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Molecular Pathways: Can Activin-Like Kinase Pathway Inhibition Enhance the Limited Efficacy of VEGF Inhibitors?

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Abstract

The vascular endothelial growth factor (VEGF) pathway is critical for tumor angiogenesis. However, VEGF pathway inhibition has been limited by intrinsic and acquired resistance. Simultaneously targeting multiple steps involved in tumor angiogenesis is a potential means of overcoming this resistance. Activin like kinase 1 (ALK1) and endoglin (ENG) have effects on angiogenesis that are distinct from VEGF. While VEGF is important for vessel initiation, ALK1 and endoglin are involved in vessel network formation. Thus, ALK1 and endoglin pathway inhibitors are attractive partners for VEGF-based combination anti-angiogenic therapy. Genetic evidence supports a role for this receptor family and its ligands, bone morphogenetic proteins (BMP) 9 and 10, in vascular development. Patients with genetic alterations in ALK1 or endoglin develop hereditary hemorrhagic telangiectasia, a disorder characterized by abnormal vessel development. There are several inhibitors of the ALK1 pathway advancing in clinical development for treatment of various tumor types including renal cell, and ovarian carcinomas. Targeting of alternate angiogenic pathways, particularly in combination with VEGF pathway blockade, holds the promise of optimally inhibiting angiogenic driven tumor progression.

Background

Molecular signaling of the ALK1/ENG pathway

Activin like kinase (ALK)-1 is a type I transforming growth factor (TGF)β serine/threonine kinase receptor that binds to bone morphogenetic protein (BMP) 9 and 10 (1). These cytokines are members of the TGFβ super family of ligands that includes TGFβ, activins, growth and differentiation factors (GDFs), and the other BMPs. The functional BMP9/10 signaling complex contains the type I receptor (ALK1) and a type II TGFβ receptor (BMP Receptor II, Activin receptor IIA (ActR11A) or ActRIIB). Upon ligand binding, the type II TGFβ receptor phosphorylates the type I receptor which leads to the phosphorylation and

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activation of SMADs 1, 5 and 8 (2–5). SMAD phosphorylation then leads to expression of downstream genes including the DNA binding protein inhibitor ID-1 and transmembrane protein 100 (TMEM100)(6,7). Another member of the TGFβsuperfamily, TGFβ1, utilizes a similar receptor complex, TGFβRII (a type II receptor) and ALK5 (a type I receptor) and activates SMAD2, 3 signaling. ENG is a type I integral membrane protein with a large extracellular domain and a short cytoplasmic tail lacking a kinase signaling motif. While there are some reports of signaling by endoglin(8,9), in general it has been regarded as a co-receptor in this family. Endoglin binds BMP9 and an anti-endoglin antibody has been shown to regulate BMP9 induced signaling(8). ENG expression is upregulated by hypoxia and TGFβ(10). A soluble form of ENG can be generated via cleavage at the membrane, releasing sENG (11).

ALK1 and ENG are involved in development of vascular networks

Extensive genetic evidence in humans and mice supports the essential role of the ALK/ENG pathway in the development of vascular networks. Hereditary hemorrhagic telangectasia (HHT, Osler-Weber-Rendu syndrome)(12,13) is an autosomal dominant disorder seen in individuals with mutations in either *ACVRL1* (the gene encoding ALK1) or *ENG* genes. Patients with (HHT) type 1 (ENG mutation) and HHT type 2 (ALK1 mutation) develop vascular abnormalities including telangectasias and arterial venous malformations (AVMs). Telangectasias are clusters of abnormally dilated thin-walled blood vessels, typically found in the skin and mucous membranes. Patients with HHT commonly develop recurrent epistaxis or nosebleeds and gastrointestinal bleeding from telangectasias in the nasal and gastrointestinal muscosa frequently later in life. AVMs are characterized by abnormal connections between arteries and veins and are commonly found in the internal organs such as liver, lung and brain of patients with HHT.

Murine genetic studies also support the role of ALK1 and ENG in vascular network formation. Two germline ALK1 (*ACVRL1)* mutations have been studied in mice. One mutation disrupts transcriptional and translational initiation (14), and the other disrupts exon 8 that encodes the kinase subdomain V of ALK1(15). Mice lacking *ACVRL1* expression die at midgestation around embryonic day 11.5 with abnormal development of vascular networks. One of the earliest steps in the development of the vascular system is the specification of arteries and veins, leading to distinction of vascular beds. Mice lacking *ACVRL1* develop large shunts between arteries and veins resulting in AVM formation. Additionally, the vascular smooth muscle cells that develop around vessels fail to develop after AVM formation and expression of an early molecular marker of arteries, ephrinB2, is reduced in the *ACVRL1*-/- embryos. Conditional deletion of *ACVRL1* in restricted vascular endothelia also results in severe vascular malformations (16). *ACVRL1* heterozygous mice develop cutaneous lesions in the ear, tongue and AVMs in liver, lung, spleen and brain(17). Additionally, disruption of ALK1 in zebrafish leads to an abnormal circulation pattern which is characterized by dilated vessels which fail to perfuse the trunk (violet beauregarde) (18).

Mice lacking *ENG* expression also die at midgestation with defective vascular development. ENG-/- mice die around embryonic day 11.5 with immature disorganized vascular plexi that

fail to undergo remodeling and lack vascular branching and sprouting. Lack of vascular smooth muscle development is also seen in these mice(19). Mice harboring a nonsense mutation in *ENG* also die early in embryogenesis and in addition to abnormalities in vascular development exhibit abnormal yolk sac development and evidence of cardiac defects(20). The abnormal yolk sacs have reduced TGFβ signaling, demonstrating cross-talk between the ENG/ALK1 and TGFβ pathways (21). In contrast to *ACVRL1* -/- mice, *ENG* -/- mice do not develop profound vessel dilation or decrease in ephrinb2 expression(22).

