

Hereditary hemorrhagic telangiectasia: from signaling insights to therapeutic advances

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Hereditary hemorrhagic telangiectasia (HHT) is an inherited vascular disorder with highly variable expressivity, affecting up to 1 in 5,000 individuals. This disease is characterized by small arteriovenous malformations (AVMs) in mucocutaneous areas (telangiectases) and larger visceral AVMs in the lungs, liver, and brain. HHT is caused by loss-of-function mutations in the BMP9-10/ENG/ALK1/SMAD4 signaling pathway. This Review presents up-to-date insights on this mutated signaling pathway and its crosstalk with proangiogenic pathways, in particular the VEGF pathway, that has allowed the repurposing of new drugs for HHT treatment. However, despite the substantial benefits of these new treatments in terms of alleviating symptom severity, this not-so-uncommon bleeding disorder still currently lacks any FDA- or European Medicines Agency-approved (EMA-approved) therapies.

Genetic basis of HHT

Hereditary hemorrhagic telangiectasia (HHT) or Osler-Weber-Rendu syndrome is a hereditary disease that is transmitted in an autosomal dominant manner and is caused by loss-of-function (LOF) mutations in certain components of the predominantly endothelial BMP9-10/ENG/ALK1/SMAD4 signaling pathway, which is an important mediator of vascular quiescence (1). Specifically, mutations in *ENG* (chromosomal locus 9q34.11, encoding the coreceptor endoglin) and *ACVRL1* (chromosomal locus 12q13.13, encoding the type I receptor ALK1) are nearly equally responsible for the majority of HHT cases and give rise to the two major forms of the disease, HHT1 (OMIM 187300) and HHT2 (OMIM 600376), respectively (2–4). Collectively, over 600 different HHT-associated pathogenic mutations in *ENG* and *ACVRL1*, spanning the entire coding sequences of both genes, have been documented in the ClinVar database (5). While reported *ENG* mutations were mostly nonsense or frameshift mutations leading to premature termination codons, a higher prevalence of missense mutations was observed for *ACVRL1*. Mutations in the *SMAD4* gene (chromosomal locus 18q21.2) were also found in HHT patients, although more rarely than *ENG* and *ACVRL1* mutations, resulting in a combined syndrome of HHT and juvenile polyposis (JP-HHT; OMIM 175050) (4, 6). Based on the *SMAD4* mutation repository developed by Wooderchak et al. (7), at least 26 different mutations in *SMAD4* have been described in patients with the combined JP-HHT syndrome, most of which are missense mutations and deletions that are mainly concentrated at the MH2 domain of SMAD4.

Together, mutations in *ENG*, *ACVRL1*, and *SMAD4* are responsible for more than 90% of HHT cases, leaving a minority of clinically diagnosed individuals with an unknown genetic basis. In some cases, this can be attributed to limitations of current sequencing technologies in detecting certain variants in the previously identified loci or to the exclusion of testing noncoding regions (8); however, additional chromosomal loci could also be implicated in HHT development. As such, mutations in *GDF2* (chromosomal locus 10q11.22, encoding BMP9) were described in few cases displaying an HHT-like syndrome (9–13). This rare form of HHT was annotated as HHT5 (OMIM #615506). In addition, mutations in *RASAI*, which are commonly associated with hereditary capillary malformations with or without arteriovenous malformations (AVMs) (14, 15), have been reported in few patients presenting HHT symptoms (16–18). More recently, LOF variants in *EPHB4* (encoding ephrin receptor B4) were reported in a few individuals exhibiting atypical HHT symptoms and HHT-like hepatic abnormalities (19). Furthermore, one study described an overrepresentation of rare mutations in *DROSHA*, a key enzyme involved in miRNA processing, among clinically diagnosed HHT patients who did not carry any mutations in the typical HHT-associated genes (20). Interestingly, this study also demonstrated that zebrafish and mice with endothelial-specific *DROSHA* deficiency developed vascular defects resembling those observed in HHT patients (20).

Clinical manifestations of HHT

AVMs are the hallmark of HHT. According to the international classification of vascular anomalies, AVMs are characterized by malformed arteries, veins, and capillaries with direct arteriovenous communications resulting in arteriovenous shunting (21) (Figure 1). The stepwise development of AVMs in the context of HHT was histologically documented in cutaneous biopsies by Braverman et al. in 1990 (22). This process was found to commence with focal dilations of the postcapillary venules that progressively encompass

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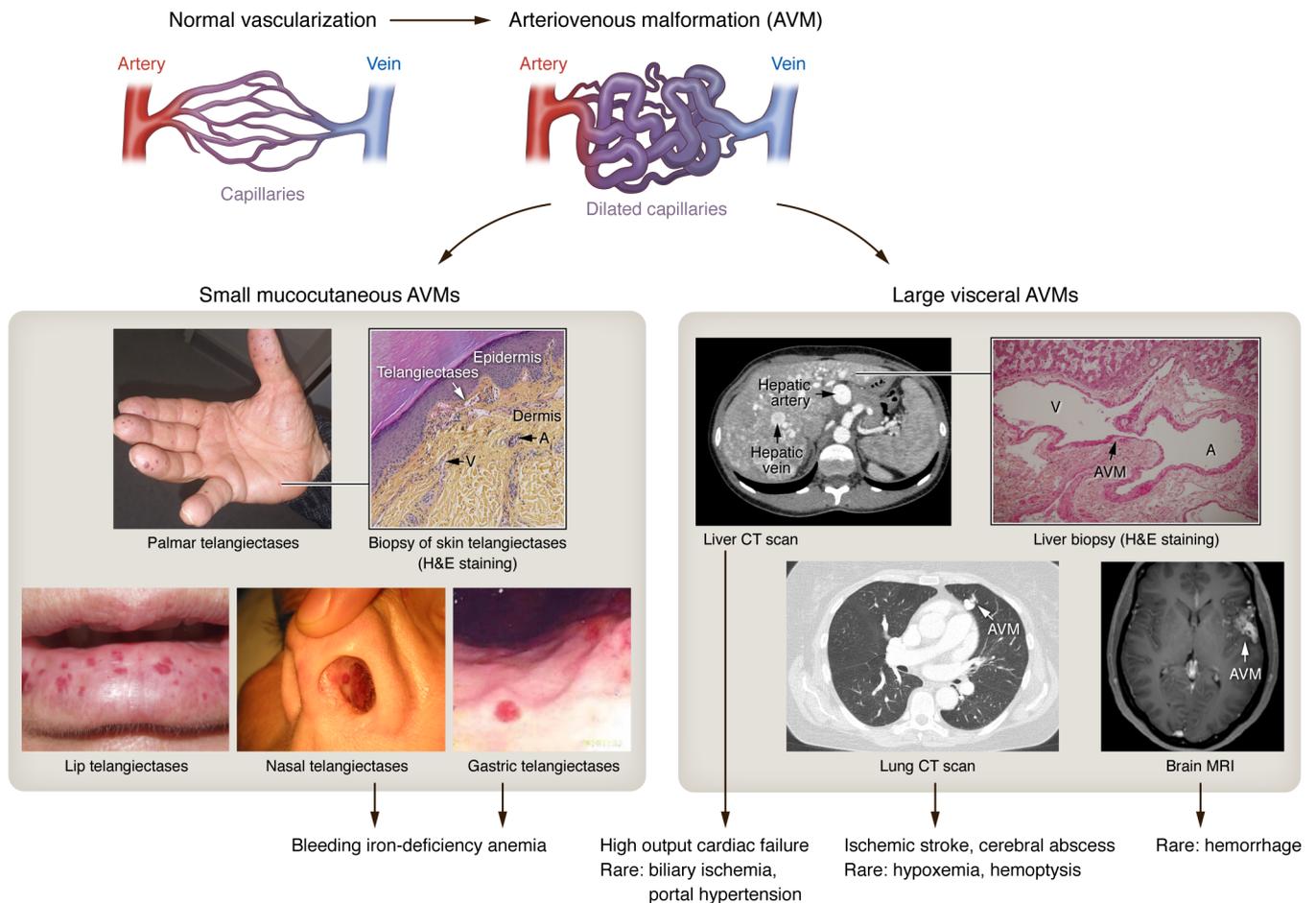


Figure 1. AVMs: The hallmark of HHT and its clinical consequences. In HHT, focal dilations of the postcapillary venules (indicated on images by V) progressively encompass the normal capillaries and establish a direct connection with dilated arterioles (indicated by A), leading to AVM formation, muscularization of large vessels, and dilation of capillary beds, which are often surrounded by inflammatory cells. At the microvascular level, AVMs appear as telangiectases on specific mucocutaneous areas (finger pads, lips, tongue, nasal and digestive mucosa). They are responsible for spontaneous and recurrent epistaxis/bleeding, leading to bleeding iron-deficiency anemia. At the macrovascular level, large AVMs mainly affect the liver, leading to high-output cardiac failure, and more rarely biliary ischemia and portal hypertension; lung AVMs can provoke ischemic stroke and cerebral abscess and, more rarely, hypoxemia and hemoptysis. Central nervous system AVM is rarely complicated by hemorrhage. Skin and liver sections were stained for H&E. Photo of the liver section was provided by J.Y. Scoazec (Institut Gustave Roussy, University Paris-Saclay, Paris, France).

the normal capillaries, finally establishing a direct connection with dilated arterioles. The venous origin of AVMs was later supported by studies specifically deleting *Alk1* or *Eng* from venous and capillary beds, which was sufficient to obtain AVMs in retinas (23, 24). In addition, the process seemed to involve an immune arm, as perivascular lymphocytic infiltrates were evident at the site of the AVM (22). It is noteworthy that all clinical signs of HHT result from AVMs of varying size and number affecting different organ systems.

At the mucocutaneous surfaces, HHT AVMs appear as telangiectases, after which the disease is named. HHT-associated telangiectases are typically small, red, flat, or slightly elevated spots that blanch under pressure. They are classically present on the palmar faces of finger pads and hands, the lips, the tongue, and mucosal areas inside of the mouth, the nasal cavity, and the gastrointestinal (GI) tract (25, 26) (Figure 1). On the nasal mucosa, telangiectases are responsible for the spontaneous and recurrent epistaxis leading to iron-deficiency anemia in about 50% of patients (27). Similarly, GI telangiectases, which can affect any part of the tract, occasionally result in occult bleeding.

