Volume 4 | Number 1

HHT FRONTLINES

BRINGING SCIENCE FROM BENCH TO BEDSIDE

CLINICAL RESEARCH

Welcome to the HHT Frontlines newsletter! Twice a year we will be bringing you select publications about HHT based on the importance of each study for patients, clinicians and scientists. The full abstracts are available in the journal of publication as noted in each article.

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Efficacy and Safety of a 0.1% Tacrolimus Nasal Ointment as a Treatment for Epistaxis in Hereditary Hemorrhagic Telangiectasia: A Double-Blind, Randomized, Placebo-Controlled, Multicenter Trial

Sophie Dupuis-Girod, Anne-Emmanuelle Fargeton, Vincent Grobost, Sophie Rivière, Marjolaine Beaudoin, Evelyne Decullier, Lorraine Bernard, Valentine Bréant, Bettina Colombet, Pierre Philouze, Sabine Bailly, Frédéric Faure
J Clin Med 2020; 16;9(5):1262; doi: 10.3390/jcm9051262

The authors of this article evaluated the effect of tacrolimus nasal ointment as treatment for epistaxis in HHT.

Objective: to evaluate, six weeks after the end of the treatment, the efficacy on the duration of nosebleeds of tacrolimus nasal ointment, administered for six weeks to patients with hereditary hemorrhagic telangiectasia complicated by nosebleeds.

Methods: a prospective, multicenter, randomized, placebo-controlled, double-blinded, ratio 1:1 phase II study. Patients were recruited from three French Hereditary Hemorrhagic Telangiectasia (HHT) centers between May 2017 and August 2018, with a six-week follow-up, and we included people aged over 18 years, diagnosed with hereditary hemorrhagic telangiectasia and epistaxis (total duration > 30 min/6 weeks prior to inclusion). Tacrolimus ointment 0.1% was self-administered by the patients twice daily during 6 weeks. About 0.1 g of product was to be administered in each nostril with a cotton swab.

Results: a total of 50 patients was randomized and treated. Mean epistaxis duration before and after treatment + 6 weeks in the tacrolimus group were 324.64 and 249.14 min, respectively, and in the placebo group 224.69 and 188.14 min, respectively. Epistaxis duration improved in both groups, with no significant difference in our main objective comparing epistaxis before and after treatment ($p = 0.77$); however, there was a significant difference in evolution when comparing epistaxis before and during treatment ($p = 0.04$). Toxicity was low and no severe adverse events were reported

Implications: Tacrolimus nasal ointment, administered for six weeks, did not improve epistaxis in HHT patients 6 weeks after the end of the treatment. However, the good tolerance, associated with a significant improvement in epistaxis duration during treatment, encouraged the authors to plan a phase 3 trial on a larger patient population with a main outcome of epistaxis duration during treatment and a longer treatment time.

Topical Propranolol Improves Epistaxis Control in Hereditary Hemorrhagic Telangiectasia (HHT): A Randomized Double-Blind Placebo-Controlled Trial

Meir Mei-Zahav, Yulia Gendler, Elchanan Bruckheimer, Dario Prais, Einat Birk, Muhamad Watad, Neta Goldschmidt, Ethan Soudry
J Clin Med 2020; 9:3130; doi: 10.3390/jcm9103130

The authors of this article evaluated the effect of topical propranolol as treatment for epistaxis in HHT.

Objective: the aim of the study was to assess the safety and efficacy of nasal propranolol gel as treatment of HHT-related epistaxis.

Methods: twenty participants with moderate-severe HHT-related epistaxis were randomized to eight weeks of propranolol gel 1.5%, or placebo 0.5 cc, applied to each nostril twice daily; and continued propranolol for eight weeks in an open-label study.

Results: within the propranolol group, the epistaxis severity score (ESS) improved significantly (-2.03 +/- 1.7 as compared with -0.35 +/- 0.68 for the placebo group, p = 0.009); hemoglobin levels improved significantly (10.5 +/- 2.6 to 11.4 +/- 2.02 g/dL, p = 0.009); and intravenous iron and blood transfusion requirement decreased. The change in nasal endoscopy findings was not significant. During the open-label period, the ESS score improved significantly in the former placebo group (-1.99 +/- 1.41, p = 0.005). The most common adverse event was nasal mucosa burning sensation. No cardiovascular events were reported.

Implications: The results of this study suggest that topical propranolol gel is safe and effective in HHT-related epistaxis.

Pazopanib for severe bleeding and transfusion-dependent anemia in hereditary hemorrhagic telangiectasia

Joseph G Parambil, James R Gossage, Keith R McCrae, Troy D Woodard, K V Narayanan Menon, Kasi L Timmerman, Douglas P Pederson, Dennis L Sprecher, Hanny Al-Samkari
Angiogenesis 2021; Jul 22:1-11; doi: 10.1007/s10456-021-09807-4

Pazopanib is an oral multi-kinase angiogenesis inhibitor with promise to treat bleeding in HHT. The authors of this article investigated the effect of pazopanib on bleeding in HHT.

Objective: to analyze outcomes of HHT patients with the most severe bleeding causing blood transfusion dependence, treated on a predefined institutional pazopanib treatment pathway. The primary endpoint was achievement of transfusion independence. Secondary endpoints included hemoglobin, epistaxis severity score, RBC transfusion and iron infusion requirements, number of local hemostatic procedures, ferritin and transferrin saturation, compared using paired and repeated measures mean tests.

Results: thirteen transfusion-dependent HHT patients received pazopanib [median (range) dose 150 (25-300) mg daily)] for a median of 22 months. All patients achieved transfusion independence. Compared with pretreatment, pazopanib increased mean hemoglobin by 4.8 (95% CI, 3.6-5.9) g/dL (7.8 vs. 12.7 g/dL, P < 0.0001) and decreased mean epistaxis severity score by 4.77 (3.11-6.44) points (7.20 vs. 2.43 points, P < 0.0001) after 12 months of treatment. Compared with 3 months of pretreatment, RBC transfusions decreased by 93% (median of 16.0 vs. 0.0 units, P < 0.0001) and elemental iron infusion decreased by 92% (median of 4500 vs. 0 mg, P = 0.005) during the first 3 months of treatment; improvements were maintained over time. Pazopanib was well-tolerated: hypertension, lymphocytopenia, and fatigue were the most common TEAEs

Implications: Treatment with pazopanib was safe and effective to manage severe bleeding in HHT, liberating all patients from transfusion dependence and normalizing hematologic parameters at doses lower than used to treat malignancies. These findings require confirmation in a randomized trial.