While ALK1 is expressed at sites of angiogenesis during development, its expression is suppressed in the adult. It can be re-induced during events requiring neoangiogenesis including tumor angiogenesis (23–25). A study of ALK1 expression in mice in which ALK1 is replaced with the beta-galactosidase gene showed that ALK1 is predominantly expressed in developing arterial endothelium. Expression decreases in adult arteries, but is induced in preexisting feeding arteries and newly forming arterial vessels during wound healing and tumor angiogenesis(26). Similarly, ENG is expressed only at low levels in adult human tissues. However, during inflammatory disease and in wound healing models, ENG expression is strongly upregulated and is consistently associated with an infiltrate of inflammatory cells(27). ALK1 is also expressed in several human tumors including renal cell, ovarian and head and neck carcinoma (28,29).

ALK1 and ENG share ligands

ALK1 and ENG bind to members of the BMP family. BMP9/10 are secreted in an active form and have context dependent roles in angiogenesis (30,31). While there is clear evidence that BMP9/10 are angiogenic factors (32), their effect on angiogenesis appears to be dependent on timing of expression and whether they are being studied in developmental angiogenesis or tumor angiogenesis. In vitro, BMP9 has been shown to inhibit proliferation and migration of several cultured endothelial cell lines (32–34) and has negative effects on angiogenesis in the mouse sponge assay and the chick CAM assay (35,36). Recent genetic evidence in BMP9 and BMP10 knockout mice revealed that they are functionally redundant ALK1 ligands required for early postnatal vascular development (37). In the adult, the role of BMP10 is primarily in cardiac development. BMP9 mutations were recently identified in three patients with clinical manifestations of HHT but with no mutations in ENG or ALK1 (38) supporting the role of BMP9 in angiogenesis.

ALK1/Eng and VEGF affect distinct stages in angiogenesis

The development of arteriovenous networks is a multistep process in which Vascular Endothelial Growth Factor (VEGF) plays a critical role (Figure 1) (39,40). VEGF is an initiator of angiogenesis, along with Fibroblast Growth Factor (FGF), stimulating proliferation and migration of endothelial cells. VEGF and Notch pathway proteins then signal to initiate and support sprouting of endothelial tubes (41). Subsequent basement membrane remodeling by extracellular matrix proteinases continues to support sprouting and branching of vessels. Maturation and stabilization of early branched vessels with subsequent development of functional vascular beds is a complex process supported by several factors including angiopoietins, platelet derived growth factor, sphingosine phosphate receptors, and the ALK1/ENG pathway (31). Much of the information known

about vessel maturation comes from genetic studies and some in vivo angiogenesis models. The exact function of these molecules in tumors is not fully understood due to lack of adequate models of tumor angiogenesis. In vitro studies using cultured endothelial cell lines and in vivo studies such as Matrigel and chick chorioallantoic membrane (CAM) assays often fail to recapitulate tumor-host interactions. Thus, optimal sustained inhibition of tumor angiogenesis may require coordinated inhibition of multiple components of the angiogenic program.

Clinical-Translational Advances

Inhibitors of ALK1/ENG signaling

The growing understanding of the important contributions of the ALK1/ENG pathway to angiogenesis has led to efforts to develop functional inhibitors of the pathway. The role of ALK1 and ENG in cancer has been studied largely in the context of these inhibitors. Currently, there are 2 classes of inhibitors under development: inhibitors of ALK1 and of ENG (Figure 1). Several of these agents have already entered into clinical development. Two ALK1 inhibitors have entered clinical trials, ACE-041 (dalantercept, Acceleron Pharmaceuticals) and PF-03446962 (Pfizer). ACE-041is a fusion protein of the extracellular domain of ALK1 fused to the IgG1 human Fc. It binds both BMP9 and 10 and acts as a ligand trap of BMP9/10 (32). ALK1-Fc blocked BMP9 signaling in cultured endothelial cells as shown by a reporter assay of SMAD binding elements and downregulation of Id-1. This inhibitor also reduced endothelial cord formation(32,36), and neovascularization in the CAM angiogenesis model. In an orthotopic breast cancer model (MCF-7) ALK1-Fc treated mice displayed a ~70% reduction in tumor burden vs vehicle treated mice ($P<0.01$)(32). Our group has also shown that ALK1-Fc decreased tumor growth in two renal cell carcinoma models and that large dilated vascular structures were present in tumors of treated mice (42).

In a RIPTag2 model of endocrine pancreatic tumorigenesis BMP9 and TGBβ were upregulated during tumor progression. Neutralization of BMP9 with ALK1-Fc inhibited angiogenic sprouting and led to dose-dependent growth inhibition of both small early tumors (73% reduction in mean tumor burden; $P<0.001$) and larger established tumors. Treatment also resulted in decreased vascular density and decreased perfusion as assessed by detection of an injected labeled lectin. ALK1-Fc also decreased sprouting and migration of endothelial cells in cultured explanted of angiogenic pancreatic islets(36).

