

Review

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Review

Recent Advances in Hereditary Hemorrhagic Telangiectasia Pathogenesis and Therapies

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Abstract: Hereditary Hemorrhagic Telangiectasia (HHT), also known as Osler-Weber-Rendu syndrome, is a rare and inherited vascular disorder, characterized by the development of arteriovenous malformations (AVMs) in various organs and telangiectasia (small AVM) in the mucocutaneous. The majority of HHT patients have haploinsufficiency of genes involved in the transforming growth factor-beta (TGF β) signaling pathway, including endoglin (ENG), activin receptor-like kinase 1 (ALK1, also known as ACVRL1), or SMAD4. Active angiogenesis is also required for telangiectasia and AVM development. Anti-angiogenic strategies have been tested in patients and animal models extensively. However, the exact mechanisms for telangiectasia and AVM development remain unclear. In this review, we discuss recent advances in identifying disease mechanisms, and potential therapeutic targets.

Keywords: hereditary hemorrhagic telangiectasia; arteriovenous malformations; pathogenesis; therapeutics

1. Introduction

Hereditary hemorrhagic telangiectasia (HHT) is a rare disorder characterized by arteriovenous malformations (AVMs) in multiple organs telangiectasia (small AVM) in the mucocutaneous [1]. This condition affects about 1 in 5000-8000 worldwide [2]. Telangiectasias in mucocutaneous can cause repeated gastrointestinal and nasal bleeding. AVMs in major organs, like the lungs, liver, and brain, can cause life-threatening hemorrhage and heart failure. Approximately 90% of HHT cases carry a heterozygous mutation in either the endoglin gene (ENG, HHT-1) or the activin-like receptor kinase 1 (ALK1, also known as ACVRL1, HHT-2). These pathogenic variants follow an autosomal dominant inheritance pattern. A subset of patients with HHT (about 2% of cases) carry a mutation in the SMAD4 gene, leading to a condition known as Juvenile Polyposis Syndrome (JPS). In this syndrome, the predominant clinical features include juvenile polyps and anemia [3]. Alteration of growth differentiation factor 2 (GDF2, also known as BMP-9) has lately been identified as a cause of HHT [4]. This rare form of HHT is also known as HHT5 [5]. Loss of function variants in EPHB4 (encoding ephrin receptor B4) were reported in a few individuals exhibiting atypical HHT symptoms and HHT-like hepatic abnormalities [6]. It has been shown that endothelial-specific DROSHA, a key enzyme for microRNA (miRNA) biogenesis, regulates the TGF- β and BMP pathway through interaction with SMADs. Inactivation of Drosha induced vascular malformation similar to HHT in zebrafish and mice, indicating a link between Drosha dysfunction and HHT [7,8].

In addition to a few randomized trials, currently, there are no standard medical therapies for HHT patients [4,9]. Therefore, the development of effective drug therapy is urgently needed. In recent years, various teams have collaboratively demonstrated several new disease mechanisms and potential therapeutic targets. This review aims to discuss the new advances within this field.

2. Pathophysiology



The pathophysiology of HHT involves haploinsufficiency of *ALK1*, *ENG* and *SMAD4* genes [10]. However, this theory cannot explain several aspects of the pathogenesis and phenotypes of HHT patients. Recent studies show that somatic second hit, environmental second hit, and alternation of *BMP9* and *BMP10* function all play important roles in HHT pathogenesis.

2.1. Second Hit Hypothesis

The prevailing theory for the progression of HHT is the haploinsufficiency model [10], which reduces the production of proteins from the altered genes. Over 900 pathogenic variants have been identified in the *ENG* and *ALK1* genes, including deletions, insertions, missense, and splice site mutations [11]. The reduction of the protein of the affected gene impairs the normal signaling pathways mediated by *ENG* or *Alk1*. However, the haploinsufficiency model cannot explain why AVMs/telangiectasia only develop in a few organs or part of the mucocutaneous, even if all cells in the body carry the same mutation. The haploinsufficiency model also fails to explain why all members carry the same mutations in one family have symptomatic HHT. This enigma has led researchers to propose the existence of a "second hit" hypothesis (also known as the Knudson hypothesis), suggesting that additional factors interact with HHT gene heterozygosity to trigger the formation of vascular lesions [10].

