), diarrhea (19%), nausea (19%), dysgeusia (14%), and headache (14%), all of which were \leq grade II (42). AMG-780, an antibody that binds both Ang1 and Ang2, is also in phase I development (NCT01137552) (39). In addition, Roche has a preclinical stage CrossMab, which is a bispecific antibody that binds and neutralizes both Ang2 and VEGF-A. This bifunctional approach is notable, as several preclinical studies have shown an advantage to dual inhibition of both VEGF/VEGFR and Ang/Tie2 pathways, including the study by Daly and colleagues, in a recent issue of *Cancer Research* (29, 42).

Outstanding Questions and Future Directions for Rational Intervention

The prominent upregulation of Ang2 in the vasculature of most solid tumors makes Ang2 an attractive candidate for therapeutic intervention. Preclinical and clinical studies suggest that Ang2 may augment the activity of VEGF-targeted therapies without clear evidence of enhanced toxicity. However, the pleiotropic activities of Ang2 in blood, lymphatic, and myeloid biology, as well as complexities in receptor use and contextual presentation demand further investigation to clarify the critical aspects of Ang2 that are drivers in cancer progression. Regardless of which effects turn out to be dominant in cancer, current evidence supports the therapeutic rationale to antagonize Ang2.

In tumor angiogenesis, Ang2 appears to function as a potent Tie2 agonist based on the ability of Ang1 to counter the efficacy of anti-Ang2 antibodies. However, modulation of Tie2 activation in tumor vessels remains to be explored (29). Ectopic administration of Ang1 can substitute for Ang2 function, raising the question of the difference between autocrine Ang2 (released by endothelial cells subjected to stress) or paracrine Ang2. This may be particularly relevant in some tumor settings where Ang2 is produced by tumor cells. Ang2 has also been associated with tumor metastases. High Ang2 expression in primary human breast cancer specimens correlates with an increased metastatic phenotype (44). Furthermore, overexpression of Ang2 in MCF-7 promoted their metastasis to lymph nodes and lungs, an effect postulated to result from direct interaction between Ang2 and beta1 integrins on tumor cells (much the way Ang2 was reported to facilitate sprouting of Tie2-

negative tip cells; refs. 11, 27). The delineation of autocrine versus paracrine presentation of Ang2 still needs to be carefully investigated.

The partial agonist concept developed by Daly and colleagues raises important questions as to when and where angiopoietin ligands and Tie2 receptor are expressed in different tumor settings (Fig. 1; ref. 1). Endothelial cells in mature tumor-associated vessels express Tie2 in an Ang1-rich/Ang2-poor microenvironment. When this balance is disturbed by increased expression of Ang2 (a weak agonist), the net result is a paradoxical decrease in Tie2 phosphorylation. Conversely, endothelial cells in the most immature tumor vasculature downregulate Tie2 expression, while at the same time upregulating Ang2 and favoring proangiogenic Ang2 function through integrin signaling. The contextual presentation of Ang1 in tumors is not well-defined, but an intermediate zone of Tie2- and Ang2-expressing endothelial cells in an Ang1-low microenvironment could likely be present in the actively proliferating stalk cell zone (Fig. 1).

Extrapolating from rapidly growing mouse tumors with a very immature vasculature, it is widely believed that the tumor vasculature is low in Ang1-producing pericytes. However, this is most likely an overstatement as microvessels in human tumors are often well-invested by pericytes. These pericytes may not be as tightly associated with endothelial cells as in resting blood vessels but are widely detected in human tumors (45). In fact, there are distinct differences in the pericyte coverage of different human tumor blood vessels. It is tempting, therefore, to speculate that pericyte-mediated differences in tumor blood vessel maturation could account for differences in the efficacy of immature blood vessels targeted by anti-VEGF/VEGFR therapies. These concepts strongly support a rationale for inducing vascular maturation as part of the treatment of tumors.

The development of Ang/Tie pathway drugs and the deciphering of the intricate details of Ang/Tie signaling occur at a time when the goals of antiangiogenic intervention are under serious consideration. Understanding the cross-talk between vessel maturation and remodeling (i.e., Ang/Tie) and primarily sprouting angiogenesis-inducing pathways (i.e., VEGF) holds great promise to rationally develop safe and improved antiangiogenic cancer therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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