PF-03446962 is a human monoclonal antibody against ALK1 (Anti-ALK1) that interferes with BMP9 and 10 signaling(29,43). Anti-ALK1 interferes with BMP9/10 signaling in HUVEC cells and with endothelial sprouting and tube formation. The ALK1 antibody also blocks ENG recruitment to the receptor complex and competes with BMP9 for binding to ALK1. Anti-ALK1 antibody treatment produced 59% tumor growth inhibition in the MDA-MB-231 human breast cancer xenograft tumor model ($P<0.05$) (29). In treated tumors, microvessel density was decreased and lymphatic vessel density was moderately decreased. Reduction in microvessel density was also seen in anti-ALK1 treated human melanoma murine xenografts. Anti-ALK1 administration also inhibited the development of functional vessels as measured by contrast enhanced ultrasound (CE-US) (29).

TRC105 (Tracon Pharmaceuticals) is a chimeric human IgG1 anti-ENG antibody. It is derived from a monoclonal mouse anti-human ENG antibody (SN6j)(44). This antibody has been shown to inhibit proliferation of HUVEC cells in vitro and vessel formation in Matrigel (44,45) In the 4T1 mouse breast cancer model TRC105 slowed tumor growth (tumor size at day 24 post treatment with control IgG 888.8+/- 427.5 mm3 vs anti-ENG 662.6 +/- 285.8 mm3 ($P<0.05$)) (44). Further it was more effective in immunocompentent mice in a T cell dependent manner as depletion of CD4 or CD8 cells abrogated the effect of ENG inhibition (46). This suggests that a component of its activity may be due to ADCC. Anti-ENG also prevented metastatic tumor spread in the 4T1 breast cancer and the colon26 models where a reduction in liver metastatic colonies was observed (control 10.1 +/- 7.2 vs anti-ENG 3.2 +/- 1.4 ($P<0.05$) ($P<0.03$)) (44). However, in the genetic model, ENG loss in tumor vessels, led to increased tumor metastases(47). The effects of ALK1/ENG pathway inhibitors on tumor vasculature remain to be fully understood. Simply measuring vessel density of treated tumor xenografts may not reflect the complex roles of these pathways in angiogenesis. More studies and models are needed to understand the differences among these inhibitors.

Phase I studies

In a Phase I study of dalantercept toxicities included fatigue, peripheral edema, anemia and nausea. The dose limiting toxicity was fluid overload/edema. Interestingly patients treated at the higher dose levels developed skin telangectasias. Some patients showed evidence of tumor response including decrease in tumor FDG uptake on Positron Emission Tomography (PET) imaging (48). In a Phase I study of PF-03446962 in patients with advanced solid tumors the most common toxicities included thrombocytopenia and fatigue with no dose limiting toxicities observed. Grade 1 telangectasias were seen in 8.3% of patients. There was also some evidence of clinical activity. (49). Anti-ENG (TRC105) was tested in patients with advanced solid tumors in a phase I clinical trial. Gastrointestinal hemorrhage was an observed toxicity. Telangectasias were seen in some patients. Promising clinical activity has led to further testing of TRC105 (50).

Although these three inhibitors do not seem to show overlapping toxicities, a common finding in all three phase I trials was the treatment emergence of telangectasias in a small subset of patients treated at higher doses. As HHT is characterized by telangectasias, likely due to defects in ALK1/ENG signaling, the emergence of these skin manifestations in the clinical trials may indicate that each agent is capable of blocking its respective target. By contrast, none of the agents was associated with the common toxicities seen with VEGF pathway inhibitors including hypertension and proteinuria. Future studies of the relationship of telangiectasia development to dose, blood level of each agent, treatment exposure and clinical outcome will likely provide insights into the optimal dosing and relevance of this pathway in patients with specific tumor types. Another important finding from all three trials was the evidence of clinical activity (partial responses and stable disease) even in patients whose disease had developed resistance to VEGF pathway inhibitors. Importantly, evaluating the effects of these inhibitors on tumor blood flow and other parameters of tumor vasculature will help assess target engagement and function. Thus, it will be important to incorporate vascular imaging into future trials.

Importance of combination therapy

VEGF is one of the critical mediators of angiogenesis, however the clinical activity of VEGF pathway inhibitors is limited by short duration of benefit even in the more sensitive tumor types (e.g. clear cell RCC). Determining how to augment and extend the activity of VEGF pathway inhibitors is an area of intensive investigation. ALK1 or ENG pathway inhibition has shown the ability to enhance the antitumor activity of VEGF pathway inhibitors in preclinical models (9,29,32). Anti-ALK1 was shown to improve the activity of bevacizumab in a murine melanoma model where the combination showed a 58% tumor growth inhibition with the addition of bevacizumab ($P<0.05$)(29). Combination therapy led to disruption of pericyte-endothelial contacts in this model. Similarly, ENG+/- RIP-TAG mice demonstrated a more marked sensitivity to the VEGFR TKI, AG-028262, and to the VEGFR2 antibody, DC101, than ENG+/+ mice, suggesting benefit from dual pathway inhibition (47).