In visceral organs, AVMs can reach a larger size in the lungs, the central nervous system, and the liver and result in life-threatening complications (Figure 1). Pulmonary AVMs can cause direct complications, such as hemorrhage/hemoptysis, due to rupture and hypoxemia due to shunting, but can also cause indirect complications by allowing systemic embolic events resulting in brain abscesses and embolic strokes (28). On the other hand, hepatic AVMs can lead to high-output cardiac failure, biliary ischemia, and portal hypertension (29), warranting liver transplantation. Interestingly, hepatic relapse of the vascular lesions has been described in some HHT patients receiving liver transplants (30), suggesting a potential role of circulating endothelial precursors, which bear the HHT-causal mutation, in populating the newly transplanted liver and driving the formation of new vascular lesions. Cerebral and spinal AVMs are less frequent and generally asymptomatic. Although they can cause serious complications, such as hemorrhagic stroke or epilepsy, the risks associated with treatment are currently higher than those of natural progression and unruptured

cerebral AVMs are generally not treated. Widespread screening of asymptomatic HHT patients is therefore still controversial (31).

Cohort studies have demonstrated interorgan differences in the natural history of AVMs. Mucocutaneous telangiectases and liver AVMs appear progressively over the life span, with complications generally occurring in late adulthood. The majority of lung AVMs are probably present from childhood, but some of them become detectable only during adulthood. Most brain AVMs appear in utero or in early childhood and generally remain stable later in life (32). Although HHT1 and HHT2 patients are clinically indistinguishable, *ACVRL1* mutations are associated with higher rates of liver AVMs and digestive telangiectases, while *ENG* mutations are more predominantly linked to pulmonary and cerebral AVMs (33).

In addition to the well-documented HHT symptoms, i.e., epistaxis, telangiectases, and AVMs, HHT patients exhibit a heightened infectious risk in soft tissues, bones, and joints involving *Staphylococcus aureus* as well as cerebral infections involving bacteria from the orodigestive flora (34). HHT is also associated with immunological abnormalities, mainly characterized by a T-helper lymphopenia, although these abnormalities lack clear correlation with the aforementioned infectious risks (35).

The second-hit hypothesis

The LOF nature of HHT causal mutations and the autosomal dominant inheritance of the disease led to the longstanding belief that HHT is caused by haploinsufficiency of the mutated gene product. This was corroborated by several reports demonstrating a reduction in endoglin levels in HHT1-derived cells (36–38) and in ALK1 levels in some HHT2-derived cells compared with control counterparts (37–39). In line with these studies, mice heterozygous for mutations in *Eng* or *Acvrl1* (*Eng*^{+/-} or *Alk1*^{+/-}) display reduced expression levels of the affected gene and develop some HHT-like lesions at the adult stage, albeit with a low penetrance, including telangiectases, nosebleeds, and dilated vessels with reduced vascular smooth muscle cell (VSMC) coverage (40–42).

However, the haploinsufficiency model does not explain why HHT vascular lesions develop focally in characteristic vascular beds despite the presence of the germline HHT causal mutation in all endothelial cells (ECs) of the body (43). This model also fails to elucidate the differences in disease expressivity that are observed even between related patients carrying the same mutation (44). These disparities put forth the second-hit hypothesis in HHT, which is becoming more and more accepted by the HHT scientific community (45). This hypothesis implies that the germline mutation (first hit) predisposes the endothelium to vascular defects that strictly develop in the presence of additional, local factors (second hit) that could be either environmental or genetic. Along these lines, several preclinical studies have demonstrated the role of proangiogenic and proinflammatory triggers in driving HHT pathogenesis (46). For instance, *Eng*^{+/-} or *Alk1*^{+/-} mice acquired cerebrovascular dysplasia 8 to 10 weeks after intracranial administration of viral vectors encoding VEGF, as opposed to normal angiogenesis in WT mice receiving the same treatment (47, 48). In addition, unlike WT mice, inducible *Eng*- or *Alk1*-knockout mice readily and consistently developed AVMs in their brains only upon local VEGF overexpression (49–52) as well as dermal AVMs strictly after wounding or upon local VEGF

or LPS treatment (53–56). Interestingly, blocking VEGF in inducible *Alk1*^{-/-} mice partially reversed or blocked the development of AVMs induced by VEGF, LPS, or wounding (51, 55), in line with the well-documented effectiveness of the humanized anti-VEGF antibody bevacizumab in alleviating HHT symptoms (Table 1) (57, 58). In addition to angiogenic and inflammatory stimuli, several other environmental triggers have been proposed as potential second hits in HHT, including mechanical stress and sun exposure. A study on 103 HHT patients revealed a higher number of telangiectases on the dominant hand and on the lower lip, which are expected to be more frequently exposed to mechanical stimuli, than the other hand and the upper lip (59). The number of telangiectases on the lips was also found to be positively correlated with sunlight exposure (59).

Another study supporting the two-hit hypothesis in HHT patients was released in 2019, but this time involving a genetic second hit (60). Using next-generation sequencing, Snellings et al. detected low-frequency somatic mutations, mostly in trans configuration, leading to biallelic loss of *ENG* or *ACVRL1* in cutaneous telangiectases on the hands of some HHT patients (60). Consequently, HHT vascular malformations were proposed to develop when specific endothelial clones acquire somatic mutations leading to loss of heterozygosity (60). This is a well-known pathogenic mechanism in cancer, known as the Knudsonian two-hit mechanism (61), that was also shown in some heritable vascular anomalies, including venous, glomuvenous, and cerebral cavernous malformations (62, 63). It remains to be validated whether this hypothesis holds true for HHT-related AVMs. As sunlight comprises mutagenic ultraviolet radiation, it is plausible that prolonged exposure to sunlight may trigger somatic mutations that potentially support the development of dermal telangiectases on the hands. This hypothesis might explain the late-onset development of some AVMs (skin, liver) (32), but cannot explain the ones that appear early in life (cerebral and pulmonary AVMs) (32, 64). Therefore, the somatic second-hit hypothesis could indeed be responsible for some HHT-related vascular defects, but might not represent a universal pathogenic mechanism in HHT.

Signaling pathways involved in HHT

BMP9-10/ENG/ALK1/SMAD4 pathway. Endoglin and ALK1 are two transmembrane receptors mainly expressed on ECs, explaining why mutations in this pathway result in vascular abnormalities. BMP9 and BMP10 are the high-affinity ligands of the receptors ALK1 and endoglin (65, 66). Endoglin is a coreceptor for BMP9 and BMP10 and serves as a reservoir of these ligands on the surface of ECs (67), enhancing ligand-induced responses (65). In line with endoglin's role as an upstream coreceptor of the ALK1 pathway, it was shown that AVMs induced by endothelial loss of *Eng* could be corrected by overexpression of ALK1, whereas endoglin overexpression could not compensate for the loss of *Alk1* (68). BMP9 or BMP10 recruits a heterocomplex of two type II receptors (BMPRII or ActRIIA, which are the main type II receptors expressed on ECs) and two type I receptors (ALK1) (1, 69). Upon BMP9 or BMP10 binding, the serine/threonine kinase type II receptor phosphorylates the serine/threonine kinase type I receptor ALK1, leading to its activation. Subsequently, activated ALK1 phosphorylates the transcription factors SMAD1/5, allowing their binding to SMAD4, which is a common downstream signaling mediator shared with the TGF-β

Table 1. Therapeutic strategies targeting the VEGF pathway

Therapeutic group	Evidence in preclinical models and HHT patients			
	Drug used	References	Study type	Key findings
Anti-VEGF-A monoclonal antibodies	Bevacizumab	E.J. Walker, 2012 (51)	Mouse model	VEGF blockade reduces vascular density in a brain AVM-induced mouse model (<i>Alk1</i> -KO+VEGF)
		S. Dupuis-Girod, 2012 (86)	Prospective phase 2 open label (<i>n</i> = 25)	Decrease in cardiac output and reduction of the duration and number of episodes of epistaxis
		A. Chavan, 2013 (113)	Prospective case series (<i>n</i> = 3)	Reduction in epistaxis frequency, quantity and duration of bleeding per episode; normalization of cardiac output
		A.B. Thompson, 2014 (114)	Prospective open label (<i>n</i> = 6)	Low bevacizumab doses improve epistaxis severity and frequency
		A. Guilhem, 2017 (115)	Retrospective (<i>n</i> = 46)	Improvement of 74% of the cohort
		A. Chavan, 2017 (116)	Prospective (<i>n</i> = 21)	Clinical improvement (pain, dyspnea, fatigability, and general performance) without significant toxicity in 18 patients
		V.N. Iyer, 2018 (117)	Retrospective (<i>n</i> = 34)	Reduction in epistaxis severity score and RBC transfusion requirements
		H. Al-Samkari, 2020 (118)	Survey based (<i>n</i> = 291)	Bevacizumab reported to be effective in reducing bleeding symptoms and improving hematologic parameters
		C. Vázquez, 2020 (119)	Retrospective (<i>n</i> = 20)	Reduction in RBC transfusion requirements, increase in hemoglobin levels
		H. Al-Samkari, 2021 (58)	Retrospective (<i>n</i> = 238)	Reduction in RBC transfusion requirements and in i.v. iron supplementation; increase in hemoglobin levels
		S. Dupuis-Girod, 2023 (120)	Prospective randomized trial (<i>n</i> = 24)	Reduction in RBC transfusion requirements and in i.v. iron supplementation; increase in hemoglobin levels
		NCT04404881	Ongoing trial (TRUST) (<i>n</i> = 33, estimated)	Not yet available
		(Various)	28 singular case reports	
G6-31 antibody	C. Han, 2014 (55)	Mouse model	Reduces wound-induced skin AVMs and hemorrhages in <i>Alk1</i> -KO adult mice	
		D.S. Ardelean, 2014 (105)	Mouse model	Normalizes the lung peripheral microvessel density and attenuates the secondary right ventricular hypertrophy in <i>Eng</i> ^{-/-} and <i>Alk1</i> ^{+/-} mice
Extracellular receptor trap for VEGF-A, VEGF-B, and PlGF	Aflibercept	B. Villanueva, 2023 (121)	Case report	Reduction in RBC transfusion requirements; increase in hemoglobin levels
Anti-VEGFR2 antibody	D5B1	J.H. Thalgott, 2018 (88)	Mouse model	Reduces telangiectases in trachea of <i>Alk1</i> -KO mice infected with <i>M. pulmonis</i>
		S. Tual-Chalot, 2020 (106)	Mouse model	Reduces pelvic AVMs and HOHF in adult in <i>Eng</i> -ieKO mice

pathway (Figure 2). The trimeric SMAD complex then migrates to the nucleus, where it interacts with other transcription factors to regulate the transcription of many target genes (1, 69). Accordingly, endothelium-specific *Smad1/5* or *Smad4* deletions resulted in AVM formation in the retina (70–72). In addition, few studies show that BMP9 and BMP10 can activate noncanonical BMP signaling pathways, including P38, ERK, Wingless (Wnt), and NOTCH signaling (73). It is widely accepted that BMP9 and BMP10 lead to vascular maturation and quiescence (74). The current working model suggests that BMP9-10/ENG/ALK1/SMAD4 signaling maintains vascular homeostasis via attenuation of proangiogenic pathways and, in particular, the VEGF signaling pathway. However, the mechanisms underlying this attenuation are still not fully characterized.