Intranasal Efudix reduces epistaxis in hereditary hemorrhagic telangiectasia

D V C de Jel, F J M Disch, S Kroon, J J Mager, F J Verdam
Angiogenesis 2020; Aug;23(3): 271-274; doi: 10.1007/s10456-020-09712-2

Local application of fluorouracil (Efudix, 5-FU) induces sclerosis in patients with sinonasal tumors and superficial basocellular skin carcinoma.The authors of this article investigated the effect of intranasal Efudix on epistaxis in HHT.

Objective: to evaluate the effect of nasally applied 5-FU on (severe) epistaxis in patients with hereditary hemorrhagic telangiectasia (HHT).

Methods: HHT patients with severe and frequent epistaxis, subsequent anemia and a necessity for blood and/or iron infusions were treated with a nasal tampon with 5-FU. This tampon was placed unilaterally in the nasal cavity on the side of the most severe epistaxis and replaced once weekly during 4 weeks. Outcome measures were safety and side effects, the aspect of the nasal mucosa measured with the mucosal HHT score, the epistaxis severity score (ESS), hemoglobin and ferritin plasma levels, and quality of life assessment pre-treatment, one and three months post-treatment.

Results: six HHT patients participated. During treatment and follow-up, the nasal mucosa turned more pale and sclerotic and the number of telangiectases diminished. The mucosal HHT score improved and the ESS declined (p = 0.01). The decline of ESS persisted up to 3 months post-5-FU treatment. Moreover, mean hemoglobin levels increased from 6.0 pre-5-FU to 6.8 after one month post-5-FU.

Conclusion: Unilateral application of 5-FU on a nasal tampon diminished the severity and frequency of epistaxis in all 6 HHT patients. This effect sustained up to three months post-treatment, despite the fact that the contralateral side remained untreated. Subsequently, hemoglobin levels increased. Intranasal 5-FU is a promising entity for further research on epistaxis treatment in HHT patients.

Oral itraconazole for epistaxis in hereditary hemorrhagic telangiectasia: a proof of concept study

S Kroon, R J Snijder, A E Hosman, V M M Vorselaars, F J M Disch, M C Post, J J Mager
Angiogenesis 2021; May 24(2): 379-386; doi: 10.1007/s10456-020-09758-2

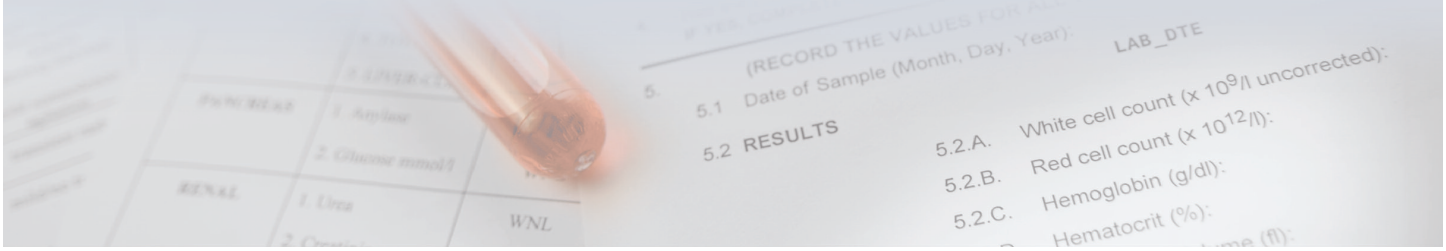
The inhibiting effects of itraconazole (an antifungal drug) on vascular endothelial growth factor (VEGF) have recently been discovered. The authors of this article investigated the effect of itraconazol as treatment for epistaxis in HHT.

Objective: to evaluate the effect of itraconazole on epistaxis in patients with HHT and severe epistaxis.

Methods: patients were treated with daily 200 mg orally administered itraconazole for sixteen weeks.

Results: twenty-one HHT patients, 8 females (38%), 13 males (62%), median age of 59 years (interquartile range (IQR) 55-69) were enrolled. Of these patients, 13 (62%) were diagnosed with HHT type 1, seven (33%) with HHT type 2 and in one patient (5%), no pathognomonic HHT mutation was found. Four patients (19%) prematurely terminated the study (3 due to mild or moderate side-effects) resulting in 17 patients included in the analyses. The median epistaxis severity score significantly decreased during treatment from 6.0 (IQR 5.1-7.2) to 3.8 (IQR 3.1-5.2) (p = 0.006). The monthly epistaxis frequency decreased from 56 to 38 epistaxis episodes (p = 0.004) and the monthly duration from 407 to 278 minutes (p = 0.005). Hemoglobin levels did not significantly change. The quality of life showed a small but significant improvement.

Implications: Oral itraconazole significantly improved epistaxis in HHT patients. The potential benefit of itraconazole in HHT should be further investigated.



Sclerotherapy Versus Cautery/Laser Treatment for Epistaxis in Hereditary Hemorrhagic Telangiectasia

Troy D Woodard, Kathleen B Yappel-Sinkko, Xiaofeng Wang, Keith R McCrae, Joseph G Parambil
Laryngoscope 2021; Jun 23; doi: 10.1002/lary.29701

Surgical interventions for epistaxis management in hereditary hemorrhagic telangiectasia (HHT) demonstrate short-term success and require repeated procedures for disease control. The authors of this article compared the effect of sclerotherapy with cautery/laser treatment.

Objective: to investigate if tetradecyl sclerotherapy (STS) is more effective than cautery/laser treatment as treatment for epistaxis in HHT.

Methods: the authors retrospectively assessed 67 patients with HHT with moderate and severe epistaxis that were treated periodically with C ± L (34 patients) versus STS (33 patients). The primary outcome was the number of procedures needed to maintain the epistaxis severity score (ESS) as mild. Secondary outcomes assessed for differences in postoperative complications, hemoglobin levels, iron stores, hematologic support, and quality-of-life (QoL) scores.