The combination of VEGFR tyrosine kinase inhibition and ALK1 inhibition has also been investigated in RCC xenograft models. The VHL defect seen in the majority of clear cell RCC leads to a unique dependence of RCC on VEGF driven angiogenesis. VHL loss in RCC leads to upregulation of HIF and its downstream factors (including VEGF) even in the absence of hypoxia contributing to the sensitivity of RCC to VEGFR TKIs such as sunitinib, sorafenib, pazopanib and axitinib. Each of these agents has produced tumor responses in a large proportion of patients with RCC and prolonged median progression free survival, leading to their approval by the FDA for this indication. However, responses are virtually always partial and treatment resistance invariably develops at a median of 6-12 months. Resistance to these agents has been shown to be related to ability of tumors to use alternative pro-angiogenic mechanisms to restore angiogenesis. In a VHL deficient RCC murine xenograft model of VEGFR TKI resistance, VEGFR TKI therapy in combination with ALK1 inhibition (using ALK1-Fc) led to prolonged disease stabilization (42) suggesting that ALK1-Fc may inhibit a mechanism of angiogenic escape. Another rationale for combined VEGFR and ALK1 inhibition is that the two pathways affect sequential steps in angiogenesis. Our group has shown that the combination of VEGFR and ALK1 inhibition is more effective at slowing tumor growth with a 52% decrease in tumor burden in combination ALK1-Fc + sunitinib treated mice vs single agent sunitinib treatment ($P<0.005$). Additionally, combination VEGFR and ALK1 inhibition led to greater reduction in tumor blood flow than either agent alone (42). Based on these data, it appears that combined inhibition of both early and later angiogenic processes can provide more effective antiangiogenic activity than either alone.

This relationship of VEGF and ALK1/ENG has been studied in vascular formation models. Walker et al. have developed a model of brain AVM formation using brain-specific conditional deletions of ALK1(51) and Choi et al have performed similar studies with ENG (52). When mice harboring deletion of ALK1 or ENG in the brain are injected with an adenovirus expressing VEGF, enlarged vessels are seen. These vessels are AVMs as evidenced by altered arterio/venous molecular markers and evidence of blood shunting. Thus, the combination of VEGF stimulation and ALK1/ENG deficiency leads to large

dysplastic vessels, but in the absence of VEGF signaling the number of abnormal vessels is reduced.

This relationship of ALK1/ENG and VEGF is also seen in HHT patients, who can benefit from VEGF pathway inhibition (53–55). Patients with HHT exhibit enlarged abnormal blood vessels that are prone to bleeding. The clinical picture is consistent with unopposed endothelial cell proliferation in a setting where there are defects in the regulation of vessel proliferation and in vessel maturation. Thus, it was hypothesized that blocking VEGF driven angiogenesis could limit the abnormal vessel development and reduce the resultant clinical complications(53,56). Early clinical reports suggest that patients with HHT treated with bevacizumab could experience amelioration of HHT related bleeding (57,58). Currently bevacizumab is being studied in clinical trials in HHT patients with recurrent epistaxis (59,60).

We hypothesize that tumors treated with ALK1 inhibitors have vessels that resemble the pathology seen in HHT and that dual VEGFR/ALK1 inhibition mimics anti-VEGF treated HHT in producing multistep inhibition of tumor angiogenesis. This pathophysiology helps shed further light on the potential value of combination VEGF and AIK-1 pathway inhibition.

Two phase II trials of ALK1/ENG pathway inhibitors are planned in patients with metastatic RCC who have failed prior VEGFR TKI therapy.. In each case, patients will be treated with combination dalantercept or TRC105 and axitinib or axitinib alone. This design is based on the assumption that tumors developing resistance to anti-VEGF therapy can respond to ALK1/ENG inhibition and that continued VEGFR inhibition is important for optimal anti-angiogenesis. This is likely due to the fact that blood vessels that are able to form in the absence of VEGF would still require BMP9/10 for vessel development. Table 1 summarizes all the ongoing phase II trials of ALK1/ENG pathway inhibiting agents.

Future trials

The next 3-5 years will be critical for validation of the importance of targeting this pathway and identification of the optimal patient population and setting for these therapies. With the wide range of therapeutic choices, it will be important to identify biomarkers that inform in which patients and at what point during their course of treatment, certain agents will be useful. For example, circulating BMP9/10 may be an indicator of activation of the ALK1/ENG pathway, but these cytokines are often bound to extracellular matrix and, thus, plasma levels are not likely to represent local tumor BMP signaling. Imaging techniques that assess tumor blood flow may be useful in the future but cannot identify which angiogenic molecules are driving angiogenesis at a given time point. Tumor tissue from resected primary tumors is often used as a surrogate for the metastatic disease being treated, but may not necessarily reflect all the changes a tumor undergoes over the course of multiple therapies. Tumor biopsies of metastatic lesions will likely need to be incorporated into future studies and despite current technical limitations of biopsy procurement and tumor heterogeneity may be the most useful means of assessing pathway activation in order to select treatments for specific patients.

Additionally, the molecular study of the exact mechanisms of action of ALK1/ENG inhibitors has been challenging and complicated by the heterogeneity of angiogenic model systems. Given the complexity of vessel development in vivo, cultured endothelial cell studies yield context dependent results that are difficult to interpret. Other models such as chick CAM and Matrigel assays are also challenging to interpret especially since these have been performed in the setting of abundant VEGF. Better models of tumor angiogenesis are needed for mechanistic studies. Additionally, mechanistic studies may lead to identification of better markers of sensitivity to this pathway and of target engagement.

In conclusion, the ALK1/ENG pathway appears to play a pivotal role in angiogenesis. Years of molecular pathway analysis and genetic studies have paved the way for development of several pathway inhibitors. Although it seems that all three currently studied inhibitors are similar and will yield similar clinical results, the differences among the molecular pathways inhibited may be interesting to consider in the future. The ALK1 and endoglin pathway inhibitors represent a novel class of antiangiogenic agents that have the potential to enhance the treatment of a number of tumor types and improve outcomes of VEGFR TKI therapy.