The immunosuppressor tacrolimus (FK506) has been identified in two independent screens as a potent activator of SMAD1/5 signaling using a reporter assay based on the Id1 promoter (75, 76). However, the mechanism by which tacrolimus activates this pathway is not clearly understood. Tacrolimus is a macrolide with immunomodulatory and antiangiogenic properties commonly

used in patients who have undergone organ transplantation (77). It inhibits the phosphatase calcineurin, which dephosphorylates NFAT proteins (Figure 3) (78, 79). Activation of calcineurin has also been shown to be downstream of VEGF (80) (Figure 2). Interestingly, we have previously shown that BMP9 regulates the calcineurin/NFAT pathway in lymphatic differentiation (81). Tacrolimus can also activate the BMP signaling pathway by blocking 12 kDa FK506 (FKBP12) (Figure 3) (82), which is known to bind and suppress ALK1 activation (76). In ECs, it was shown that tacrolimus activated SMAD1/5 signaling and inhibited AKT and P38 phosphorylation induced by VEGF (75). The same group showed that tacrolimus injection in BMP9/BMP10-immunodepleted postnatal retinas prevented hypervascularization (Table 2). However, the molecular mechanisms involved in this protective effect of tacrolimus are not yet fully elucidated.

VEGF/VEGFR2 pathway. VEGFs, which include VEGF-A, VEGF-B, and PlGF (placenta growth factor), are some of the most potent and extensively studied angiogenic factors. VEGF signaling occurs through its binding to the receptor tyrosine kinase VEGFR2,

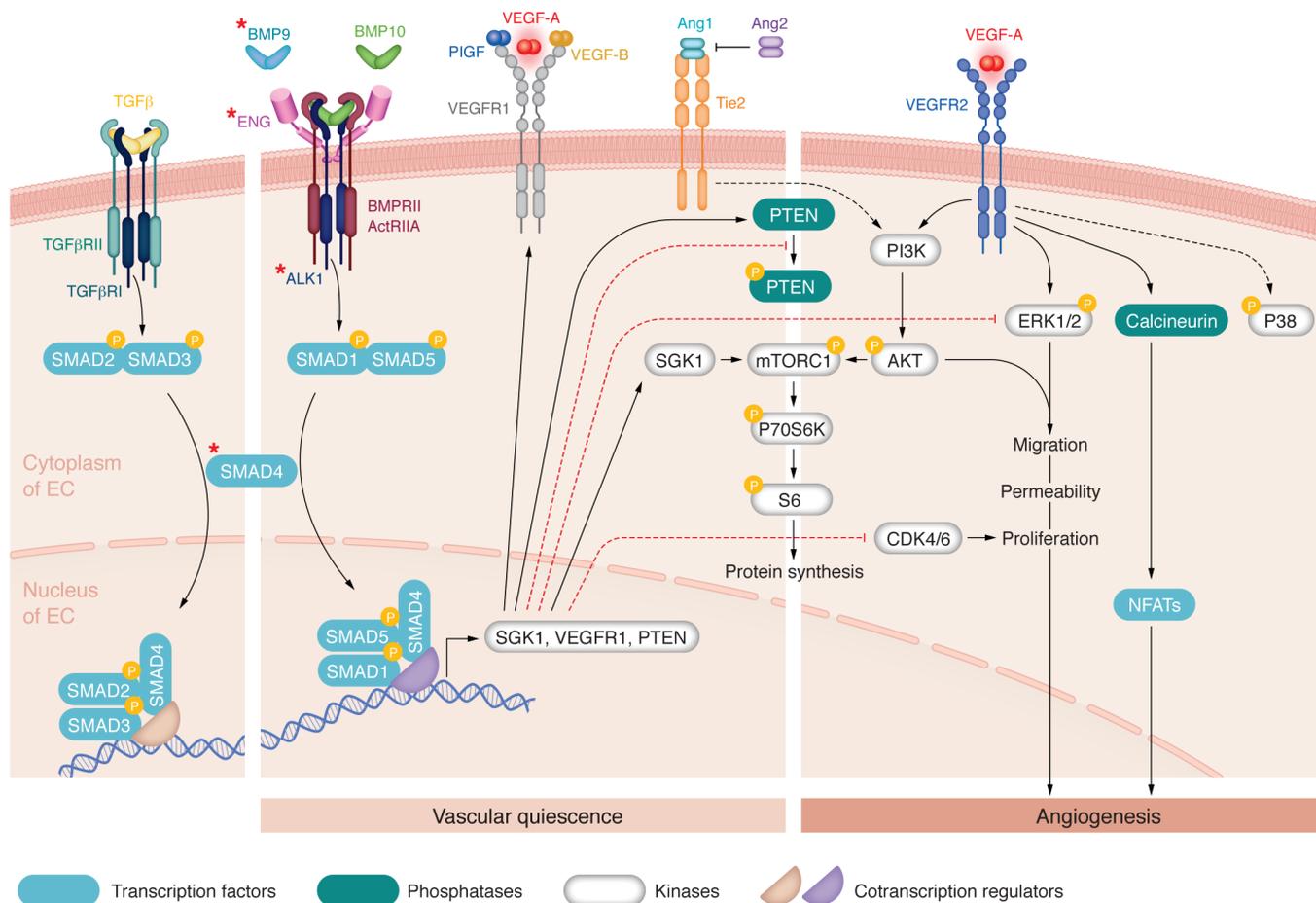


Figure 2. BMP9-10/ENG/ALK1/SMAD4 signaling pathway maintains vascular quiescence by repressing angiogenic pathways. HHT occurs due to LOF mutations in *ENG*, *ALK1*, *SMAD4*, and, more rarely, *BMP9* (respective proteins indicated with red asterisks), which are all in the same signaling pathway. On endothelial cells, BMP9 or BMP10 recruits a heterocomplex composed of two type II receptors (BMPRII or ActRIIA, which are the main type II receptors expressed on ECs, and two similar type I receptors (ALK1), and the coreceptor ENG (endoglin). Upon BMP binding, the type II receptor phosphorylates ALK1, which subsequently phosphorylates the transcription factors SMAD1/5. SMAD1/5 bind SMAD4, which is shared with the TGF- β pathway, to regulate transcription of many genes (in association with other transcription factors). BMP9 and BMP10 maintain vascular quiescence (middle panel) via repression of angiogenesis pathways (right panel). VEGF-A (red) binds to VEGFR2, which activates the ERK1/2 and P38 MAPK pathways and the PI3K/AKT/mTORC1 pathway. In turn, the PI3K/AKT/mTORC1 pathway activates the signaling cascade P70S6K/S6. VEGF can also activate the calcineurin phosphatase, which activates, via dephosphorylation, the NFAT transcription factor family. The PI3K/AKT/mTOR pathway is negatively regulated by the phosphatase PTEN, which is active when unphosphorylated. VEGF-A can also bind to VEGFR1, but this will not generate a signal. Two other members of the VEGF family, VEGF-B (yellow) and PlGF (blue), also bind to VEGFR1. BMP9 induces the expression of VEGFR1, thus inhibiting VEGF signaling. BMP9 also induces PTEN expression and phosphorylation, which inhibit AKT activity as well as the expression of SGK1 kinase, which can activate the mTORC1/P70S6K/S6 pathway. Moreover, BMP9 inhibits ERK activation and CDK4/6 kinases through not-yet-characterized mechanisms. Ang1 activates the TIE2 receptor to maintain vascular quiescence, and this pathway can be antagonized by Ang2.

which activates several downstream pathways (Figure 2). These include the ERK1/2 pathway, the PI3K/AKT/mTOR pathway, the SRC and small GTPases pathways, and others that are poorly understood, including the p38 MAPK pathway (80, 83) (Figure 2). The PI3K/AKT/mTOR pathway is negatively regulated by the phosphatase and tensin homolog (PTEN), which is active when unphosphorylated. VEGF-A, VEGF-B, and PlGF can also bind VEGFR1 with higher affinity than VEGFR2, but the former exhibits low kinase activity, making VEGFR1 a decoy receptor (80). VEGF signaling is thus modulated by the different relative expression levels of VEGFR2 versus VEGFR1 in ECs.

Awareness of the beneficial effect of blocking VEGF signaling in HHT patients dates back to 2006, when an HHT patient suffering from a malignant mesothelioma unexpectedly showed amelioration of HHT symptoms following antiangiogenic cancer treat-

ment with an anti-VEGF-A monoclonal antibody (bevacizumab) (84). The following year, BMP9 and BMP10 were identified as two high-affinity ligands for the receptors ALK1 and endoglin (65, 66). It was shown that these two ligands inhibited angiogenesis in vitro (EC proliferation and migration) and in ex vivo models (66, 85). A few years later, the first clinical trial using bevacizumab showed very positive results on 25 patients suffering from HHT (Table 1) (86). Since then, preclinical HHT models have been developed in order to test different therapeutic approaches. The main model used is the murine retina, a two-dimensional-like vascular structure that forms via angiogenesis during early postnatal days. It has been shown that the loss of *Alk1*, *Eng*, *Smad1/5*, *Smad4*, or *Bmp9/Bmp10* led to spontaneous AVMs in the retina (46). Using these models, it was shown that blocking VEGF signaling reduced the