2.1.1. Genetic Two-Hit

Recently, the second somatic mutations were identified in dermal telangiectasia of both HHT1 and HHT2 patients (Figure 1). The second somatic mutation was first discovered by Snellings et al. in 2019 in skin telangiectasia [12]. Hence, local somatic mutations are present and may play a role in initiating focal vascular malformation [13]. In these studies, the authors checked (1) if there's a mutation in the same gene as the one causing germline HHT; (2) if both the germline and somatic mutations occur in pairs (bi-allelic); and (3) if both the mutations lead to loss of function (LoF). Capture-based library preparations were used to sequence 19 telangiectasia samples, focusing on three genes associated with HHT (*ENG*, *ALK1*, and *SMAD4*), along with an additional 13 genes related to vascular malformations. They discovered that among 19 telangiectasia samples, nine have had somatic mutations in the same gene of the pathogenic germline mutation [12]. Importantly, these mutations are not present in the constitutional (germline) DNA. To determine the biallelic nature of the mutations, they investigated whether these mutations were arranged in cis or trans configuration by sequencing amplicons encompassing positions of both somatic and germline mutations in a single molecule. They found that all seven mutation pairs are arranged in a trans configuration corresponds to a p-value of 0.008, indicating a significant bias toward a trans arrangement. This observation supports the idea that both somatic and germline mutations tend to occur in pairs, exhibiting a bi-allelic pattern. It also confirms that all bi-allelic germline and somatic mutations are highly likely to result in LoF [12]. Collectively, these data suggest that the occurrence of bi-allelic loss of function of *ENG*, *ALK1*, or *SMAD4* genes may be a crucial step in the development of telangiectasia.

Animal studies have also supported the two-hit model. There is a significant impact of LoF of the remaining wild-type allele on HHT phenotype development, regardless of how the LoF occurs (e.g. loss of heterozygosity or loss of protein during inflammation) [14]. Elimination of one allele of genes implicated in HHT, like *Eng* or *Alk1*, mimics particular characteristics of the human disease in animal models, primarily in older animals [15,16]. However, loss of both alleles of any HHT-causative gene is embryonically lethal in mice [17,18]. Further, conditional homozygous deletion of *Eng* [14] or *Alk1* [19,20] in mice results in striking vascular malformations, resembling the AVMs found in HHT patients. It is important to acknowledge that, while the genomic second-hit hypothesis is a reasonable explanation for the development of vascular malformation in HHT patients, further research is needed to fully understand how bi-allelic loss of function of *ENG* or *ALK1* in a few cells lead to the development of telangiectasia or AVM lesions, [21].