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References

1. David L, Mallet C, Mazerbourg S, Feige J-J, Bailly S. Identification of BMP9 and BMP10 as functional activators of the orphan activin receptor-like kinase 1 (ALK1) in endothelial cells. Blood. 2007; 109:1953–61. [PubMed: 17068149]
2. Attisano L, Wrana JL, Montalvo E, Massagué J. Activation of signalling by the activin receptor complex. Mol Cell Biol. 1996; 16:1066–73. [PubMed: 8622651]
3. Wrana JL, Attisano L, Wieser R, Ventura F, Massague J. Mechanism of activation of the TGF-β receptor. Nature. 1994; 370:341–7. [PubMed: 8047140]
4. Massagué J. TGF-β signal transduction. Annu Rev Biochem. 1998; 67:753–91. [PubMed: 9759503]
5. Koenig BB, Cook JS, Wolsing DH, Ting J, Tiesman JP, Correa PE, et al. Characterization and cloning of a receptor for BMP-2 and BMP-4 from NIH 3T3 cells. Mol Cell Biol. 1994; 14:5961–74. [PubMed: 8065329]
6. Korchynskyi O, ten Dijke P. Identification and functional characterization of distinct critically important bone morphogenetic protein-specific response elements in the Id1 promoter. J Biol Chem. 2002; 277:4883–91. [PubMed: 11729207]
7. Somekawa S, Imagawa K, Hayashi H, Sakabe M, Ioka T, Sato GE, et al. Tmem100, an ALK1 receptor signaling-dependent gene essential for arterial endothelium differentiation and vascular morphogenesis. Proc Natl Acad Sci U S A. 2012; 109:1–6.
8. Nolan-Stevaux O, Zhong W, Culp S, Shaffer K, Hoover J, Wickramasinghe D, et al. Endoglin requirement for BMP9 signaling in endothelial cells reveals new mechanism of action for selective anti-endoglin antibodies. PLoS One. 2012; 7:e50920. [PubMed: 23300529]
9. Castonguay R, Werner E, Matthews R, Presman E, Mulivor A, Solban N, et al. Soluble endoglin specifically binds bone morphogenetic proteins 9 and 10 via its orphan domain, inhibits blood vessel formation, and suppresses tumor growth. J Biol Chem. 2011; 286:30034–46. [PubMed: 21737454]

10. Tian F, Zhou A-X, Smits AM, Larsson E, Goumans M-J, Heldin C-H, et al. Endothelial cells are activated during hypoxia via endoglin/ALK-1/SMAD1/5 signaling in vivo and in vitro. Biochem Biophys Res Commun. 2010; 392:283–8. [PubMed: 20060813]
11. López-Casillas F, Cheifetz S, Doody J, Andres JL, Lane WS, Massagué J. Structure and expression of the membrane proteoglycan betaglycan, a component of the TGF-beta receptor system. Cell. 1991; 67:785–95. [PubMed: 1657406]
12. McAllister K, Grogg K, Johnson D, Gallione C, Baldwin M, Jackson C, et al. Endoglin, a TGF-beta binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1. Nat Genet. 1994; 8:345–51. [PubMed: 7894484]
13. Johnson D, Berg J, Baldwin M, Gallione C, Marondel I, Yoon S, et al. Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2. Nat Genet. 1996; 13:189–95. [PubMed: 8640225]
14. Urness L, Sorensen L, Li D. Arteriovenous malformations in mice lacking activin receptor-like kinase-1. Nat Genet. 2000; 26:328–31. [PubMed: 11062473]
15. Oh SP, Seki T, Goss KA, Imamura T, Yi Y, Donahoe PK, et al. Activin receptor-like kinase 1 modulates transforming growth factor-β1 signaling in the regulation of angiogenesis. Proc Natl Acad Sci U S A. 2000; 97:2626–31. [PubMed: 10716993]
16. Park S, Lee Y, Seki T, Hong K-H, Fliess N, Jiang Z, et al. ALK5- and TGFBR2-independent role of ALK1 in the pathogenesis of hereditary hemorrhagic telangiectasia type 2. Blood. 2008; 111:633–42. [PubMed: 17911384]
17. Srinivasan S, Hanes M, Dickens T, Porteous M, Oh S, Hale L, et al. A mouse model for hereditary hemorrhagic telangiectasia (HHT) type 2. Hum Mol Genet. 2003; 12:473–82. [PubMed: 12588795]
18. Roman BL, Pham VN, Lawson ND, Kulik M, Childs S, Lekven AC, et al. Disruption of acvrl1 increases endothelial cell number in zebrafish cranial vessels. Development. 2002; 129:3009–19. [PubMed: 12050147]
19. Li D, Sorensen L, Brooke B, Urness L, Davis E, Taylor D, et al. Defective angiogenesis in mice lacking endoglin. Science. 1999; 284:1534–7. [PubMed: 10348742]
20. Arthur H, Ure J, Smith A, Renforth G, Wilson D, Torsney E, et al. Endoglin, an ancillary TGFbeta receptor, is required for extraembryonic angiogenesis and plays a key role in heart development. Dev Bio. 2000; 217:42–53. [PubMed: 10625534]
21. Carvalho RLC, Jonker L, Goumans M-J, Larsson J, Bouwman P, Karlsson S, et al. Defective paracrine signalling by TGFbeta in yolk sac vasculature of endoglin mutant mice: a paradigm for hereditary haemorrhagic telangiectasia. Development. 2004; 131:6237–47. [PubMed: 15548578]
22. Sorensen LK, Brooke BS, Li DY, Urness LD. Loss of distinct arterial and venous boundaries in mice lacking endoglin, a vascular-specific TGFβ coreceptor. Dev Biol. 2003; 261:235–50. [PubMed: 12941632]
23. Seki T, Hong K-H, Oh S. Nonoverlapping expression patterns of ALK1 and ALK5 reveal distinct roles of each receptor in vascular development. Lab Invest. 2006; 86:116–29. [PubMed: 16344855]
24. Roelen B, Rooijen M, Mummery C. Expression of ALK-1, a type 1 serine/threonine kinase receptor, coincides with sites of vasculogenesis and angiogenesis in early mouse development. Dev Dyn. 1997; 209:418–30. [PubMed: 9264265]
25. Panchenko MP, Williams MC, Brody JS, Yu Q. Type I receptor serine-threonine kinase preferentially expressed in pulmonary blood vessels. Am J Physiol. 1996; 270:L547–L558. [PubMed: 8928814]
26. Seki T, Yun J, Oh SP. Arterial endothelium-specific activin receptor-like kinase 1 expression suggests its role in arterialization and vascular remodeling. Circ Res. 2003; 93:682–9. [PubMed: 12970115]
27. Torsney E, Charlton R, Parums D, Collis M, Arthur HM. Inducible expression of human endoglin during inflammation and wound healing in vivo. Inflamm Res. 2002; 51:464–70. [PubMed: 12365720]