Results: To maintain ESS in the mild range, 1.6 STS procedures (range, 1-4) were performed versus 3.6 C ± L procedures (range, 1-8) (P = .003). Significant postoperative differences included reduction in nasal crusting (3% vs. 32%, P = .001), foul odor (3% vs. 35%, P < .001), and septal perforation (3% vs. 29%, P = .006) after STS. There were no significant differences between the two treatments in hemoglobin levels, iron stores, hematologic support, or QoL scores.

Implications: STS is able to attain satisfactory epistaxis control with significantly fewer procedures and lower postoperative complications than C ± L. STS should be considered as the initial surgical intervention for epistaxis in patients with HHT.

Development and Validation of the Nasal Outcome Score for Epistaxis in Hereditary Hemorrhagic Telangiectasia (NOSE HHT)

Andrew M Peterson, Dorina Kallogjeri, Edward Spitznagel, Murali M Chakinala, John S Schneider, Jay F Piccirillo
JAMA Otolaryngol Head Neck Surg 2020; 146(11): 999-1005; doi: 10.1001/jamaoto.2020.3040

Epistaxis is the greatest cause of morbidity in patients with hereditary hemorrhagic telangiectasia (HHT); because of this, a validated epistaxis-specific quality-of-life instrument for HHT should be made available. The authors of this article validated an epistaxis-specific quality-of-life patient-reported outcome survey.

Objective: to develop and validate an epistaxis-specific quality-of-life patient-reported outcome measure for HHT.

Methods: this survey study focused on the development and validation of the Nasal Outcome Score for Epistaxis (NOSE) in HTT (NOSE HHT) outcome measure with data prospectively collected from December 10, 2019, to March 15, 2020. A total of 401 patients were recruited from within the Cure Hemorrhagic Telangiectasia online patient advocacy social media network, the Washington University HHT Center of Excellence, and a randomized clinical trial investigating an intranasal timolol gel for HHT-associated epistaxis.

Main outcomes and measures: face and content validity, factor analysis, internal consistency as measured through Cronbach α, construct validity, responsiveness to change, and minimal clinically important difference.

Results: The NOSE HHT was developed and validated with a possible score ranging discretely from 0 to 4 for each of the 29 items and a total score ranging continuously from 0 to 4 after dividing by the total number of items answered. A total of 401 participants completed the NOSE HHT. Factor analysis identified 3 factors that matched the a priori specified subgroups of particular aspects of life affected by HHT-associated epistaxis: physical problems (mean [SD] magnitude, 1.59 [0.83]), functional limitations (mean [SD] magnitude, 1.28 [0.84]), and emotional consequences (mean [SD] magnitude, 1.95 [1.02]). The instrument had high internal consistency with an overall Cronbach α of 0.960. Convergent validity determined the total NOSE HHT score to be a strong predictor of disease severity; total NOSE HHT score can be split up into the following epistaxis severity categories: mild (0-1), moderate (1.01-2), and severe (>2). The instrument was found to be sensitive to change, and the minimal clinically important difference for the total NOSE HHT score was 0.46.

Implications: Evaluation of the consistency, reliability, and responsiveness of the NOSE HHT survey found it to be a valid instrument to assess severity and change in epistaxis. Study results suggest that the NOSE HHT survey is clinically applicable and useful as an outcome measure of future HHT-associated epistaxis trials.

An international, multicenter study of intravenous bevacizumab for bleeding in hereditary hemorrhagic telangiectasia: the InHIBIT-Bleed study

Hanny Al-Samkari, Raj S. Kasthuri, Joseph G. Parambil, Hasan A. Albitar, Yahya A. Almodallal, Carolina Vázquez, Marcelo M. Serra, Sophie Dupuis- Girod, Craig B. Wilsen, Justin P. McWilliams, Evan H. Fountain, James R. Gossage, Clifford R. Weiss, Muhammad A. Latif, Assaf Issachar, Meir Mei-Zahav, Mary E. Meek, Miles Conrad, Josanna Rodriguez-Lopez, David J. Kuter, Vivek N. Iyer
Haematologica 2021 Volume 106(8): 2161-2169

Hereditary Hemorrhagic Telangiectasia (HHT) is a multisystem vascular disorder that causes chronic gastrointestinal bleeding, epistaxis, and severe anemia. Bevacizumab, an anti-vascular endothelial growth factor antibody, may be effective to treat bleeding in HHT. The authors of this article describe the results of an international, multicenter, retrospective study in which they evaluated the use of systemic bevacizumab to treat HHT-associated bleeding and anemia at 12 HHT treatment centers.

Objective: to evaluate the efficacy and safety of treatment with bevacizumab for HHT-associated bleeding and anemia. Methods: hemoglobin, Epistaxis Severity Score (ESS), red cell units transfused, and intravenous iron infusions before and after treatment were evaluated using paired means testing and mixed-effects linear models.

Results: bevacizumab was given to 238 HHT patients for a median of 12 (range, 1-96) months. Compared with pretreatment, bevacizumab increased mean hemoglobin by 3.2 g/dL (95% confidence interval: 2.9-3.5 g/dL); i.e., from a mean hemoglobin of 8.6 (8.5- 8.8) g/dL to 11.8 (11.5-12.1) g/dL; P<0.0001) and decreased the ESS by 3.4 (3.2-3.7) points (mean ESS 6.8 [6.6-7.1] versus 3.4 [3.2-3.7]; P<0.0001) during the first year of treatment. Compared with 6 months before treatment, the number of red blood cell units transfused decreased by 82% (median of 6.0 [interquartile range, 0.0-13.0] units versus 0 [0.0-1.0] units; P<0.0001) and iron infusions decreased by 70% (median of 6.0 [1.0-18.0] infusions versus 1.0 [0.0-4.0] infusions, P<0.0001) during the first 6 months of bevacizumab treatment. Outcomes were similar regardless of the underlying pathogenic mutation. Following initial induction infusions, continuous/scheduled bevacizumab maintenance achieved higher hemoglobin and lower ESS than intermittent/as-needed maintenance but with more drug exposure. Bevacizumab was well tolerated: hypertension, fatigue, and proteinuria were the most common adverse events. Venous thromboembolism occurred in 2% of patients.