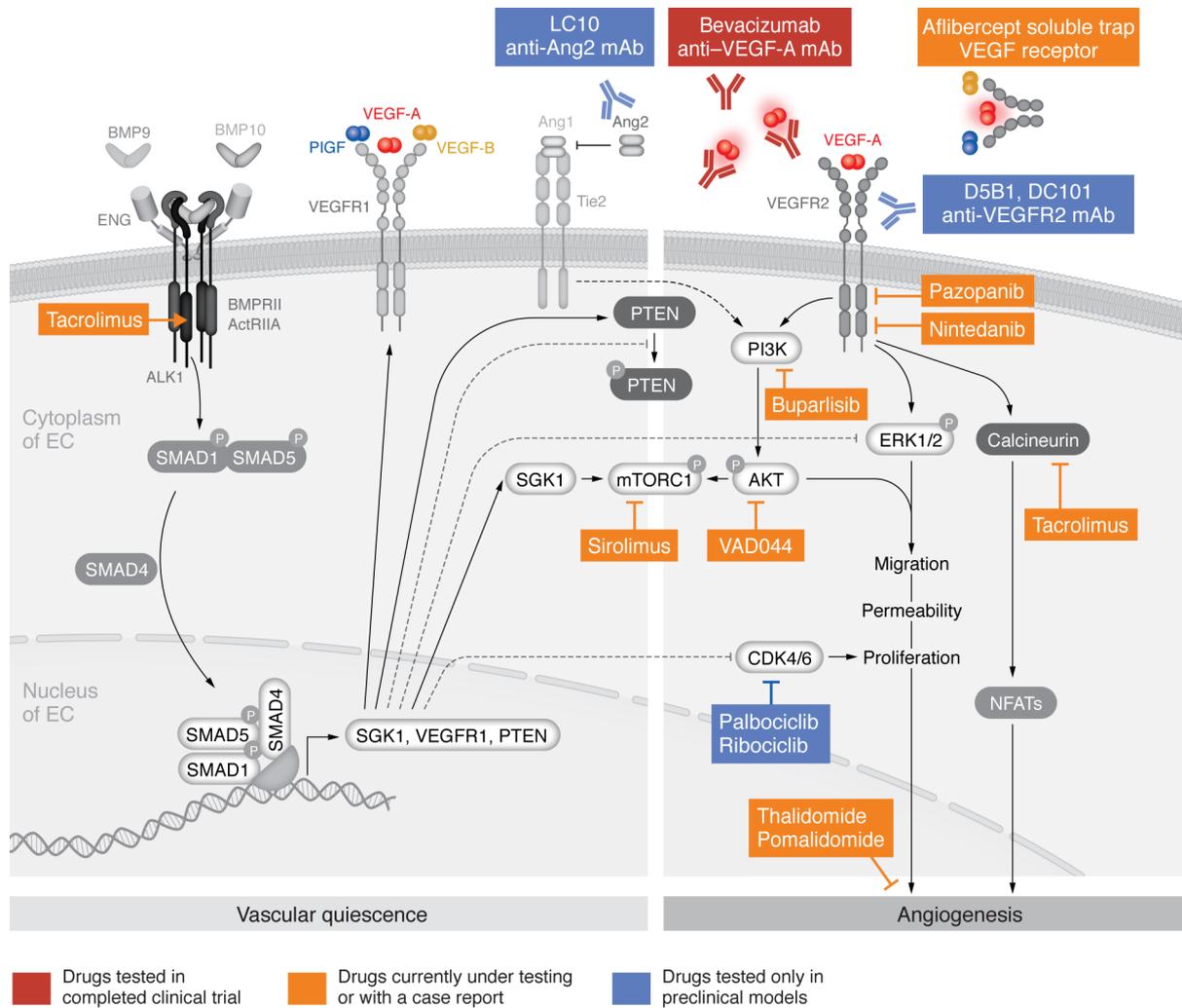


Figure 3. Therapeutic targets of antiangiogenic drugs tested in preclinical models and in HHT patients. Figure shows the targets of drugs tested in completed clinical trials (red), drugs currently under testing or with a case report (orange), and drugs tested only in preclinical models (blue). For further details, see Table 1 and Table 2. Drugs have been developed to block VEGF-A signaling using neutralizing anti-VEGF-A mAbs (bevacizumab) or soluble trap/decoy receptors that bind VEGF-A (red), VEGF-B (yellow), and PIGF (blue) (aflibercept), or neutralizing anti-VEGFR2 antibodies (D5B1 and DC101). Drugs developed to block intracellular signaling, such as tyrosine kinase receptor inhibitors that block VEGFR2 activity but also other receptors, are currently undergoing testing: pazopanib (VEGFR, PDGFR, c-KIT, and FGFR) and nintedanib (VEGFR, PDGFR, and FGFR). Drugs have also been developed to block PI3K and AKT (VAD044), as well as mTORC1 (sirolimus) and calcineurin and FKBP12 (tacrolimus). Immunomodulatory imide drugs (IMiDs), such as thalidomide and pomalidomide, have been tested in HHT patients. Other drugs have been tested so far only in preclinical models, such as the neutralizing anti-Ang2 monoclonal antibodies (LC10) and inhibitors of CDK4/6 (palbociclib and ribociclib).

development of vascular malformations (51, 55). These mouse models supported that AVM formation involved aberrant EC responses, including enhanced proliferation, impaired flow-migration coupling, and abnormal cellular behavior in response to angiogenic signals such as VEGF (46).

To date, there is a limited understanding of how BMP9 or BMP10 mitigates VEGF signaling. It has been found that BMP9 induces the expression of VEGFR1, thus restricting downstream VEGF signaling (87) (Figure 2). Conversely, inhibiting ALK1 signaling in ECs using ALK1 ligand trap (ALK1-Fc) promoted an elevation of several key proangiogenic regulators (*DLL4*, *ANGPT2* [encoding angiopoietin 2], and *KDR* [encoding VEGFR2]) (75). Accordingly, it was shown that *Alk1*^{-/-} mice presented a reduced level of VEGFR1 expression and that VEGFR1 levels were reduced in skin biopsies from HHT patients (88).

Concerning the molecular mechanisms involved downstream of the VEGF signaling pathway, studies in mice have shown that the loss of *Alk1* or *Smad4* resulted in the activation of the PI3K/AKT pathway (70, 89, 90). Similar results were obtained in vitro in HUVECs using siRNA against ALK1 (70, 90). Inversely, BMP9 treatment for 2 hours in HUVECs was found to increase PTEN expression and activity, leading to a decrease in AKT activity (Figure 2) (89, 90). Activated AKT subsequently activates the mTORC1 complex, which in turn activates the signaling cascade P70S6K/S6 (Figure 2). Interestingly, this pathway has been found activated in skin telangiectases from HHT patients (89, 91). In HUVECs, BMP9 was also found to induce the expression of SGK1 kinase, which can also activate the mTORC1/P70S6K/S6 pathway (92). In this work, it was proposed that activation of this pathway would play a role in regulating protein synthesis. Additionally, BMP9 was also

shown to inhibit ERK activation, although the specific mechanism behind this inhibition remains unclear (Figure 2). In parallel, BMP9 and BMP10 have been shown to inhibit endothelial cell proliferation, but the underlying mechanism is not yet clearly characterized. One study showed that BMP9-induced inhibition of EC proliferation was SMAD1/5 dependent and required the expression of the CDK4/6 inhibitor P27^{KIP} (93). In contrast, *Smad4* loss enhanced flow-mediated KLF4/TIE2/PI3K/AKT signaling, leading to cell-cycle progression, which was inhibited using CDK4/6 inhibitors (94). Accordingly, the CDK4/6 inhibitors and ribociclib inhibited the formation of retinal AVMs and the knockdown of CDK6 prevented the development of retinal AVMs (Table 2) (94, 95).

Angiopoietin/Tie2 pathway. Genetic HHT mouse models have also shown an increase in Angiopoietin 2 (Ang2) levels (96, 97). The angiopoietin/Tie2 pathway is critical for maintaining vessel stability by regulating, as for VEGF, the PI3K/AKT pathway (Figure 2). Angiopoietin 1 (Ang1), produced by mural cells, activates the receptor tyrosine kinase TIE2 to maintain VSMC coverage. An increase in TIE2 antagonist Ang2 produced by ECs inhibits Ang1-mediated TIE2 activation, leading to destabilization of VSMC-EC interaction and a decrease in vascular quiescence (83, 98). Accordingly, it was recently shown that blocking Ang2 (using LC10 monoclonal antibody) can prevent or reduce AVMs and other HHT-associated abnormalities in mice (96, 97) (Figure 3). However, in contrast with these mouse models, Ang2 circulating levels in HHT patients tend to be reduced compared with those in healthy individuals (99, 100). Thus, although Ang2 seems to be a promising new therapeutic target, further work is needed before pursuing clinical trials (101).

Preclinical data, clinical trials, and perspectives in HHT

Since 2009, antiangiogenic drugs developed in oncology have been increasingly used in drug-repurposing strategies in patients with HHT (102, 103). Today, numerous preclinical (51, 55, 70, 75, 88, 90, 94–97, 104–112) and clinical studies (58, 86, 104, 113–136) targeting angiogenesis in the scope of HHT have been carried out and are summarized in Table 1 and Table 2. Drugs used in the aforementioned studies and their distinct sites of action are depicted in Figure 3.

Today, with our current knowledge of the pathophysiological mechanisms of HHT owing to preclinical models and clinical trials, the main therapeutic lines of action to be considered include the following: first, avoiding angiogenic and inflammatory stimuli by all means, in line with the second hit hypothesis. Whenever possible, “mechanical” prevention, such as sun protection, application of nasal ointments, and avoidance of nasal cauterization during childhood, is recommended to reduce the development of cutaneous and mucosal telangiectases. In addition, it is essential to prevent and treat anemia and iron deficiency, whose repercussions are probably not limited to hematology alone, but also include the risk of infection and stroke (43, 137).

Another important point is moving toward personalized medicine for each patient. While studies across 14 years that involved more than 400 patients ensured the safety of bevacizumab, they demonstrated that the response varied depending on its elimination rate (58, 86). Indeed, pharmacokinetic studies highlighted a correlation between a progressive decrease in response to the drug and low plasma bevacizumab concentration. Thus, as in

cancer, there is a concentration threshold for efficacy. So taking into account the metabolisms of different patients and adapting the dosage accordingly would allow us to maintain its efficacy and optimize the risk-benefit balance. It is therefore essential to monitor clinical improvement as a function of bevacizumab levels during maintenance therapy in order to maintain bioactive levels within the therapeutic window, especially if efficacy appears to be declining (120). In case of treatment failure, it is mandatory to verify that bevacizumab residual levels are above 25 mg/L (120, 138).

Another aspect that should be considered more carefully is tailoring treatment to disease severity in order to limit drug toxicity. To reduce systemic adverse effects, antiangiogenic treatments applied locally have been studied. For example, 0.1% tacrolimus ointment applied twice daily has promising outcomes (139) and could be a good option for patients with moderate epistaxis, although this awaits further confirmation by larger studies. Moreover, continued use of tacrolimus in HHT patients could be called into question, as a few HHT patients using this regimen after liver transplantation developed new hepatic vascular lesions and needed a second transplantation (30). Other antiangiogenic drugs with local use are more debated. Two randomized trials showed that intranasal bevacizumab was not effective (140, 141). This result was confirmed in a systematic review (142) conducted on 13 studies. Some studies evaluated submucosal administration alone (143, 144), with electrocautery (145) or cyanoacrylate glue (146). In addition, nonselective β -adrenergic receptor blockers with antiangiogenic properties that reduce VEGF and matrix metalloproteinase-9 (MMP-9) tissue expression have been studied (147). For instance, four randomized studies using topical timolol in the form of a nasal spray or gel for epistaxis and an ophthalmic solution for cutaneous telangiectases reported discordant results in cohorts ranging from 6 to 58 patients (148–151). This is consistent with a systematic review from 2022 (152) on topical beta blockers suggesting that propranolol is more promising than timolol. Topical propranolol was reported to increase hemoglobin levels (153, 154), but its use warrants monitoring for bradycardia occurrence, even when administered locally.