28. Chien C-Y, Chuang H-C, Chen C-H, Fang F-M, Chen W-C, Huang C-C, et al. The expression of activin receptor-like kinase 1 among patients with head and neck cancer. Otolaryngol Neck Surg. 2013; 148:965–73.
29. Hu-Lowe DD, Chen E, Zhang L, Watson KD, Mancuso P, Lappin P, et al. Targeting activin receptor-like kinase 1 inhibits angiogenesis and tumorigenesis through a mechanism of action complementary to anti-VEGF therapies. Cancer Res. 2011; 71:1362–73. [PubMed: 21212415]
30. Ricard N, Ciais D, Levet S, Subileau M, Mallet C, Zimmers T, et al. BMP9 and BMP10 are critical for postnatal retinal vascular remodeling. Blood. 2012; 119:6162–71. [PubMed: 22566602]
31. Lamouille S, Mallet C, Feige J-J, Bailly S. Activin receptor-like kinase 1 is implicated in the maturation phase of angiogenesis. Blood. 2002; 100:4495–501. [PubMed: 12453878]
32. Mitchell D, Pobre E, Mulivor A, Grinberg A, Castonguay R, Monnell T, et al. ALK1-Fc inhibits multiple mediators of angiogenesis and suppresses tumor growth. Mol Cancer Ther. 2010; 9:379–88. [PubMed: 20124460]
33. Upton PD, Davies RJ, Trembath RC, Morrell NW. Bone Morphogenetic Protein (BMP) and Activin Type II Receptors Balance BMP9 Signals Mediated by Activin Receptor-like Kinase-1 in Human Pulmonary Artery Endothelial Cells. J Biol Chem. 2009; 284:15794–804. [PubMed: 19366699]
34. Scharpfenecker M, Van Dinther M, Liu Z, Van Bezooijen RL, Zhao Q, Pukac L, et al. BMP-9 signals via ALK1 and inhibits bFGF-induced endothelial cell proliferation and VEGF-stimulated angiogenesis. J Cell Sci. 2007; 120:964–72. [PubMed: 17311849]
35. David L, Mallet C, Keramidas M, Lamandé N, Gasc J-M, Dupuis-Girod S, et al. Bone morphogenetic protein-9 is a circulating vascular quiescence factor. Circ Res. 2008; 102:914–22. [PubMed: 18309101]
36. Cunha S, Pardali E, Thorikay M, Anderberg C, Hawinkels L, Goumans M-J, et al. Genetic and pharmacological targeting of activin receptor-like kinase 1 impairs tumor growth and angiogenesis. J Exp Med. 2010; 207:85–100. [PubMed: 20065063]
37. Chen H, Brady Ridgway J, Sai T, Lai J, Warming S, Chen H, et al. Context-dependent signaling defines roles of BMP9 and BMP10 in embryonic and postnatal development. Proc Natl Acad Sci U S A. 2013; 110:11887–92. [PubMed: 23812757]
38. Wooderchak-Donahue WL, McDonald J, O'Fallon B, Upton PD, Li W, Roman BL, et al. BMP9 Mutations Cause a Vascular-Anomaly Syndrome with Phenotypic Overlap with Hereditary Hemorrhagic Telangiectasia. Am J Hum Genet. 2013; 93:530–7. [PubMed: 23972370]
39. Sitohy B, Nagy J a, Dvorak HF. Anti-VEGF/VEGFR therapy for cancer: reassessing the target. Cancer Res. 2012; 72:1909–14. [PubMed: 22508695]
40. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature. 2011; 473:298–307. [PubMed: 21593862]
41. Tung JJ, Tattersall IW, Kitajewski J. Tips, stalks, tubes: notch-mediated cell fate determination and mechanisms of tubulogenesis during angiogenesis. Cold Spring Harb Perspect Med. 2012; 2:a006601. [PubMed: 22355796]
42. Wang X, Solban N, Bhasin MK, Bahamon B, Zhang L, Signoretti S, et al. ALK1-Fc inhibits tumor growth in a VEGF pathway resistance model of renal cell carcinoma. Cancer Res. 2012; 72:LB–313.
43. Van Meeteren LA, Thorikay M, Bergqvist S, Pardali E, Stampino CG, Hu-Lowe D, et al. Anti-human activin receptor-like kinase 1 (ALK1) antibody attenuates bone morphogenetic protein 9 (BMP9)-induced ALK1 signaling and interferes with endothelial cell sprouting. J Biol Chem. 2012; 287:18551–61. [PubMed: 22493445]
44. Uneda S, Toi H, Tsujie T, Tsujie M, Harada N, Tsai H, et al. Anti-endoglin monoclonal antibodies are effective for suppressing metastasis and the primary tumors by targeting tumor vasculature. Int J cancer. 2009; 125:1446–53. [PubMed: 19533687]
45. She X, Matsuno F, Harada N, Tsai H, Seon BK. Synergy between anti-endoglin (CD105) monoclonal antibodies and TGF-beta in suppression of growth of human endothelial cells. Int J cancer. 2004; 108:251–7. [PubMed: 14639611]