Implications: Systemic bevacizumab is safe and effective for managing chronic bleeding and anemia in HHT. While data from large, randomized prospective studies are needed to confirm these findings, in this large observational study, bevacizumab was associated with significant improvements in hemoglobin and ESS, along with significant reductions in the need for RBC transfusion and intravenous iron infusion. Improvement was similar regardless of the underlying pathogenic HHT mutation. Following an initial sequence of induction treatments, continuous scheduled maintenance therapy and intermittent as-needed maintenance therapy were both reasonable to maintain treatment effect. Hypertension, fatigue, proteinuria, and myalgia/arthralgia were the most common side effect; venous thromboembolism and treatment discontinuation for adverse events were rare, occurring only in 2% and 5% of patients, respectively.

Hereditary Hemorrhagic Telangiectasia (HHT) and Survival: The Importance of Systematic Screening and Treatment in HHT Centers of Excellence

Els M de Gussem, Steven Kroon, Anna E Hosman, Johannes C Kelder, Martijn C Post, Repke J Snijder, Johannes J Mager
J Clin Med 2020 Nov 6; 9(11): 3581; doi: 10.3390/jcm9113581

The authors of this study investigated the life expectancy of patients with HHT, screened and treated (if indicated) in an HHT center.

Objective: to investigate if HHT patients, systematically screened for HHT-related organ involvement and treated if needed, have a similar survival as persons without HHT.

Methods: all individuals screened for HHT in the Dutch HHT Center between 2004 and 2016 with a genetically or clinically confirmed diagnosis (HHT group) or excluded diagnosis (non-HHT control group) we included in the study. The social security number was used to confirm status as dead or alive in December 2019. 717 HHT patients and 471 controls (mostly family members) were included in the study.

Results: there was no difference in survival between the HHT and the non-HHT control group. The HHT group had a life expectancy of 75.9 years (95% confidence interval (CI) 73.3-78.6), comparable to the control group (79.3 years, 95% CI 74.8-84.0, Mantel-Cox test: p = 0.29).

Implications: The life expectancy of HHT patients systematically screened for HHT-related organ involvement and treated if needed in an HHT center of excellence, appeared to be similar compared to their family members without HHT, justifying systematic screening and treatment in HHT patients.

Several significant basic science papers of relevance to HHT were published in 2020-2021, and we provide a summary of some of these.

Two papers examined signaling pathways activated during formation and expansion of vascular malformations (VMs) and the effects of pharmacologically targeting these pathways in mouse models of HHT. Phillipe Marambaud's team published a 2020 *JCI* paper investigating activation of the mTOR/S6 kinase and VEGFR2/PI3K pathways within neonatal retina VMs in mouse models of HHT. They found synergistic suppression of AVM formation between sirolimus/rapamycin, an mTOR inhibitor, and nintedanib, a VEGFR inhibitor, using two different models of HHT; inducible *Alk1-/-* and anti-BMP9/10 antibody-depletion. Anne Eichmann's team (*Circulation* 2021) more recently demonstrated activation of both the integrin and YAP/TAZ signaling pathways in VMs of endothelial cell (EC)-specific *Alk1-/-* mice and demonstrated that VMs could be suppressed using cilengitude or ATN161, bot integrin signaling inhibitors, or by inhibition of YAP/TAZ signaling using verteporfin, offering possible alternative avenues for pharmacological intervention for HHT.

In late 2019, a team led by Doug Marchuk published evidence that somatic mutations in the wild type allele of the causative HHT gene might contribute to telangiectasia in patients (*Am. J. Hum. Genetics*) In sequencing gDNA isolated from telangiectases, loss of function mutations in the wild type HHT allele were found in 9 out of 19 VM lesions isolated from both HHT1 and HHT2 patients. In patients with multiple telangiectases, each lesion carried a different mutation, suggesting local somatic mutation and expansion *in situ*, and on average ~ 2.5% of cells within each lesion carried that mutation. The percentage of lesional cells carrying the mutation is low because not all cells in the lesion are endothelial, moreover, earlier studies show that not every EC within a lesion requires homozygous loss of the HHT gene. Hua Su's group had previously shown, using mouse models, that ECs with homozygous loss of *Alk1* or *Eng* can drive proliferation and expansion of adjacent wildtype, *Eng+/-* or *Alk1+/-* ECs through non-cell autonomous mechanisms such as secretion of cytokines/growth factors or alterations in cell – cell and cell-matrix interactions. More recently, her team published another paper in *J. Transl. Stroke*, 2021 supporting this concept and showing that bone marrow-derived *Alk1-/-* ECs cells can initiate brain AVM formation, and that *Alk1-/-* ECs can undergo clonal outgrowth within vascular lesions, with the incidence and severity of brain AVMs being proportional to the number of initiating *Alk1-/-* cells.

On the other side of the coin, Paul Oh's team demonstrated that overexpressing ALK-1 in ECs of HHT1 and HHT2 mouse models can rescue mice from AVM development. Thus, drugs that
upregulate ALK-1 gene expression would be predicted to be protective against AVMs in both HHT1 and HHT2. Notably Doug Marchuk's work on somatic LOF mutations in the wild type allele of the HHT gene would argue against trying to hyper-activate this allele since cells within lesions may not possess such a wildtype allele. Nevertheless, elevating expression of the wild type HHT allele may be helpful as a prophylactic strategy. Notably, Ruiz et al 2020 found that elevated ALK-2 expression might be capable of substituting for loss of ALK1 in their studies on mechanism of action of sirolimus in suppressing HHT AVMs, since ALK2-also activates pSmad1/5/8 signaling. Certain drug strategies might therefore circumnavigate a requirement for ALK1 or endoglin.