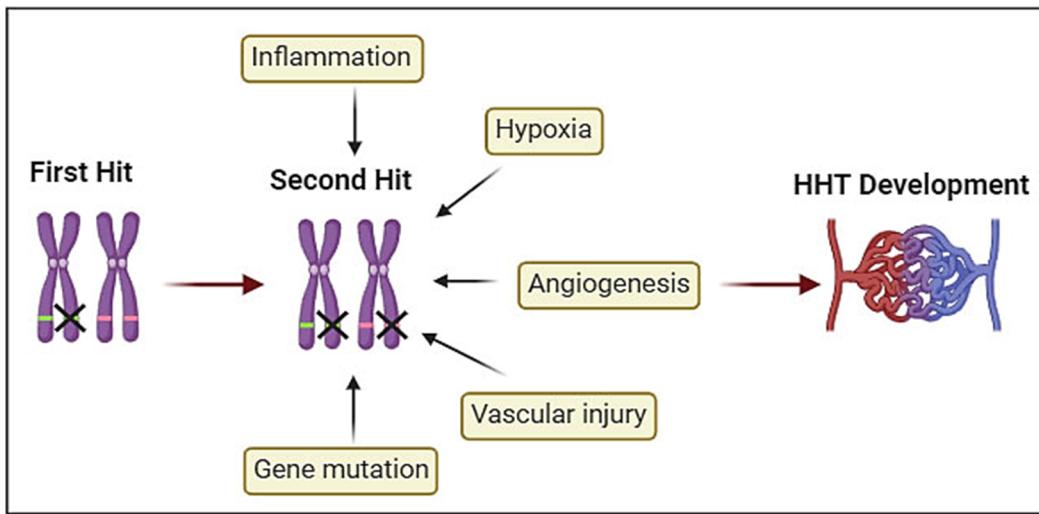


Figure 1. Schematic representation of Hypothetical Two-hit model in HHT: The germline heterozygous mutation in the HHT gene cause haploinsufficiency (First hit). The second hits including inflammation, hypoxia, neo- angiogenesis, vascular injury, and somatic mutations in the previously unaffected normal allele contribute to HHT phenotype development and progression.

2.1.2. Angiogenesis

Pathogenic variants in *Alk1* or *Eng* can initiate the development of AVMs in fetuses and neonates. During embryonic stages in mice, *Alk1* is prominently expressed in arterial ECs. However, in adult mice, *Alk1* expression is consistently high in lung arterial ECs only and during processes like wound healing and tumor angiogenesis [22].

The importance of angiogenesis in AVM formation has been shown by many studies. Conditional global or EC-specific *Alk1* deletion is lethal in neonates and adults, resulting in AVMs and hemorrhage in various organs that have active angiogenesis [20,23,24]. However, homozygous loss of *Alk1* alone does not trigger cutaneous arteriovenous (AV) shunt formation in adults. Dermal wounding in *Alk1*-deficient mice led to the formation of AVMs in the skin [20,25]. Local application of angiogenic or inflammatory stimulators, like VEGF or Lipopolysaccharide (LPS), triggers skin AVM formation, while VEGF neutralizing antibody reduces wound-induced AVMs. Additionally, angiogenic stimulation is required for the development of brain AVM in adult mice [26–28]. Deletion of *Eng* in neonates leads to brain AVM development spontaneously when brain angiogenesis is still active. However, in adults, skin and brain AVMs only developed in mice with global or EC-specific *Eng* deletion when subject to wound or angiogenic stimulation [27]. Together, these data indicate that angiogenesis is necessary for AVM development.

2.1.3. Inflammation

In addition to angiogenesis, inflammation is also a second-hit event in the development of vascular malformation in HHT patients. Down regulation of *ALK1* in human umbilical ECs (HUVECs) or *Eng* in mouse ECs upregulated pro-inflammatory and innate immune signaling [29], which may facilitate leukocyte infiltration and extraction [30]. An abnormally high number of macrophages are present in and around vascular walls in human brain AVM lesions, with or without hemorrhage [31–33], a pattern also observed in brain AVMs in mice with *Eng* or *Alk1* deletion [27,28,34]. A persistent infiltration and pro-inflammatory differentiation of monocytes have also been observed in mouse brains with *Eng* deletion, indicating the effect of inflammation on HHT pathophysiology [35].

Notably, both *ENG* and *ALK1* are expressed not only in ECs, but also in mononuclear cells (MNCs). They play essential roles in the maturation of MNCs within the bone marrow and their subsequent migration into the circulation [30,36–39]. Alterations in signaling through *ENG* and *ALK1* in MNCs have been suggested for modifying immune responses in HHT patients, which present with heightened incidence of infections or leukopenia [40]. The observed alterations in the migration and release of various interleukins and mediators in HHT cases suggest an immunodeficiency in these

patients [41]. Deletion of *Eng* in macrophages impaired mouse immune response [3,42]. However, deletion of *Eng* in macrophages did not cause bAVM development in mice, suggesting that inflammation could not cause AVM formation, but can act as a second-hit to promote AVM progression [3,27].

In addition, inflammation can alter the collagen I and III ratios and render brain AVMs prone to rupture. The collagen I/ collagen III ratios ratio was positively correlated with the number of CD68⁺ microglia/macrophages and GPNMB⁺ monocytes in Alk1 and Eng deficient mouse bAVMs. Microglia, one of the primary immune cells in the central nervous system, play a crucial role in neuroinflammation. Increased microglial accumulation was noted in bAVMs in both human and mouse models [31,43,44]. Studies revealed heightened macrophage infiltration in bAVMs with hemorrhage compared to those without [45]. However, the connection between microglial activation and hemorrhage remains unclear.