46. Tsujie M, Tsujie T, Toi H, Uneda S, Shiozaki K, Tsai H, et al. Anti-tumor activity of an anti-endoglin monoclonal antibody is enhanced in immunocompetent mice. Int J cancer. 2008; 122:2266–73. [PubMed: 18224682]
47. Anderberg C, Cunha SI, Zhai Z, Cortez E, Pardali E, Johnson JR, et al. Deficiency for endoglin in tumor vasculature weakens the endothelial barrier to metastatic dissemination. J Exp Med. 2013; 210:563–79. [PubMed: 23401487]
48. Bendell JC, Gordon MS, Hurwitz HI, Jones SF, Mendelson DS, Blobe GC, et al. Safety, Pharmacokinetics, Pharmacodynamics and Antitumor Activity of Dalantercept, an Activin Receptor-Like Kinase-1 Ligand Trap, in Patients with Advanced Cancer. Clin cancer Res. 2014; 20:480–9. [PubMed: 24173543]
49. Simonelli M, Zucali PA, Thomas MB, Brisendine A, Berlin J, Denlinger CS, et al. Phase I study of PF-03446962 (anti-ALK-1 mAb) in hepatocellular carcinoma (HCC). J Clin Oncol. 2013; 31 abstr 4121.
50. Rosen LS, Hurwitz HI, Wong MKK, Goldman J, Mendelson DS, Figg WD, et al. A Phase 1 First-in-Human Study of TRC105 (Anti-Endoglin Antibody) in Patients with Advanced Cancer. Clin Cancer Res. 2012; 18:4820–9. [PubMed: 22767667]
51. Walker EJ, Su H, Shen F, Choi E, Oh SP, Chen W, et al. NIH Public Access. Ann Neurol. 2011; 69:954–62. [PubMed: 21437931]
52. Choi E, Walker EJ, Oh SP, Arthur M. Minimal Homozygous Endothelial Deletion of Eng with VEGF Stimulation Is Sufficient to Cause Cerebrovascular Dysplasia in the Adult Mouse. Cerebrovasc Dis. 2012; 33:540–7. [PubMed: 22571958]
53. Kanellopoulou T, Alexopoulou A. Bevacizumab in the treatment of hereditary hemorrhagic telangiectasia. Expert Opin Biol Ther. 2013; 13:1315–23. [PubMed: 23815519]
54. Shovlin CL. Hereditary haemorrhagic telangiectasia: pathophysiology, diagnosis and treatment. Blood Rev. 2010; 24:203–19. [PubMed: 20870325]
55. Pardali E, Goumans M-J, ten Dijke P. Signaling by members of the TGF-beta family in vascular morphogenesis and disease. Trends Cell Biol. 2010; 20:556–67. [PubMed: 20656490]
56. Dupuis-Girod S, Ginon I, Saurin J-C, Marion D, Guillot E, Decullier E, et al. Bevacizumab in patients with hereditary hemorrhagic telangiectasia and severe hepatic vascular malformations and high cardiac output. J Am Med Assoc. 2012; 307:948–55.
57. Flieger D, Hainke S, Fischbach W. Dramatic improvement in hereditary hemorrhagic telangiectasia after treatment with the vascular endothelial growth factor (VEGF) antagonist bevacizumab. Ann Hematol. 2006; 85:631–2. [PubMed: 16807748]
58. Bose P, Holter JLSG. Bevacizumab in Hereditary Hemorrhagic Telangiectasia. N Engl J Med. 2009; 360(20):2143–4. [PubMed: 19439755]
59. Chen S, Karnezis T, Davidson TM. Safety of intranasal Bevacizumab (Avastin) treatment in patients with hereditary hemorrhagic telangiectasia-associated epistaxis. Laryngoscope. 2011; 121:644–6. [PubMed: 21344447]
60. Dheyauldeen S, Østertun Geirdal A, Osnes T, Vartdal LS, Dollner R. Bevacizumab in hereditary hemorrhagic telangiectasia-associated epistaxis: effectiveness of an injection protocol based on the vascular anatomy of the nose. Laryngoscope. 2012; 122:1210–4. [PubMed: 22565282]