Finally, three papers from the teams of Beth Roman, Helen Arthur, and Sabine Baily, in addition to providing greater insight into the molecular mechanisms regulating BMP9, BMP10 and endoglin action in the adult, also provide new models of High Output Heart Failure, an increasingly r ecognized problem in aging HHT patients. In *Eng-/-* mice, the Arthur group suggest that the presence of AVMs biophysically alters flow through the cardiovascular system to decrease aortic pressure ultimately leading to ventricular enlargement and heart failure. The Baily group showed that in mice under hypoxic conditions, BMP9 promotes expression of the vasoconstrictor, endothelin-1, as well as supporting pulmonary arterial muscularization, such that in *Bmp9-/-* mice these features are attenuated. BMP10, on the other hand prevents cardiac remodeling. Under hypoxic conditions, genetic loss of BMP9 and BMP10 leads to reduced vascular resistance, reduced blood pressure, elevated cardiac filling and eventually to HOHF.

Correcting Smad1/5/8, mTOR, and VEGFR2 treats pathology in hereditary hemorrhagic telangiectasia models

Ruiz S, Zhao H, Chandakkar P, Papoin J, Choi H, Nomura-Kitabayashi A, Patel R, Gillen M, Diao L, Chatterjee PK, He M, Al-Abed Y, Wang P, Metz CN, Oh SP, Blanc L, Campagne F, Marambaud P
J Clin Invest. 2020 Feb 3;130(2):942-957. doi: 10.1172/JCI127425.PMID: 31689244

A Hereditary hemorrhagic telangiectasia (HHT), a genetic bleeding disorder leading to systemic arteriovenous malformations (AVMs), is caused by loss-of-function mutations in the ALK1/ENG/Smad1/5/8 pathway. Evidence suggests that HHT pathogenesis strongly relies on overactivated PI3K/Akt/mTOR and VEGFR2 pathways in endothelial cells (ECs). In the BMP9/10-immunoblocked (BMP9/10ib) neonatal mouse model of HHT, we report here that the mTOR inhibitor, sirolimus, and the receptor tyrosine kinase inhibitor, nintedanib, could synergistically fully block, but also reversed, retinal AVMs to avert retinal bleeding and anemia. Sirolimus plus nintedanib prevented vascular pathology in the oral mucosa, lungs, and liver of the BMP9/10ib mice, as well as significantly reduced gastrointestinal bleeding and anemia in inducible ALK1-deficient adult mice. Mechanistically, in vivo in BMP9/10ib mouse ECs, sirolimus and nintedanib blocked the overactivation of mTOR and VEGFR2, respectively. Furthermore, we found that sirolimus activated ALK2-mediated Smad1/5/8 signaling in primary ECs - including in HHT patient blood outgrowth ECs - and partially rescued Smad1/5/8 activity in vivo in BMP9/10ib mouse ECs. These data demonstrate that the combined correction of endothelial Smad1/5/8, mTOR, and VEGFR2 pathways opposes HHT pathogenesis. Repurposing of sirolimus plus nintedanib might provide therapeutic benefit in patients with HHT.

Implications: This paper shows that combinatorial delivery of an mTOR inhibitor, rapamycin, with a VEGFR2 tyrosine kinase inhibitor, nintedanib, suppresses AVM development and induces regression of established AVMs in mouse models of HHT. The authors used a non-genetic mouse model of HHT, namely trans-mammary delivery of BMP9/10 blocking antibodies to suckling pups, that results in neonatal vascular defects in the retina, oral mucosa, tongue, lung, liver, and intestine, as well as hemorrhaging and anemia. Data were validated in EC-specific *Alk1-/-* mice. The drugs elevated pSmad1/5/8 signaling due to upregulation of ALK2 expression. The data support the use of combinatorial sirolimus/rapamycin plus nintedanib for therapeutic application in patients with HHT.

Defective Flow-Migration Coupling Causes Arteriovenous Malformations in Hereditary Hemorrhagic Telangiectasia

Park H, Furtado J, Poulet M, Chung M, Yun S, Lee S, Sessa WC, Franco CA, Schwartz MA, Eichmann A
Circulation. 2021 Sep 7;144(10):805-822. doi: 10.1161/CIRCULATIONAHA.120.053047. Epub 2021 Jun 29.PMID: 34182767

Background: Activin receptor-like kinase 1 (ALK1) is an endothelial transmembrane serine threonine kinase receptor for BMP family ligands that plays a critical role in cardiovascular development and pathology. Loss-of-function mutations in the ALK1 gene cause type 2 hereditary hemorrhagic telangiectasia, a devastating disorder that leads to arteriovenous malformations. Here, we show that ALK1 controls endothelial cell polarization against the direction of blood flow and flow-induced endothelial migration from veins through capillaries into arterioles.

Methods: Using Cre lines that recombine in different subsets of arterial, capillary-venous, or endothelial tip cells, we show that capillary-venous Alk1 deletion was sufficient to induce arteriovenous malformation formation in the postnatal retina.

Results: ALK1 deletion impaired capillary-venous endothelial cell polarization against the direction of blood flow in vivo and in vitro. Mechanistically, ALK1-deficient cells exhibited increased integrin signaling interaction with vascular endothelial growth factor receptor 2, which enhanced downstream YAP/TAZ nuclear translocation. Pharmacologic inhibition of integrin or YAP/TAZ signaling rescued flow migration coupling and prevented vascular malformations in Alk1-deficient mice.

Conclusions: Our study reveals ALK1 as an essential driver of flow-induced endothelial cell migration and identifies loss of flow-migration coupling as a driver of arteriovenous malformation formation in hereditary hemorrhagic telangiectasia disease. Integrin-YAP/TAZ signaling blockers are new potential targets to prevent vascular malformations in patients with hereditary hemorrhagic telangiectasia.

Implications: ALK1 is activated by flow and required for EC polarization and migration against the direction of blood flow. This paper shows that loss of ALK1 elevates expression of integrins and Yap/Taz signaling. Inhibition of these pathways, using cilengitude or ATN161 for integrins or verteporfin for YAP/TAZ, suppressed vascular malformations in EC-specific *Alk1-/-* mice. These drug classes may therefore be of therapeutic use for patients with HHT.