Future challenges in HHT

Although great progress has been made in the treatment of HHT patients, there are still some remaining challenges. Perhaps the greatest challenge is determining whether large AVMs observed in HHT patients would respond to antiangiogenic therapies. Indeed, several antiangiogenic drugs, particularly those administered orally, including tyrosine kinase inhibitors, AKT inhibitors, pomalidomide, and mTOR inhibitors, are currently being tested on visceral AVMs, but no regression of visceral AVMs has been observed so far. Only skin or mucosal telangiectases have been found to be reduced in a few cases (125, 128). In the Metafore study (86), cardiac index and hepatic blood flow were decreased after bevacizumab treatment, but large liver AVMs on CT scan were unchanged, and despite a marked improvement in digestive bleeding, the number of GI telangiectases was not reduced. Although some preclinical studies reported that antiangiogenic treatments, such as PI3K inhibitors, could revert established AVMs in preclinical models (90), these AVMs were only seen in neonatal retinas of mice. Similarly, it was shown that in zebrafish *Eng* mutants, the HHT-like phenotype was abrogated by inhibiting VEGF signaling with drugs targeting VEGFR2, but only in embryos (112). In both models, the treatment was effective during

Table 2. Therapeutic strategies targeting intracellular pathways

Therapeutic group	Evidence in preclinical models and HHT patients			
	Drug used	References	Study type	Key findings
TKI	Pazopanib	J.G. Parambil, 2018 (122)	Case report	Normalization of iron levels; increase in hemoglobin levels
		M. Faughnan, 2019 (123)	Prospective open label (<i>n</i> = 7)	Improvement in hemoglobin levels and/or epistaxis in all treated patients
		J.G. Parambil, 2022 (124)	Retrospective (<i>n</i> = 13)	RBC transfusion independence within 12 months of treatment; increase in hemoglobin levels
		J.Y. Moon, 2022 (125)	Case report	Improvement in cutaneous HHT
		NCT03850964	Ongoing trial of Paz (70 enrolled)	Data not yet available
	Sorafenib, pazopanib analog, erlotinib, sunitinib	Y.H. Kim, 2017 (107)	Mouse model	Sorafenib and pazopanib analog improves, while erlotinib worsens anemia and gastrointestinal bleeding; no effect on wound-induced skin AVMs in adult <i>Alk1</i> -ieKO mice
	SU5416	Y. Jin, 2017 (108)	Mouse model	Reduces retinal AVMs in <i>Eng</i> -ieKO
	Nintedanib	S. Ruiz, 2020 (109)	Mouse model	Prevents and reverses retinal AVMs in BMP9/10ib
		E. Kovacs-Sipos, 2017 (126)	Case report	Decrease in frequency and duration of epistaxis
		NCT03954782	Ongoing trial (EPICURE) (60 enrolled)	Data not yet available
	NCT04976036	Ongoing trial (EPISTOP) (48 enrolled)	Data not yet available	
PI3K and AKT inhibitors	Wortmannin	R. Ola, 2016 (90)	Mouse models	Prevents and reverses the formation of retinal AVMs in <i>Alk1</i> -ieKO, BMP9/10ib, and <i>Smad4</i> -ieKO mice
		R. Ola, 2018 (70)		
	Pictilisib	R. Ola, 2016 (90)	Mouse model	Reduces retinal AVMs in <i>Alk1</i> -ieKO
	BKM120 (buparlisib)	U.W. Geithoff, 2014 (127)	Case report	Decrease in the frequency of epistaxis, which stopped after 6 weeks
	VAD044	NCT05406362	Ongoing trial (80 enrolled)	Data not yet available
mTOR inhibitor	Sirolimus	S. Ruiz, 2020 (109)	Mouse model	Prevents and reverses retinal AVMs in BMP9/10ib
		A.I. Skaro, 2006 (128)	Case report	Regression of telangiectases
		NCT05269849	Ongoing trial (10 enrolled)	Data not yet available
MEK1/2 inhibitor	PD0325901	R.O. Snodgrass, 2023 (112)	Zebrafish model	Associated with sirolimus: normalizes enlarged vessels in <i>Engmu130</i> zebrafish embryos
	Trametinib	C.L. Shovlin, 2023 (136)	Case report	Decrease in frequency and duration of epistaxis
Calcineurin inhibitor/ FKBP12 competitor	Tacrolimus (FK506)	S. Ruiz, 2017 (75)	Mouse model	Reduces retinal AVMs in BMP9/10ib mice
		N. Sommer, 2019 (135)	Case report	Decrease in frequency and duration of epistaxis
		J.M. Pruijssen, 2021 (129)	Case report	Decrease in gastrointestinal bleeding, RBC transfusion
		J. Hessels, 2022 (130)	Prospective open label (<i>n</i> = 25)	Reduction in RBC transfusion requirements; increase in hemoglobin levels
IMiDs	Thalidomide	F. Lebrin, 2010 (104)	Mouse model	Inhibits angiogenesis by increasing mural cell coverage of the vasculature of <i>Eng</i> ^{+/−} mice
		H.-L. Peng, 2015 (110)	Zebrafish model	Reverses telangiectases, controls nosebleeds, and leads to vascular remodeling in the zebrafish model
		F. Lebrin, 2010 (104)	Case series (7 patients)	Reduction in RBC transfusion requirements; increase in hemoglobin levels
		R. Invernizzi, 2015 (131)	Prospective open label (<i>n</i> = 31)	Reduction in RBC transfusion, increase in hemoglobin levels, decrease of all epistaxis parameters
		J. Fang, 2017 (132)	Prospective open label (<i>n</i> = 7)	Reduction in ESS scores
		M. Baysal, 2019 (133)	Retrospective (<i>n</i> = 6)	Reduction in ESS scores; increase in hemoglobin levels
	Thalidomide, lenalidomide	W. Zhu, 2018 (111)	Mouse model	Inhibition of brain AVM development and improvement of vascular integrity of existing brain AVM in adult <i>Alk1</i> -ieKO mice
	Pomalidomide	M. Samour, 2016 (134)	Phase I (<i>n</i> = 6)	Reduction in ESS scores, increase in hemoglobin, reduction in iron infusions
	NCT03910244	Ongoing trial (145 enrolled)	Data not yet available	
Anti-Ang2 antibody	LC10	A.M. Crist, 2019 (97)	Mouse model	Prevents and reverses retinal AVM in <i>Smad4</i> -ieKO mice
		X. Zhou, 2023 (96)	Mouse models	Limits and prevents brain AVM and blood vessel enlargement in <i>Eng</i> -, <i>Alk1</i> - and <i>Smad4</i> -KO mice
CDK4/6 inhibitors	Palbociclib, ribociclib	S. Dinakaran, 2023 (95)	Mouse model	CDK4/6 inhibition decreases the formation of retinal AVMs and brain vascular defects in BMP9/10ib mice
		K. Banerjee, 2023 (94)	Mouse model	CDK4/6 inhibition decreases the formation of retinal AVMs in <i>Smad4</i> -ieKO mice
Drug combination	TKI and mTOR inhibitor	S. Ruiz, 2020 (109)	Mouse models	Nintedanib and sirolimus prevent and reverse retinal AVMs
	MEK and mTOR inhibitors	R.O. Snodgrass, 2023 (112)	Zebrafish models	Subtherapeutic MEK inhibitor and sirolimus normalize enlarged vessels in <i>Engmu130</i> zebrafish embryos

ESS, epistaxis severity score; TKI, tyrosine-kinase inhibitor.

active angiogenesis (embryonic or postnatal stages), which is not the case after remodeling of vascular structure in large AVMs. Thus there is a real need for testing the efficacy of drugs in preclinical models on large AVMs or AVMs at adult stages. Accordingly, recent preclinical HHT models that better mimic HHT symptoms have been developed in adult mice and zebrafish (55, 106, 107, 112). However, to maximize the potential of currently available models for monitoring the response of AVMs to tested drugs, advancements in visualization methodologies are required for the deeper investigation of AVM formation, progression, and regression. Such advancements might also allow the revelation of previously undetected visceral AVMs in HHT mouse models, necessitating further investigation of these models using advanced visualization tools in the future.

Another challenge is to identify the best target to inhibit angiogenesis in HHT patients. VEGF signaling is complex and drives numerous downstream pathways, so adverse complications can be caused by inhibiting the VEGF pathway as a whole (155). Thus, one could imagine that targeting specific downstream pathways of VEGF could reduce these complications. A recent study addressed this point by using an adult endoglin-mutant zebrafish model that developed several HHT symptoms (112). They found that inhibiting mTORC1 (using rapamycin, also known as sirolimus) or MEK pathways prevented vascular abnormalities (Table 2), whereas inhibiting P38 or nitric oxide synthase pathways had no effect. Combined subtherapeutic mTORC1 and MEK inhibitors demonstrated synergistic effects in treating HHT. However, it remains unclear at this stage whether it will be more beneficial in the future to only target particular downstream targets of VEGF (PI3K, MEK), which are activated by many signaling pathways, or to target a specific proangiogenic growth factor, such as VEGF.

In the present Review, we focus on therapeutic approaches that aim to block activated signaling pathways due to LOF of the BMP9-10/ALK1 signaling pathway, but another possibility would be to increase the deficient signaling pathway (more ligands, more receptors, activation of the downstream signaling pathway). However, the validity of such a therapeutic option fundamentally relies on whether HHT vascular lesions are driven by haploinsufficiency or biallelic loss of the affected gene.