Several other environmental triggers have also been proposed as potential second-hits in HHT pathogenesis, including mechanical stress and sun exposure. A study on 103 HHT patients revealed a higher number of telangiectasias on the dominant hand and on the lower lip, which are expected to be more frequently exposed to mechanical stimuli, than the undominant hand and the upper lip [46].

Above evidence indicates that haploinsufficiency of HHT causative genes alone is not sufficient to induce AVM lesion formation. Additional insult (second hit), such as somatic mutation of the normal allele, angiogenesis and aforementioned factors are needed to trigger or promote AVM formation and development.

2.2. Role of BMP9 and BMP10

BMP 9 and 10, the principal ligands within the TGF- β family, are involved in the HHT development [47]. Mutations in BMP-9 have been reported to cause a vascular anomaly syndrome with phenotypic overlap with HHT [48]. BMP10 is the closest family member of BMP9, sharing many biochemical properties with BMP9, including regulating ALK1 downstream signals, such as SMAD1/5 phosphorylation and Id1 expression [49]. BMP10-null mice died at embryonic day 10.5 with phenotypes like *Alk1* null embryos [50]. HHT-like phenotypes have also been shown in zebrafish with both *bmp10* and *bmp10-like* genes knocked down, which phenocopied ALK1 mutants [51] and *bmp10*-null adult fish [52]. These ligands exert their impact through a heteromeric transmembrane receptor of serine/threonine type I (RI) and type II (RII), followed by the activation of the SMAD cascades [53]. It has been found that BMP9 induces the expression of VEGFR1, thus reduces downstream VEGF signaling [54]. In addition, BMP9 and BMP10 promote vascular maturation and quiescence [49].

Numerous studies (Table 1) have highlighted functional overlaps between BMP9 and BMP10. Both BMP9 and BMP10 are physiological ligands for ENG and ALK1 signaling and contribute to the proper formation of arteriovenous networks [55]. Knockout of *Bmp9* and *Bmp10* induced AVM development in mice [56].

Table 1. Summary of recent investigations of BMP9-10/ ENG/Alk1/SMAD signaling pathways.

Subject	Title	Description	Ref.
Zebrafis	BMP10-mediated ALK1 signaling is continuously required for vascular development and maintenance.	The authors have found that loss of both <i>bmp10</i> and <i>bmp10-like</i> genes leads to embryonic lethal cranial AVMs, which are distinguishable from <i>alk1</i> mutants and concluded that BMP10 is vital for the maintenance of post-embryonic vascular development as a non-redundant ligand of Alk1.	[52]
Mice	Context-dependent signaling defines roles of BMP9 and BMP10 in embryonic and postnatal development.	The study examined BMP9 and BMP10 in embryonic and postnatal development. The authors found that BMP9 is indispensable for postnatal vascular development in mice. BMP9 and BMP10 are ALK1's natural ligands.	[57]

	This study showed that administration of a BMP9 and BMP10 are neutralizing anti-BMP10 antibody to juvenile <i>Bmp9</i> -critical for postnatal retinal vascular remodeling.	[58]
	A mouse model of HHT generated by trans-mammary-delivery of anti-BMP9/10 antibodies.	[59]
Human functional activators cell lines of the orphan activin receptor-like kinase 1 (ALK1) in ECs.	This study induced AVMs in postnatal retina through trans-mammary delivery of anti-BMP9/10 antibodies. This could be a practical and non-invasive method for the induction of HHT vascular pathology in the retina of postnatal mice.	[49]
Human and BMP10 in ECs from hereditary hemorrhagic telangiectasia and pulmonary arterial hypertension donors	Identification of BMP9 and BMP10 as functional activators of the orphan activin receptor-like kinase 1 (ALK1) in ECs. Impact of Heterozygous <i>ALK1</i> mutations on the transcriptomic response to BMP9 and BMP10 in ECs from hereditary hemorrhagic telangiectasia and pulmonary arterial hypertension donors	[60]
	The authors demonstrated that BMP9/BMP10 activate SMAD1/5/8 pathways and concluded that BMP9 and BMP10 serve as distinct ALK1 ligands and potentially elicit ALK1-mediated angiogenic effects. Endothelial colony-forming cells (ECFCs) and microvascular ECs (HMVECs) were isolated from new-born HHT and adult PAH donors, and the impact of <i>ALK1</i> mutations on BMP9 and BMP19 transcriptomic responses in ECs was consequently observed. RNA-sequencing was performed on these cells following an 18h stimulation with BMP9 or BMP10. The data showed that ALK1 heterozygosity modified a few of the BMP9/BMP19 regulated genes which are comparable to the controls.	[60]