Somatic Mutations in Vascular Malformations of Hereditary Hemorrhagic Telangiectasia Result in Bi-allelic Loss of ENG or ACVRL1

Snellings DA, Gallione CJ, Clark DS, Vozoris NT, Faughnan ME, Marchuk DA
Am J Hum Genet. 2019 Nov 7;105(5):894-906. doi: 10.1016/j.ajhg.2019.09.010. Epub 2019 Oct 17.PMID: 31630786

Hereditary hemorrhagic telangiectasia (HHT) is a Mendelian disease characterized by vascular malformations (VMs) including visceral arteriovenous malformations and mucosal telangiectasia. HHT is caused by loss-of-function (LoF) mutations in one of three genes, ENG, ACVRL1, or SMAD4, and is inherited as an autosomal-dominant condition. Intriguingly, the constitutional mutation causing HHT is present throughout the body, yet the multiple VMs in individuals with HHT occur focally, rather than manifesting as a systemic vascular defect. This disconnect between genotype and phenotype suggests that a local event is necessary for the development of VMs. We investigated the hypothesis that local somatic mutations seed the formation HHT-related telangiectasia in a genetic two-hit mechanism. We identified low-frequency somatic mutations in 9/19 telangiectasia through the use of next-generation sequencing. We established phase for seven of nine samples, which confirms that the germline and somatic mutations in all seven samples exist in trans configuration; this is consistent with a genetic two-hit mechanism. These combined data suggest that bi-allelic loss of ENG or ACVRL1 may be a required event in the development of telangiectasia, and that rather than haploinsufficiency, VMs in HHT are caused by a Knudsonian two-hit mechanism.

Implications: This study shows for the first time that acquired loss of function (LOF) mutations within the wildtype allele of the HHT locus can occur within vascular lesions. Using "deep sequencing" of gDNA from cutaneous telangiectases they found evidence for such an event in 9 of 19 samples analyzed from HHT1 and HHT2 patients. For technical reasons, they could not establish what percentage of ECs within the lesion showed such genetic events, nor could they detect cases of "Loss of Heterozygosity" whereby the entire wild type allele is lost. The findings have therapeutic implications since some drug design strategies aim to enhance expression of the wildtype allele of the causative gene. Since the wild type allele may be lost within lesional ECs, this strategy may be limited to prevention rather than cure.

Bone Marrow-Derived Alk1 Mutant Endothelial Cells and Clonally Expanded Somatic Alk1 Mutant Endothelial Cells Contribute to the Development of Brain Arteriovenous Malformations in Mice

Shaligram SS, Zhang R, Zhu W, Ma L, Luo M, Li Q, Weiss M, Arnold T, Santander N, Liang R, do Prado L, Tang C, Pan F, Oh SP, Pan P, Su H
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We have previously demonstrated that deletion of activin receptor-like kinase 1 (Alk1) or endoglin in a fraction of endothelial cells (ECs) induces brain arteriovenous malformations (bAVMs) in adult mice upon angiogenic stimulation. Here, we addressed three related questions: (1) could Alk1-mutant bone marrow (BM)-derived ECs (BMDECs) cause bAVMs? (2) are Alk1- ECs clonally expanded during bAVM development? and (3) are the number of mutant ECs correlated to bAVM severity? For the first question, we transplanted BM from PdgfbiCreER;Alk1$^{2f/2f}$ mice (EC-specific tamoxifen-inducible Cre with Alk1-floxed alleles) into wild-type mice, and then induced bAVMs by intra-brain injection of an adeno-associated viral vector expressing vascular endothelial growth factor and intra-peritoneal injection of tamoxifen. For the second question, clonal expansion was analyzed using PdgfbiCreER;Alk12f/2f; confetti+/- mice. For the third question, we titrated tamoxifen to limit Alk1 deletion and compared the severity of bAVM in mice treated with low and high tamoxifen doses. We found that wild-type mice with PdgfbiCreER;Alk1$^{2f/2f}$ BM developed bAVMs upon VEGF stimulation and Alk1 gene deletion in BMDECs. We also observed clusters of ECs expressing the same confetti color within bAVMs and significant proliferation of Alk1- ECs at early stage of bAVM development, suggesting that Alk1- ECs clonally expanded by local proliferation. Tamoxifen dose titration revealed a direct correlation between the number of Alk1- ECs and the burden of dysplastic vessels in bAVMs. These results provide novel insights for the understanding of the mechanism by which a small fraction of Alk1 or endoglin mutant ECs contribute to development of bAVMs.

Implications: This study shows that bone marrow-derived *Alk1-/-* ECs can contribute to VEGF/wounding-induced brain AVMs. The authors also used sophisticated lineage tracing tools to show that *Alk1-/-* ECs undergo clonal expansion and show higher proliferation than wildtype ECs at early but not later stages of AVM development. The authors suggest that *Alk1-/-* ECs support their own expansion through autocrine mechanisms as well as the expansion of neighboring wild type ECs through paracrine mechanisms with the implication that somatic loss of *Alk1* in a few cells may influence a larger field of cells to undergo AVM lesional expansion.

Overexpression of Activin Receptor-Like Kinase 1 in Endothelial Cells Suppresses Development of Arteriovenous Malformations in Mouse Models of Hereditary Hemorrhagic Telangiectasia

Hwan Kim Y, Vu PN, Choe SW, Jeon CJ, Arthur HM, Vary CPH, Lee YJ, Oh SP
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Rationale: Hereditary hemorrhagic telangiectasia (HHT) is a genetic disease caused by mutations in ENG, ALK1, or SMAD4. Since proteins from all 3 HHT genes are components of signal transduction of TGF- (transforming growth factor) family members, it has been hypothesized that HHT is a disease caused by defects in the ENG-ALK1-SMAD4 linear signaling. However, in vivo evidence supporting this hypothesis is scarce.

Objective: We tested this hypothesis and investigated the therapeutic effects and potential risks of induced-ALK1 or -ENG overexpression (OE) for HHT.