Summary and conclusions

Ever since the discovery that HHT is due to mutations in a single signaling pathway nearly 30 years ago, considerable progress has been made in treating HHT symptoms by blocking the VEGF angiogenic pathway. However, the molecular mechanism underlying the interaction between BMP9/BMP10 and VEGF signaling is still not fully elucidated. In addition, as described in this Review, several points remain to be better characterized, such as the development of AVMs, preclinical adult models for AVMs, biomarkers for personalized antiangiogenic treatments, the choice of therapeutic target (growth factors such as VEGF or more focused downstream signaling [AKT, MTORC1, MEK]), the possibility and efficacy of localized treatment and the benefit of preventative antiangiogenic treatments. Larger scale and real-life data are particularly difficult to obtain, since these treatments are used off-label due to a lack of better options. This is partly due to the general abstention of pharmaceutical industries from repurposing drugs in rare diseases. In the meantime, antiangiogenic drugs hold a promising potential in HHT and represent avenues worth investigating before taking on the future challenges associated with gene therapy.

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- Desroches-Castan A, et al. BMP9 and BMP10: Two close vascular quiescence partners that stand out. *Dev Dyn*. 2022;251(1):178–197.
- McAllister KA, et al. Endoglin, a TGF- β binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type. *Nat Genet*. 1994;8(4):345–351.
- Johnson DW, et al. Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2. *Nat Genet*. 1996;13(2):189–195.
- McDonald J, et al. Curaçao diagnostic criteria for hereditary hemorrhagic telangiectasia is highly predictive of a pathogenic variant in ENG or ACVRL1 (HHT1 and HHT2). *Genet Med*. 2020;22(7):1201–1205.
- National Library of Medicine. ClinVar. <https://www.ncbi.nlm.nih.gov/clinvar/>. Accessed November 9, 2023.
- Gallione CJ, et al. SMAD4 mutations found in unselected HHT patients. *J Med Genet*. 2006;43(10):793–797.
- Wooderchak WL, et al. Repository of SMAD4 mutations: reference for genotype/phenotype correlation. *J Data Mining in Genom Proteomics*. 2010;1(1):1000101.
- Wooderchak-Donahue WL, et al. Genome sequencing reveals a deep intronic splicing ACVRL1 mutation hotspot in Hereditary Haemorrhagic Telangiectasia. *J Med Genet*. 2018;55(12):824–830.
- Wooderchak-Donahue WL, et al. BMP9 mutations cause a vascular-anomaly syndrome with phenotypic overlap with hereditary hemorrhagic telangiectasia. *Am J Hum Genet*. 2013;93(3):530–537.
- Balachandar S, et al. Identification and validation of a novel pathogenic variant in GDF2 (BMP9) responsible for hereditary hemorrhagic telangiectasia and pulmonary arteriovenous malformations. *Am J Med Genet A*. 2022;188(3):959–964.
- Liu J, et al. Homozygous GDF2-related hereditary hemorrhagic telangiectasia in a Chinese family. *Pediatrics*. 2020;146(2):e20191970.
- Hodgson J, et al. Homozygous GDF2 nonsense mutations result in a loss of circulating BMP9 and BMP10 and are associated with either PAH or an “HHT-like” syndrome in children. *Molec Gen Gen Med*. 2021;9(12):e1685.
- Farhan A, et al. Clinical manifestations of patients with GDF2 mutations associated with hereditary hemorrhagic telangiectasia type 5. *Am J Med Genet A*. 2022;188(1):199–209.
- Revcu N, et al. RASA1 mutations and associated phenotypes in 68 families with capillary malformation-arteriovenous malformation. *Hum Mutat*. 2013;34(12):1632–1641.
- Orme CM, et al. Capillary malformation-arteriovenous malformation syndrome: review of the literature, proposed diagnostic criteria, and recommendations for management. *Pediatr Dermatol*.

- matol.* 2013;30(4):409–415.
16. El Hajjam M, et al. *RASA1* phenotype overlaps with hereditary haemorrhagic telangiectasia: two case reports. *J Med Genet.* 2021;58(9):645–647.
 17. Hernandez F, et al. Mutations in *RASA1* and *GDF2* identified in patients with clinical features of hereditary hemorrhagic telangiectasia. *Hum Genome Var.* 2015;2:15040.
 18. Wooderchak-Donahue WL, et al. Expanding the clinical and molecular findings in *RASA1* capillary malformation-arteriovenous malformation. *Eur J Hum Genet.* 2018;26(10):1521–1536.
 19. Guilhem A, et al. Seven cases of hereditary haemorrhagic telangiectasia-like hepatic vascular abnormalities associated with *EPHB4* pathogenic variants. *J Med Genet.* 2023;60(9):905–909.
 20. Jiang X, et al. Inactivating mutations in *Drosophila* mediate vascular abnormalities similar to hereditary hemorrhagic telangiectasia. *Sci Signal.* 2018;11(513):eaan6831.
 21. Wassef M, et al. Vascular anomalies classification: recommendations from the international society for the study of vascular anomalies. *Pediatrics.* 2015;136(1):e203–e214.
 22. Braverman IM, et al. Ultrastructure and three-dimensional organization of the telangiectases of hereditary hemorrhagic telangiectasia. *J Invest Dermatol.* 1990;95(4):422–427.
 23. Singh E, et al. Arterial endoglin does not protect against arteriovenous malformations. *Angiogenesis.* 2020;23(4):559–566.
 24. Park H, et al. Defective flow-migration coupling causes arteriovenous malformations in hereditary hemorrhagic telangiectasia. *Circulation.* 2021;144(10):805–822.
 25. McDonald J, et al. Frequency of epistaxis and telangiectasia in patients with hereditary hemorrhagic telangiectasia (HHT) in comparison with the general population: Curaçao diagnostic criteria revisited. *Genet Med.* 2023;25(8):100865.
 26. Hyldahl SJ, et al. Skin and mucosal telangiectatic lesions in hereditary hemorrhagic telangiectasia patients. *Int J Dermatol.* 2022;61(12):1497–1505.
 27. Kasthuri RS, et al. Prevalence and predictors of anemia in hereditary hemorrhagic telangiectasia. *Am J Hematol.* 2017;92(10):591–593.
 28. Dupuis-Girod S, et al. The lung in hereditary hemorrhagic telangiectasia. *Respiration.* 2017;94(4):315–330.
 29. Buscarini E, et al. Liver involvement in hereditary hemorrhagic telangiectasia. *Abdom Radiol (NY).* 2018;43(8):1920–1930.
 30. Dumortier J, et al. Recurrence of hereditary hemorrhagic telangiectasia after liver transplantation: clinical implications and pathophysiological insights. *Hepatology.* 2019;69(5):2232–2240.
 31. Eker OF, et al. European Reference Network for Rare Vascular Diseases (VASCERN) position statement on cerebral screening in adults and children with hereditary haemorrhagic telangiectasia (HHT). *Orphanet J Rare Dis.* 2020;15(1):165.
 32. Plauchu H, et al. Age-related clinical profile of hereditary hemorrhagic telangiectasia in an epidemiologically recruited population. *Am J Med Genet.* 1989;32(3):291–297.
 33. Lesca G, et al. Genotype-phenotype correlations in hereditary hemorrhagic telangiectasia: data from the French-Italian HHT network. *Genet Med.* 2007;9(1):14–22.
 34. Dupuis-Girod S, et al. Hemorrhagic hereditary telangiectasia (Rendu-Osler disease) and infectious diseases: an underestimated association. *Clin Infect Dis.* 2007;44(6):841–845.
 35. Guilhem A, et al. Immunological abnormalities associated with hereditary haemorrhagic telangiectasia. *J Intern Med.* 2013;274(4):351–362.
 36. Cymerman U, et al. Identification of hereditary hemorrhagic telangiectasia type 1 in newborns by protein expression and mutation analysis of endoglin. *Pediatr Res.* 2000;47(1):24–35.
 37. Fernandez-L A, et al. Blood outgrowth endothelial cells from Hereditary Haemorrhagic Telangiectasia patients reveal abnormalities compatible with vascular lesions. *Cardiovasc Res.* 2005;68(2):235–248.
 38. Fernandez-L A, et al. Mutation study of Spanish patients with hereditary hemorrhagic telangiectasia and expression analysis of Endoglin and ALK1. *Hum Mutat.* 2006;27(3):295.
 39. Abdalla SA. Analysis of ALK-1 and endoglin in newborns from families with hereditary hemorrhagic telangiectasia type 2. *Hum Mol Genet.* 2000;9(8):1227–1237.
 40. Bourdeau A, et al. A murine model of hereditary hemorrhagic telangiectasia. *J Clin Invest.* 1999;104(10):1343–1351.
 41. Srinivasan S. A mouse model for hereditary hemorrhagic telangiectasia (HHT) type 2. *Hum Mol Genet.* 2003;12(5):473–482.
 42. Arthur HM, et al. Endoglin, an ancillary TGFβ2 receptor, is required for extraembryonic angiogenesis and plays a key role in heart development. *Dev Biol.* 2000;217(1):42–53.
 43. Faughnan ME, et al. Second international guidelines for the diagnosis and management of hereditary hemorrhagic telangiectasia. *Ann Intern Med.* 2020;173(12):989–1001.
 44. Kritharis A, et al. Hereditary hemorrhagic telangiectasia: diagnosis and management from the hematologist's perspective. *Haematologica.* 2018;103(9):1433–1443.
 45. Bernabeu C, et al. Potential second-hits in hereditary hemorrhagic telangiectasia. *J Clin Med.* 2020;9(11):3571.
 46. Arthur HM, Roman BL. An update on preclinical models of hereditary haemorrhagic telangiectasia: Insights into disease mechanisms. *Front Med (Lausanne).* 2022;9:973964.
 47. Xu B, et al. Vascular endothelial growth factor induces abnormal microvasculature in the endoglin heterozygous mouse brain. *J Cereb Blood Flow Metab.* 2004;24(2):237–244.
 48. Hao Q, et al. VEGF induces more severe cerebrovascular dysplasia in endoglin than in Alk1 mice. *Transl Stroke Res.* 2010;1(3):197–201.
 49. Choi E-J, et al. Novel brain arteriovenous malformation mouse models for type 1 hereditary hemorrhagic telangiectasia. *PLoS One.* 2014;9(2):e88511.
 50. Choi E-J, et al. Minimal homozygous endothelial deletion of Eng with VEGF stimulation is sufficient to cause cerebrovascular dysplasia in the adult mouse. *Cerebrovasc Dis.* 2012;33(6):540–547.
 51. Walker EJ, et al. Bevacizumab attenuates VEGF-induced angiogenesis and vascular malformations in the adult mouse brain. *Stroke.* 2012;43(7):1925–1930.
 52. Walker EJ, et al. Arteriovenous malformation in the adult mouse brain resembling the human disease. *Ann Neurol.* 2011;69(6):954–962.
 53. Park SO, et al. Real-time imaging of de novo arteriovenous malformation in a mouse model of hereditary hemorrhagic telangiectasia. *J Clin Invest.* 2009;119(11):3487–3496.
 54. Garrido-Martin EM, et al. Common and distinctive pathogenetic features of arteriovenous malformations in hereditary hemorrhagic telangiectasia 1 and hereditary hemorrhagic telangiectasia 2 animal models—brief report. *Arterioscler Thromb Vasc Biol.* 2014;34(10):2232–2236.
 55. Han C, et al. VEGF neutralization can prevent and normalize arteriovenous malformations in an animal model for hereditary hemorrhagic telangiectasia 2. *Angiogenesis.* 2014;17(4):823–830.
 56. Mahmoud M, et al. Pathogenesis of arteriovenous malformations in the absence of endoglin. *Circ Res.* 2010;106(8):1425–1433.
 57. Dupuis-Girod S, et al. European Reference Network for Rare Vascular Diseases (VASCERN): When and how to use intravenous bevacizumab in hereditary haemorrhagic telangiectasia (HHT)? *Eur J Med Genet.* 2022;65(10):104575.
 58. Al-Samkari H, et al. An international, multicenter study of intravenous bevacizumab for bleeding in hereditary hemorrhagic telangiectasia: the InHIBIT-Bleed study. *Haematologica.* 2021;106(8):2161–2169.
 59. Geisthoff U, et al. Trauma can induce telangiectases in hereditary hemorrhagic telangiectasia. *J Clin Med.* 2020;9(5):1507.
 60. Snellings DA, et al. Somatic mutations in vascular malformations of hereditary hemorrhagic telangiectasia result in bi-allelic loss of ENG or ACVRL1. *Am J Hum Genet.* 2019;105(5):894–906.
 61. Maris JM, Knudson AG. Revisiting tissue specificity of germline cancer predisposing mutations. *Nat Rev Cancer.* 2015;15(2):65–66.
 62. Brouillard P, Vikkula M. Genetic causes of vascular malformations. *Hum Mol Genet.* 2007;16 Spec No. 2(r2):R140–R149.
 63. Snellings DA, et al. Developmental venous anomalies are a genetic primer for cerebral cavernous malformations. *Nat Cardiovasc Res.* 2022;1(3):246–252.
 64. Brinjikji W, et al. Prevalence and characteristics of brain arteriovenous malformations in hereditary hemorrhagic telangiectasia: a systematic review and meta-analysis. *J Neurosurg.* 2017;127(2):302–310.
 65. David L, et al. Identification of BMP9 and BMP10 as functional activators of the orphan activin receptor-like kinase 1 (ALK1) in endothelial cells. *Blood.* 2007;109(5):1953–1961.
 66. Scharpfenecker M, et al. BMP-9 signals via ALK1 and inhibits bFGF-induced endothelial cell proliferation and VEGF-stimulated angiogenesis. *J Cell Sci.* 2007;120(pt 6):964–972.
 67. Lawera A, et al. Role of soluble endoglin in BMP9 signaling. *Proc Natl Acad Sci U S A.* 2019;116(36):17800–17808.
 68. Hwan Kim Y, et al. Overexpression of activin receptor-like kinase 1 in endothelial cells suppresses development of arteriovenous malformations in mouse models of hereditary hemorrhagic