Abbreviations: BMP9-KO=Bone Morphogenetic Protein 9 Knockout, BMP10-KO= Bone Morphogenetic Protein 10 Knockout, PAH=pulmonary arterial hypertension.

3. Advancements in Developing New Therapies

The use of anti-angiogenic agents like bevacizumab (an anti-VEGF antibody) has shown promising in treating HHT patients. Anti-angiogenic approaches promise to modify the disease process and slow down the development of telangiectasias in addition to potentially causing regression of existing vascular malformations [61]. The available evidence thus far indicates a potential benefit of anti-angiogenic agents in treating small, mucosal telangiectasias, but not effective in treating larger visceral AVMs [62]. The limitations associated with the use of anti-angiogenic agents include limited efficacy and considerable adverse effects, which make them unsuitable for long-term utilization. Topical nasal spray of bevacizumab (Avastin®), a recombinant, humanized VEGF antibody, did not improve epistaxis severity in comparison with placebo [63,64]. An *in vitro* study showed that high bevacizumab concentration reduced VEGF expression but posed a higher risk of toxic effects on ECs [65]. A more recent systematic review revealed a significant gap in sociodemographic variables that identify the race and ethnicity of participants with established HHT in receiving intravenous delivery of bevacizumab treatment [66]. All current treatments for brain AVM, including surgery, radiation, or endovascular embolization, are associated with considerable risks of stroke or death. Treatment of patients with unruptured bAVMs has become increasingly controversial because the natural history of these patients may be less morbid than invasive therapies [67]. There is currently no FDA-approved treatment for HHT [68].

3.1. Enhancing ALK1 or ENG Expression

An alternative therapeutic approach for HHT patients is to enhance the expression of ENG and ALK1 genes in ECs. Kim et al. reported that overexpression of Alk1 in ECs can rescue HHT phenotype in mice with *Alk1* or *Eng* gene deleted, without untoward effects [69]. This observation indicates that overexpression of Alk1 in ECs is well tolerated by mice. Similar finding has been shown in a zebrafish model in which *alk1* is stably expressed in all ECs. [51] This zebrafish line has been continuously maintained for more than 12 years. The fact that overexpression of Alk1 rescues the HHT phenotypes in *Eng* mutant mice suggests that overexpression of Alk1 in ECs is a promising therapeutic strategy to treat both HHT2 (*ALK1* mutation) and HHT1 (*ENG* mutation) patients, which together make up more than 80% of the HHT patient population.

The absence of hepatic endothelial Alk1 changes liver sinusoidal EC differentiation, hinders the differentiation of large vessels by stimulating angiogenesis and arterialization, and cause the formation of shunts within the hepatic vascular system. Therefore, hepatic endothelial ALK1 signaling protects from development of vascular malformations by preserving organ-specific endothelial differentiation and angiocrine signaling [70]. This evidence further supports the finding that an increase in ALK1 or ENG levels can reduce HHT phenotype severity.