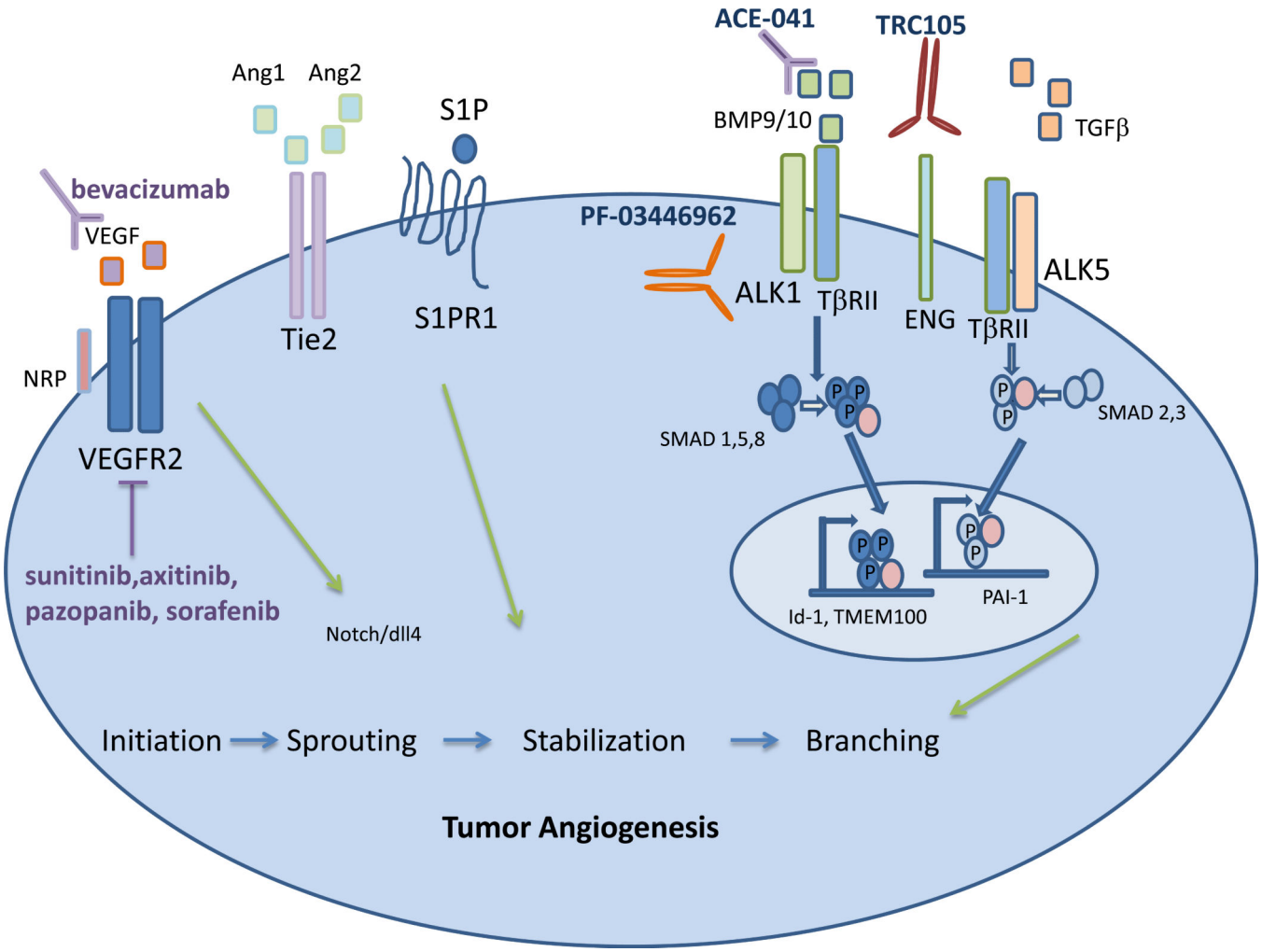

Figure 1.

Tumor angiogenesis involves several molecular pathways that affect sequential steps in vessel formation. Shown here are some representative pathways that lead to the formation of vascular networks in tumors. Also shown are the inhibitors of the ALK/ENG pathway. ACE-041 (ALK1-Fc, dalantercept), Anti-ALK1 (PF-03446962) and Anti-ENG (TRC105) are all inhibitors of this pathway and are currently in clinical development.

Table 1

Ongoing trials of ALK1/ENG pathway inhibitors

	Tumor Type	N	Phase	Status
Dalantercept	Squamous Cell Ca	45	2	Recruiting
	Endometrial Ca	52	2	Recruiting
	Renal Cell Ca	156	2	Recruiting
	Epithelial Ovarian, Fallopian tube, Primary Peritoneal Ca	43	2	Recruiting
	Advanced solid tumors, multiple myeloma	37	1	Completed
PF-03446962	Hepatocellular Ca	180	2	Not yet recruiting
	Advanced Solid Tumors	68	1	Completed
	Transitional Cell Ca of the Bladder	45	2	Not yet recruiting
TRC105	Metastatic Breast Ca	30	1B	Recruiting
	Advanced Solid Tumor	38	1B	Active, not recruiting
	Renal Cell Ca	18	1B	Recruiting
	Glioblastoma Multiforme	128	1,2	Recruiting
	Advanced Urothelial Carcinoma	13	2	Active, not recruiting
	Glioblastoma Multiforme	32	2	Recruiting
	Recurrent Ovarian Cancer, Fallopian tube Carcinoma			Active, not
	Primary Peritoneal Carcinoma	45	2	recruiting
	Hepatocellular Carcinoma (HCC)	30	2	Recruiting
	Metastatic Castrate Resistant Prostate Cancer	21	1,2	Active, not recruiting
	Hepatocellular Carcinoma (HCC)	72	1,2	Recruiting
	Advanced Solid Cancer	51	1	Completed
	Glioblastoma Multiforme	22	2	Recruiting
	Advanced Renal Cell Carcinoma	88	2	Recruiting