- telangiectasia. *Circ Res*. 2020;127(9):1122–1137.
69. Roman BL, Hinck AP. ALK1 signaling in development and disease: new paradigms. *Cell Mol Life Sci*. 2017;74(24):4539–4560.
 70. Ola R, et al. SMAD4 prevents flow induced arteriovenous malformations by inhibiting casein kinase 2. *Circulation*. 2018;138(21):2379–2394.
 71. Crist AM, et al. Vascular deficiency of Smad4 causes arteriovenous malformations: a mouse model of hereditary hemorrhagic telangiectasia. *Angiogenesis*. 2018;21(2):363–380.
 72. Benn A, et al. BMP-SMAD1/5 signaling regulates retinal vascular development. *Biomolecules*. 2020;10(3):488.
 73. García de Vinuesa A, et al. BMP signaling in vascular biology and dysfunction. *Cytokine Growth Factor Rev*. 2016;27:65–79.
 74. Ricard N, et al. The quiescent endothelium: signalling pathways regulating organ-specific endothelial normalcy. *Nat Rev Cardiol*. 2021;18(8):565–580.
 75. Ruiz S, et al. Tacrolimus rescues the signaling and gene expression signature of endothelial ALK1 loss-of-function and improves HHT vascular pathology. *Hum Mol Genet*. 2017;26(24):4786–4798.
 76. Spiekerkoetter E, et al. FK506 activates BMPR2, rescues endothelial dysfunction, and reverses pulmonary hypertension. *J Clin Invest*. 2013;123(8):3600–3613.
 77. Posadas Salas MA, Srinivas TR. Update on the clinical utility of once-daily tacrolimus in the management of transplantation. *Drug Des Devel Ther*. 2014;8:1183–1194.
 78. Horsley V, Pavlath GK. NFAT: ubiquitous regulator of cell differentiation and adaptation. *J Cell Biol*. 2002;156(5):771–774.
 79. Tocci MJ, Sigal NH. Recent advances in the mechanism of action of cyclosporine and FK506. *Curr Opin Nephrol Hypertens*. 1992;1(2):236–242.
 80. Simons M, et al. Mechanisms and regulation of endothelial VEGF receptor signalling. *Nat Rev Mol Cell Biol*. 2016;17(10):611–625.
 81. Subileau M, et al. Bone Morphogenetic protein 9 regulates early lymphatic-specified endothelial cell expansion during mouse embryonic stem cell differentiation. *Stem Cell Reports*. 2019;12(1):98–111.
 82. Chen YG, et al. Mechanism of TGFβ receptor inhibition by FKBP12. *EMBO J*. 1997;16(13):3866–3876.
 83. Graupera M, Potente M. Regulation of angiogenesis by PI3K signaling networks. *Exp Cell Res*. 2013;319(9):1348–1355.
 84. Flieger D, et al. Dramatic improvement in hereditary hemorrhagic telangiectasia after treatment with the vascular endothelial growth factor (VEGF) antagonist bevacizumab. *Ann Hematol*. 2006;85(9):631–632.
 85. David L, et al. Bone morphogenetic protein-9 is a circulating vascular quiescence factor. *Circ Res*. 2008;102(8):914–922.
 86. Dupuis-Girod S, et al. Bevacizumab in patients with hereditary hemorrhagic telangiectasia and severe hepatic vascular malformations and high cardiac output. *JAMA*. 2012;307(9):948–955.
 87. Larivière B, et al. ALK1 signaling inhibits angiogenesis by cooperating with the Notch pathway. *Dev Cell*. 2012;22(3):489–500.
 88. Thalgot JH, et al. Decreased expression of vascular endothelial growth factor receptor 1 contributes to the pathogenesis of hereditary hemorrhagic telangiectasia type 2. *Circulation*. 2018;138(23):2698–2712.
 89. Alsina-Sanchis E, et al. ALK1 loss results in vascular hyperplasia in mice and humans through PI3K activation. *Arterioscler Thromb Vasc Biol*. 2018;38(5):1216–1229.
 90. Ola R, et al. PI3 kinase inhibition improves vascular malformations in mouse models of hereditary haemorrhagic telangiectasia. *Nat Commun*. 2016;7(1):13650.
 91. Iriarte A, et al. PI3K (Phosphatidylinositol 3-Kinase) activation and endothelial cell proliferation in patients with hemorrhagic hereditary telangiectasia type 1. *Cells*. 2019;8(9):971.
 92. Medina-Jover F, et al. SGK1 is a signalling hub that controls protein synthesis and proliferation in endothelial cells. *FEBS Lett*. 2020;594(19):3200–3215.
 93. Rostama B, et al. DLL4/Notch1 and BMP9 interdependent signaling induces human endothelial cell quiescence via P27KIP1 and thrombospondin-1. *Arterioscler Thromb Vasc Biol*. 2015;35(12):2626–2637.
 94. Banerjee K, et al. SMAD4 maintains the fluid shear stress set point to protect against arterial-venous malformations. *J Clin Invest*. 2023;133(18):e168352.
 95. Dinakaran S, et al. CDK6-mediated endothelial cell cycle acceleration drives arteriovenous malformations in hereditary hemorrhagic telangiectasia [preprint]. Posted on bioRxiv September 16, 2023. <https://doi.org/10.1101/2023.09.15.554413>.
 96. Zhou X, et al. ANG2 blockade diminishes proangiogenic cerebrovascular defects associated with models of hereditary hemorrhagic telangiectasia. *Arterioscler Thromb Vasc Biol*. 2023;43(8):1384–1403.
 97. Crist AM, et al. Angiotensin-2 inhibition rescues arteriovenous malformation in a smad4 hereditary hemorrhagic telangiectasia mouse model. *Circulation*. 2019;139(17):2049–2063.
 98. Kim M, et al. Opposing actions of angiotensin-2 on Tie2 signaling and FOXO1 activation. *J Clin Invest*. 2016;126(9):3511–3525.
 99. Ojeda-Fernandez L, et al. Reduced plasma levels of Ang-2 and sEng as novel biomarkers in hereditary hemorrhagic telangiectasia (HHT). *Clin Chim Acta*. 2010;411(7–8):494–499.
 100. Fernandez-L A, et al. Gene expression fingerprinting for human hereditary hemorrhagic telangiectasia. *Hum Mol Genet*. 2007;16(13):1515–1533.
 101. Bernabeu C. Therapeutic targeting of the Ang2/tie pathway in endothelial cells as a potential treatment of hereditary hemorrhagic telangiectasia. *Arterioscler Thromb Vasc Biol*. 2023;43(8):1404–1408.
 102. Ardelean DS, Letarte M. Anti-angiogenic therapeutic strategies in hereditary hemorrhagic telangiectasia. *Front Genet*. 2015;6:35.
 103. Snodgrass RO, et al. Hereditary haemorrhagic telangiectasia, an inherited vascular disorder in need of improved evidence-based pharmaceutical interventions. *Genes (Basel)*. 2021;12(2):174.
 104. Lebrin F, et al. Thalidomide stimulates vessel maturation and reduces epistaxis in individuals with hereditary hemorrhagic telangiectasia. *Nat Med*. 2010;16(4):420–428.
 105. Ardelean DS, et al. Endoglin and activin receptor-like kinase 1 heterozygous mice have a distinct pulmonary and hepatic angiogenic profile and response to anti-VEGF treatment. *Angiogenesis*. 2014;17(1):129–146.
 106. Tual-Chalot S, et al. Loss of endothelial endoglin promotes high-output heart failure through peripheral arteriovenous shunting driven by VEGF signaling. *Circ Res*. 2020;126(2):243–257.
 107. Kim YH, et al. Selective effects of oral antiangiogenic tyrosine kinase inhibitors on an animal model of hereditary hemorrhagic telangiectasia. *J Thromb Haemost*. 2017;15(6):1095–1102.
 108. Jin Y, et al. Endoglin prevents vascular malformation by regulating flow-induced cell migration and specification through VEGFR2 signalling. *Nat Cell Biol*. 2017;19(6):639–652.
 109. Ruiz S, et al. Correcting Smad1/5/8, mTOR, and VEGFR2 treats pathology in hereditary hemorrhagic telangiectasia models. *J Clin Invest*. 2020;130(2):942–957.
 110. Peng H-L, et al. Thalidomide effects in patients with hereditary hemorrhagic telangiectasia during therapeutic treatment and in fli-EGFP transgenic zebrafish model. *Chin Med J (Engl)*. 2015;128(22):3050–3054.
 111. Zhu W, et al. Thalidomide reduces hemorrhage of brain arteriovenous malformations in a mouse model. *Stroke*. 2018;49(5):1232–1240.
 112. Snodgrass RO, et al. Therapeutic targeting of vascular malformation in a zebrafish model of hereditary haemorrhagic telangiectasia. *Dis Model Mech*. 2023;16(4):dmm049567.
 113. Chavan A, et al. Systemic therapy with bevacizumab in patients with hereditary hemorrhagic telangiectasia (HHT). *Vasa*. 2013;42(2):106–110.
 114. Thompson AB, et al. Very low dose bevacizumab for the treatment of epistaxis in patients with hereditary hemorrhagic telangiectasia. *Allergy Rhinol (Providence)*. 2014;5(2):91–95.
 115. Guilhem A, et al. Intra-venous bevacizumab in hereditary hemorrhagic telangiectasia (HHT): A retrospective study of 46 patients. *PLoS One*. 2017;12(11):e0188943.
 116. Chavan A, et al. Emerging role of bevacizumab in management of patients with symptomatic hepatic involvement in Hereditary Hemorrhagic Telangiectasia. *Am J Hematol*. 2017;92(11):E641–E644.
 117. Iyer VN, et al. Intravenous bevacizumab for refractory hereditary hemorrhagic telangiectasia-related epistaxis and gastrointestinal bleeding. *Mayo Clin Proc*. 2018;93(2):155–166.
 118. Al-Samkari H, et al. An international survey to evaluate systemic bevacizumab for chronic bleeding in hereditary haemorrhagic telangiectasia. *Haemophilia*. 2020;26(6):1038–1045.
 119. Vázquez C, et al. Bevacizumab for treating Hereditary Hemorrhagic Telangiectasia patients with severe hepatic involvement or refractory anemia. *PLoS One*. 2020;15(2):e0228486.
 120. Dupuis-Girod S, et al. Efficacy and safety of intravenous bevacizumab on severe bleeding associated with hemorrhagic hereditary telangiectasia: A national, randomized multicenter trial. *J Intern Med*. 2023;294(6):761–774.
 121. Villanueva B, et al. Aflibercept for gastrointestinal bleeding in hereditary hemorrhagic