Methods and results: We generated a novel mouse allele (ROSA26Alk1) in which HA (human influenza hemagglutinin)-tagged ALK1 and bicistronic eGFP expression are induced by Cre activity. We examined whether ALK1-OE using the ROSA26Alk1 allele could suppress the development of arteriovenous malformations (AVMs) in wounded adult skin and developing retinas of Alk1- and Eng-inducible knockout (iKO) mice. We also used a similar approach to investigate whether ENG-OE could rescue AVMs. Biochemical and immunofluorescence analyses confirmed the Cre-dependent OE of the ALK1-HA transgene. We could not detect any pathological signs in ALK1-OE mice up to 3 months after induction. ALK1-OE prevented the development of retinal AVMs and wound-induced skin AVMs in Eng-iKO as well as Alk1-iKO mice. ALK1-OE normalized expression of SMAD and NOTCH target genes in ENG-deficient endothelial cells (ECs) and restored the effect of BMP9 (bone morphogenetic protein 9) on suppression of phosphor-AKT levels in these endothelial cells. On the other hand, ENG-OE could not inhibit the AVM development in Alk1-iKO models.

Conclusions: These data support the notion that ENG and ALK1 form a linear signaling pathway for the formation of a proper arteriovenous network during angiogenesis. We suggest that ALK1 OE or activation can be an effective therapeutic strategy for HHT. Further research is required to study whether this therapy could be translated into treatment for humans.

Implications: Since overexpression of wildtype ALK-1 can suppress AVM development in both *Alk1*- and *Eng*-inducible knockout (iKO), drugs that upregulate ALK-1 gene expression may be protective against AVMs in both HHT1 and HHT2. Conversely, overexpression of endoglin could not suppress AVMs in *Alk1*-iKO mice, suggesting that drugs that increase expression of endoglin although useful for HHT1 therapy, may not be useful in HHT2. The data suggest a linear signaling pathway of BMP9-Endoglin-ALK1-Smad4 in mice. However, in humans the pathway may be more complex because ALK-1 and SMADs induce ENG expression, and the two genes, *ENG* and *ALK-1*, show differential expression in different EC types.

Arterial endoglin does not protect against arteriovenous malformations

Singh E, Redgrave RE, Phillips HM, Arthur HM
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Introduction: Endoglin (ENG) forms a receptor complex with ALK1 in endothelial cells (ECs) to promote BMP9/10 signalling. Loss of function mutations in either ENG or ALK1 genes lead to the inherited vascular disorder hereditary haemorrhagic telangiectasia (HHT), characterised by arteriovenous malformations (AVMs). However, the vessel-specific role of ENG and ALK1 proteins in protecting against AVMs is unclear. For example, AVMs have been described to initiate in arterioles, whereas ENG is predominantly expressed in venous ECs. To investigate whether ENG has any arterial involvement in protecting against AVM formation, we specifically depleted the Eng gene in venous and capillary endothelium whilst maintaining arterial expression and investigated how this affected the incidence and location of AVMs in comparison with pan-endothelial Eng knockdown.

Methods: Using the mouse neonatal retinal model of angiogenesis, we first established the earliest time point at which Apj-Cre-ERT2 activity was present in venous and capillary ECs but absent from arterial ECs. We then compared the incidence of AVMs following pan-endothelial or venous/capillary-specific ENG knockout.

Results: Activation of Apj-Cre-ERT2 with tamoxifen from postnatal day (P) 5 ensured preservation of arterial ENG protein expression. Specific loss of ENG expression in ECs of veins and capillaries led to retinal AVMs at a similar frequency to pan-endothelial loss of ENG. AVMs occurred in the proximal as well as the distal part of the retina consistent with a defect in vascular remodelling during maturation of the vasculature.

Conclusion: Expression of ENG is not required in arterial ECs to protect against AVM formation.

Implications: This team shows that despite retention of endoglin expression in arterial ECs, *Eng-/-* targeted to venous and capillary ECs cause AVMs at the same rate and position as those formed in pan-EC *Eng-/-* mice. This implies that the target cells in AVM formation of neonatal mouse retina are venous and/or capillary rather than arterial. Further studies generating arterial EC-specific *Eng-/-* would shed light on whether Eng plays any role in arterial EC biology.

Loss of Endothelial Endoglin Promotes High-Output Heart Failure Through Peripheral Arteriovenous Shunting Driven by VEGF Signaling

Tual-Chalot S, Garcia-Collado M, Redgrave RE, Singh E, Davison B, Park C, Lin H, Luli S, Jin Y, Wang Y, Lawrie A, Jakobsson L, Arthur HM
Circ Res. 2020 Jan 17;126(2):243-257. doi: 10.1161/CIRCRESAHA.119.315974. Epub 2019 Dec 6.PMID: 31805812

Rationale: ENG (endoglin) is a coreceptor for BMP (bone morphogenetic protein) 9/10 and is strongly expressed in endothelial cells. Mutations in ENG lead to the inherited vascular disorder hereditary hemorrhagic telangiectasia characterized by local telangiectases and larger arteriovenous malformations (AVMs); but how ENG functions to regulate the adult vasculature is not understood.

Objective: The goal of the work was to determine how ENG maintains vessel caliber in adult life to prevent AVM formation and thereby protect heart function.

Methods and results: Genetic depletion of endothelial Eng in adult mice led to a significant reduction in mean aortic blood pressure. There was no evidence of hemorrhage, anemia, or AVMs in major organs to explain the reduced aortic pressure. However, large AVMs developed in the peripheral vasculature intimately associated with the pelvic cartilaginous symphysis-a noncapsulated cartilage with a naturally high endogenous expression of VEGF (vascular endothelial growth factor). The increased blood flow through these peripheral AVMs explained the drop in aortic blood pressure and led to increased cardiac preload, and high stroke volumes, ultimately resulting in high-output heart failure. Development of pelvic AVMs in this region of high VEGF expression occurred because loss of ENG in endothelial cells leads to increased sensitivity to VEGF and a hyperproliferative response. Development of AVMs and associated progression to high-output heart failure in the absence of endothelial ENG was attenuated by targeting VEGF signaling with an anti-VEGFR2 (VEGF receptor 2) antibody.

Conclusions: ENG promotes the normal balance of VEGF signaling in quiescent endothelial cells to maintain vessel caliber-an essential function in conditions of increased VEGF expression such as local hypoxia or inflammation. In the absence of endothelial ENG, increased sensitivity to VEGF drives abnormal endothelial proliferation in local regions of high VEGF expression, leading to AVM formation and a rapid injurious impact on heart function.