Through screening FDA-approved drugs, Tacrolimus was found to be a potent activator of ALK1 signaling [71]. A case report demonstrated that oral administration of low-dose Tacrolimus improved HHT-related epistaxis without impacting pulmonary artery hypertension (PAH) progression in a patient with HHT2 and PAH [72]. Clinical trials using tacrolimus in HHT patients undergoing liver transplantation enhanced control of epistaxis. This medication upregulates the expression of ENG and ALK1, promotes tubulogenesis and cell migration [73]. In another study, oral administration of tacrolimus significantly increased hemoglobin levels and decreased epistaxis and/or gastrointestinal bleeding and the needs of blood transfusion in HHT patients [73]. Adverse effects however were common. At least one adverse effect occurred in 64% of patients during tacrolimus treatment. The most frequently observed adverse effects were headache, abdominal pain, diarrhea, and insomnia. 25% of patients still have adverse effects after the trial has finished [73]. Another study reported outcomes of 11 HHT patients treated with low tacrolimus doses (0.5–2 mg/day) on an off-label prescription basis and demonstrated that low doses of tacrolimus is a promising treatment for epistaxis and gastrointestinal bleeding in HHT [74]. Overall, treatment with tacrolimus significantly reduced severe bleeding, improved hemoglobin levels, and decreased transfusion needs in patients with HHT. Nevertheless, side effects of tacrolimus were prominent. Among 11 patients, two patients had to stop treatment due to gastric intolerance [74]. Despite the potential therapeutic benefit, its safety should be further investigated in a randomized controlled clinical trial. A recent study found that Neuropilin-1 interacts with ALK1 and ENG in vascular smooth muscle cells (SMCs). Neuropilin-1 deletion in vascular SMCs leads to reduced ALK1, ENG, and pSMAD1/5/8 signaling, and reduced cell death associated with HHT. Therefore, in addition to Tacrolimus, Neuropilin can be considered as a new therapeutic target for the treatment of HHT [75].

3.2. Anti-Angiogenic Gene Therapy

Anti-angiogenic approaches promise to reduce hemorrhage and modify the disease process [61]. Many agents can block VEGF signaling, e.g., anti-VEGF antibodies [26,61,76–80] and tyrosine kinase inhibitors (TKIs) [81–83]. However, these agents have considerable side effects [84–87]. In addition, TKIs showed a differential effect on skin and intestinal AVMs [88]. Genetic deletion of the angiogenic signal-transducing VEGF receptor-2 (VEGFR-2) prevented excessive angiogenesis but did not fully revert AVM formation [89]. This data suggests that blocking angiogenesis alone is not sufficient to treat AVMs. In addition, systemic therapies with antibodies and TKIs have many drawbacks and side effect [81,84,85,90,91]. Telangiectasia and brain AVMs are chronic lesionin which angiogenesis can be activated over time[87,92,93]. To control the disease progression, systemic delivery of antibodies or TKIs must be repeated for an extended period, which may be intolerable to some patients.

The soluble FMS-related tyrosine kinase 1 (sFLT1) contains the extracellular domain of VEGF receptor-1 and can bind all isoforms of VEGF, thereby inhibiting their downstream signaling. Thus, the effect of sFLT1 could be broader than VEGF antibodies that are specific to an individual VEGF isotope, and kinase inhibitors. Mice carrying two modified *Flt1* alleles which lack the cytoplasmic tyrosine kinase signaling domain but retain the ability to express the sFlt1 isoform, are viable with mild adult phenotypes [94–96]. This suggests that signaling by FLT1 is not essential and supports a

primary role for FLT1 and sFLT1 as decoy receptors, limiting the availability of VEGF to signal through its receptors.

It has been shown that in mice, intravenous (i.v.) injection of AAV9-sFLT1 (a recombinant AAV packaged in serotype 9 capsid) reversed bAVM phenotypes [97]. However, uncontrolled systemic sFLT1 expression caused minor liver inflammation and growth arrest of young mice [97]. New engineered AAV capsids and different delivery routes may minimize the side effects. AAV has many advantages over direct systemic delivery of anti-angiogenic protein because it can mediate long-term transgene expression [98–103], which makes a need for repeated dosing unlikely. In addition, many engineered AAV capsids make targeted delivery of genes possible. Therefore, AAV is a promising vector for developing specific gene therapy strategy for treating telangiectasia and AVMs.