- telangiectasia: a case report. *Medicina (Kaunas)*. 2023;59(9):1533.
122. Parambil JG, et al. Pazopanib effective for bevacizumab-unresponsive epistaxis in hereditary hemorrhagic telangiectasia. *Laryngoscope*. 2018;128(10):2234–2236.
 123. Faughnan ME, et al. Pazopanib may reduce bleeding in hereditary hemorrhagic telangiectasia. *Angiogenesis*. 2019;22(1):145–155.
 124. Parambil JG, et al. Pazopanib for severe bleeding and transfusion-dependent anemia in hereditary hemorrhagic telangiectasia. *Angiogenesis*. 2022;25(1):87–97.
 125. Moon JY, et al. Improvement of cutaneous hereditary hemorrhagic telangiectasia with pazopanib-A multikinase inhibitor. *JAMA Dermatol*. 2022;158(2):214–216.
 126. Kovacs-Sipos E, et al. Nintedanib as a novel treatment option in hereditary haemorrhagic telangiectasia. *BMJ Case Rep*. 2017;2017:bcr2017219393.
 127. Geisthoff UW, et al. Improvement in hereditary hemorrhagic telangiectasia after treatment with the phosphoinositide 3-kinase inhibitor BKM120. *Ann Hematol*. 2014;93(4):703–704.
 128. Skaro AI, et al. Regression of cutaneous and gastrointestinal telangiectasia with sirolimus and aspirin in a patient with hereditary hemorrhagic telangiectasia. *Ann Intern Med*. 2006;144(3):226–227.
 129. Pruijsen JM, et al. Tacrolimus in gastrointestinal bleeding in a young boy with hereditary hemorrhagic telangiectasia. *JPGN Rep*. 2021;2(4):e133.
 130. Hessels J, et al. Efficacy and safety of tacrolimus as treatment for bleeding caused by hereditary hemorrhagic telangiectasia: an open-label, pilot study. *J Clin Med*. 2022;11(18):5280.
 131. Invernizzi R, et al. Efficacy and safety of thalidomide for the treatment of severe recurrent epistaxis in hereditary haemorrhagic telangiectasia: results of a non-randomised, single-centre, phase 2 study. *Lancet Haematol*. 2015;2(11):e465–e473.
 132. Fang J, et al. Thalidomide for epistaxis in patients with hereditary hemorrhagic telangiectasia: a preliminary study. *Otolaryngol Head Neck Surg*. 2017;157(2):217–221.
 133. Baysal M, et al. Thalidomide for the management of bleeding episodes in patients with hereditary hemorrhagic telangiectasia: effects on epistaxis severity score and quality of life. *Turk J Haematol*. 2019;36(1):43–47.
 134. Samour M, et al. Pomalidomide in hereditary hemorrhagic telangiectasia: interim results of a phase I study. *Blood*. 2016;128(22):210.
 135. Sommer N, et al. Treatment with low-dose tacrolimus inhibits bleeding complications in a patient with hereditary hemorrhagic telangiectasia and pulmonary arterial hypertension. *Pulm Circ*. 2019;9(2):2045894018805406.
 136. Shovlin CL, et al. MEK 1 inhibition and bleeding in hereditary haemorrhagic telangiectasia [published online October 23, 2023]. *Br J Haematol*. <https://doi.org/10.1111/bjh.19167>.
 137. Shovlin CL. Pulmonary arteriovenous malformations. *Am J Respir Crit Care Med*. 2014;190(11):1217–1228.
 138. Azzopardi N, et al. Dose - response relationship of bevacizumab in hereditary hemorrhagic telangiectasia. *MAbs*. 2015;7(3):630–637.
 139. Dupuis-Girod S, et al. ELLIPSE Study: a Phase I study evaluating the tolerance of bevacizumab nasal spray in the treatment of epistaxis in hereditary hemorrhagic telangiectasia. *MAbs*. 2014;6(3):794–799.
 140. Dupuis-Girod S, et al. Effect of bevacizumab nasal spray on epistaxis duration in hereditary hemorrhagic telangiectasia: A Randomized Clinical Trial. *JAMA*. 2016;316(9):934–942.
 141. Riss D, et al. Intranasal submucosal bevacizumab for epistaxis in hereditary hemorrhagic telangiectasia: a double-blind, randomized, placebo-controlled trial. *Head Neck*. 2015;37(6):783–787.
 142. Stokes P, Rimmer J. Intranasal bevacizumab in the treatment of HHT-related epistaxis: a systematic review. *Rhinology*. 2018;56(1):3–10.
 143. Dheyauldeen S, et al. Bevacizumab in hereditary hemorrhagic telangiectasia-associated epistaxis: effectiveness of an injection protocol based on the vascular anatomy of the nose. *Laryngoscope*. 2012;122(6):1210–1214.
 144. Steineger J, et al. Long-term experience with intranasal bevacizumab therapy. *Laryngoscope*. 2018;128(10):2237–2244.
 145. Khanwalkar AR, et al. Randomized, controlled, double-blinded clinical trial of effect of bevacizumab injection in management of epistaxis in hereditary hemorrhagic telangiectasia patients undergoing surgical cauterization. *Int Forum Allergy Rhinol*. 2022;12(8):1034–1042.
 146. Khoueir N, et al. Injection of bevacizumab and cyanoacrylate glue for hereditary hemorrhagic telangiectasia. *Laryngoscope*. 2019;129(10):2210–2215.
 147. Storch CH, Hoeger PH. Propranolol for infantile haemangiomas: insights into the molecular mechanisms of action. *Br J Dermatol*. 2010;163(2):269–274.
 148. Jeon H, Cohen B. Lack of efficacy of topical timolol for cutaneous telangiectasias in patients with hereditary hemorrhagic telangiectasia: Results of a pilot study. *J Am Acad Dermatol*. 2017;76(5):997–999.
 149. Peterson AM, et al. Efficacy of timolol in a novel intranasal thermosensitive gel for hereditary hemorrhagic telangiectasia-associated epistaxis: A Randomized Clinical Trial. *JAMA Otolaryngol Head Neck Surg*. 2020;146(11):1006–1014.
 150. Dupuis-Girod S, et al. Efficacy of TIMOLOL nasal spray as a treatment for epistaxis in hereditary hemorrhagic telangiectasia. A double-blind, randomized, placebo-controlled trial. *Sci Rep*. 2019;9(1):11986.
 151. Andorfer KEC, et al. TIMolol nasal spray as a treatment for epistaxis in hereditary hemorrhagic telangiectasia (TIM-HHT)-A prospective, randomized, double-blind, controlled, cross-over trial. *Pharmaceutics*. 2022;14(11):2335.
 152. Albarki H, Rimmer J. The use of beta-blockers in hereditary hemorrhagic telangiectasia-related epistaxis: a systematic review. *Am J Rhinol Allergy*. 2022;36(6):890–896.
 153. Mei-Zahav M, et al. Topical propranolol improves epistaxis in patients with hereditary hemorrhagic telangiectasia — a preliminary report. *J Otolaryngology Head Neck Surg*. 2017;46(1):58.
 154. Mei-Zahav M, et al. Topical propranolol improves epistaxis control in hereditary hemorrhagic telangiectasia (HHT): a randomized double-blind placebo-controlled trial. *J Clin Med*. 2020;9(10):3130.
 155. Totzeck M, et al. Cardiovascular adverse events in patients with cancer treated with bevacizumab: a meta-analysis of more than 20,000 patients. *J Am Heart Assoc*. 2017;6(8):e006278.