Implications: This study shows, for the first time, that endoglin is required for maintenance of vascular architecture in the adult using an EC-specific *Eng-/-* mouse model. Pan-EC *Eng-/-* induced in adult mice leads to rapid development of large AVMs, albeit limited to the pelvic region where VEGF levels are high. These AVMs reduced vascular resistance, reduced blood pressure, increased cardiac filling, ultimately causing High Output Heart Failure. The study provides a new mouse model to study and treat adult-onset AVM and HOHF.

BMP10-mediated ALK1 signaling is continuously required for vascular development and maintenance

Capasso TL, Li B, Volek HJ, Khalid W, Rochon ER, Anbalagan A, Herdman C, Yost HJ, Villanueva FS, Kim K, Roman BL
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Hereditary hemorrhagic telangiectasia (HHT) is an autosomal-dominant vascular disorder characterized by development of high-flow arteriovenous malformations (AVMs) that can lead to stroke or high-output heart failure. HHT2 is caused by heterozygous mutations in ACVRL1, which encodes an endothelial cell bone morphogenetic protein (BMP) receptor, ALK1. BMP9 and BMP10 are established ALK1 ligands. However, the unique and overlapping roles of these ligands remain poorly understood. To define the physiologically relevant ALK1 ligand(s) required for vascular development and maintenance, we generated zebrafish harboring mutations in bmp9 and duplicate bmp10 paralogs, bmp10 and bmp10-like. bmp9 mutants survive to adulthood with no overt phenotype. In contrast, combined loss of bmp10 and bmp10-like results in embryonic lethal cranial AVMs indistinguishable from acvrl1 mutants. However, despite embryonic functional redundancy of bmp10 and bmp10-like, bmp10 encodes the only required Alk1 ligand in the juvenile-to-adult period. bmp10 mutants exhibit blood vessel abnormalities in anterior skin and liver, heart dysmorphology, and premature death, and vascular defects correlate with increased cardiac output. Together, our findings support a unique role for BMP10 as a non-redundant Alk1 ligand required to maintain the post-embryonic vasculature and establish zebrafish bmp10 mutants as a model for AVM-associated high-output heart failure, which is an increasingly recognized complication of severe liver involvement in HHT2.

Implications: During Zebrafish embryogenesis, *bmp9* is not required, and *bmp10* and *bmp10-like* are redundant in supporting cranial vascular development. In adult Zebrafish, *bmp9* and bmp10-like are not required, but *bmp10* mutants phenocopy mutant acvrl1 and human HHT. There is variable age of onset and expressivity of vascular defects, and development of High Output Heart Failure which is a complication in aging HHT patients. The Zebrafish *bmp10* mutant provides a model for study of HOHF.

Different cardiovascular and pulmonary phenotypes for single- and double-knock-out mice deficient in BMP9 and BMP10

Bouvard C, Tu L, Rossi M, Desroches-Castan A, Berrebeh N, Helfer E, Roelants C, Liu H, Ouarne M, Chaumontel N, Mallet C, Battail C, Bikfalvi A, Humbert M, Savale L, Daubon T, Perret P, Tillet E, Guignabert C, Bailly S
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Aims: *BMP9 and BMP10 mutations were recently identified in patients with pulmonary arterial hypertension (PAH), but their specific roles in the pathogenesis of the disease are still unclear. We aimed to study the roles of BMP9 and BMP10 in cardiovascular homeostasis and pulmonary hypertension using transgenic mouse models deficient in Bmp9 and/or Bmp10.*

Methods and results: *Single- and double-knockout mice for Bmp9 (constitutive) and/or Bmp10 (tamoxifen inducible) were generated. Single-KO mice developed no obvious age-dependent phenotype when compared with their wild-type littermates. However, combined deficiency in Bmp9 and Bmp10 led to vascular defects resulting in a decrease in peripheral vascular resistance and blood pressure and the progressive development of high-output heart failure (HOHF) and pulmonary hemosiderosis. RNAseq analysis of the lungs of the double-KO mice revealed differential expression of genes involved in inflammation and vascular homeostasis. We next challenged these mice to chronic hypoxia. After three weeks of hypoxic exposure, Bmp10-cKO mice showed an enlarged heart. However, although genetic deletion of Bmp9 in the single and double-KO mice attenuated the muscularization of pulmonary arterioles induced by chronic hypoxia, we observed no differences in Bmp10-cKO mice. Consistent with these results, endothelin-1 levels were significantly reduced in Bmp9 deficient mice but not Bmp10-cKO mice. Furthermore, the effects of BMP9 on vasoconstriction were inhibited by bosentan, an endothelin receptor antagonist, in a chick chorioallantoic membrane assay.*

Conclusions: *Our data show redundant roles for BMP9 and BMP10 in cardiovascular homeostasis under normoxic conditions (only combined deletion of both Bmp9 and Bmp10 was associated with severe defects) but highlight specific roles under chronic hypoxic conditions. We obtained evidence that BMP9 contributes to chronic hypoxia-induced pulmonary vascular remodeling, whereas BMP10 plays a role in hypoxia-induced cardiac remodeling in mice.*

Implications: Monogenic loss of *Bmp9* or *Bmp10* in adult mice has little effect on the cardiovascular system under normoxic conditions, but genetic loss of both genes leads to vasodilation and HOHF. Although mutations in human *BMP9* and *BMP10* predispose to PAH, this paper shows that loss of *Bmp9* in mice paradoxically protects from hypoxia-induced pulmonary hypertension by reducing pulmonary vascular muscularization. Translating findings from mouse to human can therefore be challenging due to the complexity of BMP molecular signaling pathways in different cell types. Further investigation of differences between mouse and human may provide insight into the molecular mechanisms regulating HHT and PAH.

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- **Registration will open June 1, 2022**

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Capt. Robyn Bent (RN, MS) – Director, Patient Focused Drug Development Program, Center for Drug Evaluation and Research (CDER), FDA

Nicholas Benedict – Chief Executive Officer, VADERIS Therapeutics AG

Dr. David Liu (PhD) – Director, Merkin Institute of Transformative Technologies in Healthcare, Harvard University

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