3.3. *Angiopoietin-2 (Angpt2) Antibodies*

Alk1 germline deletion increased *Angpt2* expression in rodent brain and spinal cord AVMs [19]. *Angpt2* expression was found to increase in the postnatal retina of *Smad4* mutant mice, as detected through RNA- and ChIP-sequencing on BMP9- stimulated ECs, as well as in mice with *Smad4* knocked out specifically in ECs [104]. Increased *Angpt2* expression was also detected in postnatal brain ECs of *Alk1*, *Eng*, and *Smad4* mutant mice [104,105]. Administration of *Angpt2* monoclonal antibodies prevented and resolved retinal AVMs of *Smad4* mutant mice [104] and improved brain vascular morphology in other HHT models [105]. However, other studies showed that the blood ANGPT2 levels were decreased in HHT2 patients and unchanged in HHT1 patients [106,107]. Further, cultured blood outgrowth ECs from HHT1 and HHT2 patients, along with several tissues collected from *Eng^{+/−}* mice exhibited substantial decreases in *Angpt2* levels [108,109]. Studies have also indicated that ANGPT2 expression is depended on the type of HHT, and tissue/organ assessed [107,110,111]. Nevertheless, an inductive role for ANGPT2 in both brain and retinal AVMs has been suggested [105]. In clinical trials anti-ANGPT2 antibodies have shown potential in normalizing vessel caliber in HHT patients [104]. A Robust and ectopic *Angpt2* expression in the brain ECs was evident in all the 3 mouse models that have *Smad4*, *Alk1*, or *Eng* knockout in ECs. Loss of HHT signaling in HUVECs, via ALK1-Fc, resulted in increased secretion of ANGPT2 into the media [105]. Thus, *Angpt2*-targeted therapies may represent a novel approach for treating vascular abnormalities in HHT patients.

3.4. *PI3-Kinase Inhibitor and Other Agents*

Because the PI3-kinase pathway operates downstream of both VEGF and ANGPT2, researchers have tested PI3-kinase inhibitors in HHT patients. Treatment with Buparlisib, a PI3-kinase inhibitor, reduced the frequency of epistaxis in HHT2 patient [112,113]. However, long-term utilization of this inhibitor could pose substantial challenges related to safety and tolerability. Adverse effects like depression, increased lipase, hyperglycemia, and anxiety of first-generation PI3 kinase inhibitors warrant careful consideration for prolonged use [114]. Among the AKT inhibitors, perifosine and uprosertib, VAD044 (a small molecule) exhibited superior efficacy in inhibiting retinal AVM formation by reducing phospho-S6 and AKT activity [115]. A phase I trial known as INSIGHT is currently underway to investigate the benefits and risks of VAD044 [116].

In addition to PI3-kinase inhibitors, other drugs with anti-angiogenic property have been tested. Non-selective beta-blockers, such as propranolol and timolol have been applied locally to reduce bleeding from telangiectasia [117]. Etamsylate (a nasal spray) has recently approved to treat HHT patients with high susceptibility to nosebleeds [118].

3.5. *Modulation of BMP9-10/ENG/ALK1/SMAD Pathway as an Emerging Therapeutic Target*

Based on the insights gained from genetic studies, it becomes feasible to develop tailored treatments or repurposing existing medications to modulate the BMP9-10/ENG /ALK1/SMAD pathway (Figure 2) [62]. Various therapeutic approaches targeting *Alk1* are under investigation, including monoclonal antibodies, small molecule inhibitors, and gene therapy to modulate *Alk1* expression and signaling [69,119]. Ruiz et al. found that Sirolimus and nintedanib in combination prevented vascular pathology in the oral mucosa, lungs and liver of the BMP9/BMP10- immunoblocked mice [120]. Sirolimus binds FKBP12 and inhibits mTOR downstream of PI3K and

AKT. Post-liver transplantation, Sirolimus ameliorated HHT symptoms by correcting haploinsufficiency or upregulating protein levels of ENG and ALK1. HHT2 patients with hepatic AVMs and high-output cardiac failure experienced significant improvement in epistaxis after receiving treatment of immunosuppressive agent tacrolimus or sirolimus after liver transplantation [71,121].

Ruiz et al. found that Sirolimus and nintedanib in combination prevented vascular pathology in the oral mucosa, lungs and liver of the BMP9/BMP10-immunoblocked mice [120]. Tacrolimus is a strong activator of the BMP9/ALK1/Smad signaling cascade and it has been used for treatment of animal HHT and decreased the development of retinal AVMs [71]. A case study reported a substantial decrease in epistaxis in HHT2 patients following orally administration of a low dose of tacrolimus [72].

Together, these data shown that agents that can modulate gene expression in BMP9-10/ENG /ALK1/SMAD pathway have potential to reduce bleeding from telangiectasia and AVM.

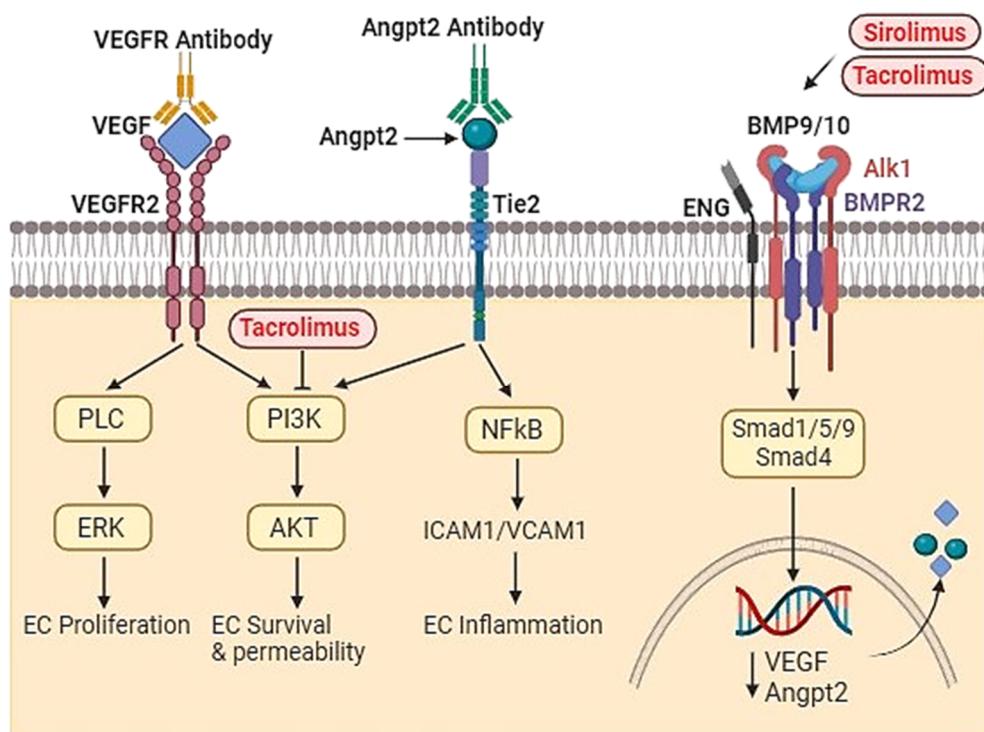


Figure 2. Targets of therapeutic agents. Anti-VEGF antibodies bind VEGF in tissue prevent it interact with its receptors and thus reduce angiogenesis and AVM severity. ANGPT2 monoclonal antibodies bind with ANGPT2 in tissue prevent it interact with its receptors and thus reduce its downstream signaling and AVM severity. Sirolimus and Tacrolimus inhibit BMP9/BMP10 downstream signaling, which resulting in reduction of AVM severity. Tacrolimus can also reduce AVM severity through inhibit PI3K signaling pathway.

4. Summary and Future Perspective

In this review, we summarized the advances in HHT pathophysiology and newly explore therapeutic strategies. The identification of second-somatic hit and enhanced ALK1 expression in ECs rescues the phenotype of *Alk1* and *Eng* knockout mice, thus opening new avenues in our understanding of HHT pathogenesis and therapeutic targets. Furthermore, the exploration of drugs like Tacrolimus and Sirolimus, which have the potential to reactivate the BMP9-10/ENG/ALK1/Smad1/5/9 signaling pathway, offers a novel direction in addressing the disrupted molecular mechanisms central to the HHT pathogenesis. ANGPT2 antibodies and AAV mediated gene therapies are also promising therapeutic strategy for reducing phenotype severity of HHT patients.

Further research should focus on exploring future potential treatments comprehensively utilizing existing data. Although the existence of somatic second hit has been proven, further investigations on how the few ECs with biallelic mutations can lead to the development of vascular malformations are needed. Although the above-mentioned therapeutic strategies are promising, they are still in the experimental and research stages. Clinical trials and further studies are necessary to determine their safety and effectiveness in treating HHT patients.

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