Volume 1 | Number 1

HHT FRONTLINES

BRINGING SCIENCE FROM BENCH TO BEDSIDE

CLINICAL RESEARCH

Welcome to the first 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.

The first two abstracts of this issue were published "back to back" in the same journal. The Cure HHT article is listed first because we know so many of you were involved. Thank you and we appreciate your feedback!

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EFFECT OF TOPICAL INTRANASAL THERAPY ON EPISTAXIS FREQUENCY IN PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA: A RANDOMIZED CLINICAL TRIAL.

Whitehead KJ, Sautter NB, McWilliams JP, Chakinala MM, Merlo CA, Johnson MH, James M, Everett EM, Clancy MS, Faughnan ME, Oh SP, Olitsky SE, Pyeritz RE, Gossage JR. JAMA 2016; 316(9):943-51.

The authors presented one of the two major studies that evaluated topical bevacizumab in HHT (see also Dupuis-Girod et al, JAMA 2106; 316(9); 934-42.

Objective: To determine whether topical therapy with any of 3 drugs with differing mechanisms of action was effective in reducing HHT-related epistaxis.

This North American Study of Epistaxis in HHT was a double-blind, placebo-controlled randomized clinical trial performed at 6 HHT centers of excellence. From August 2011 through March 2014, there were 121 adult patients who met the clinical criteria for HHT and had experienced HHT-related epistaxis with an Epistaxis Severity Score of at least 3.0.

Follow-up was completed in September 2014: Patients received twice-daily nose sprays for 12 weeks with either bevacizumab 1% (4 mg/d), estriol 0.1% (0.4 mg/d), tranexamic acid 10% (40 mg/d), or placebo (0.9% saline).

Main Outcomes: Median weekly epistaxis frequency during weeks 5 through 12. Secondary outcomes included median duration of epistaxis during weeks 5 through 12, Epistaxis Severity Score, level of hemoglobin, level of ferritin, need for transfusion, emergency department visits, and treatment failure.

Results: Among the 121 patients who were randomized (mean age, 52.8 years [SD, 12.9 years]; 44% women with a median of 7.0 weekly episodes of epistaxis [interquartile range {IQR}, 3.0-14.0]), 106 patients completed the study duration for the primary outcome measure (43 were women [41%]). Drug therapy did not significantly reduce epistaxis frequency (P = .97). After 12 weeks of treatment, the median weekly number of bleeding episodes was 7.0 (IQR, 4.5-10.5) for patients in the bevacizumab group, 8.0 (IQR, 4.0-12.0) for the estriol group, 7.5 (IQR, 3.0-11.0) for the tranexamic acid group, and 8.0 (IQR, 3.0-14.0) for the placebo group. No drug treatment was significantly different from placebo for epistaxis duration. All groups had a significant improvement in Epistaxis Severity Score at weeks 12 and 24. There were no significant differences between groups for hemoglobin level, ferritin level, treatment failure, need for transfusion, or emergency department visits.

Implications: Among patients with HHT, there were no significant between-group differences in the use of topical intranasal treatment with bevacizumab vs estriol vs tranexamic acid vs placebo and epistaxis frequency. This means the active treatments were not shown to be any more effective at reducing HHT nosebleeds than the saline (placebo) spray.

EFFECT OF TOPICAL INTRANASAL THERAPY ON EPISTAXIS FREQUENCY IN PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA: A RANDOMIZED CLINICAL TRIAL.

Dupuis-Girod S, Ambrun A, Decullier E, Fargeton AE, Roux A, Bréant V, Colombet B, Rivière S, Cartier C, Lacombe P, Chinet T, Blivet S, Blondel JH, Gilbert-Dussardier B, Dufour X, Michel J, Harle JR, Dessi P, Faure F. JAMA 2016; 316(9):934-42.

The authors presented one of the two major studies that evaluated topical bevacizumab in HHT (see also Whitehead et al, JAMA 2106; 316(9); 943-51.

Objective: To evaluate the efficacy of 3 different doses of bevacizumab administered as a nasal spray in a repeated manner for the duration of nosebleeds in patients with HHT.

Design and Participants: A randomized, multicenter, placebo-controlled, phase 2/3 clinical trial with dose selection at an intermediate analysis and prespecified stopping rules (non-binding stopping for futility) was conducted. Patients aged 18 years or older with a diagnosis of HHT were recruited from 5 French centers from April 2014 to January 2015 with a 6-month follow-up after the end of treatment. Participants had a history of self-reported nosebleeds with a monthly duration of more than 20 minutes in at least the 3 months prior to inclusion corroborated by epistaxis grids completed during the same preinclusion period.

Eighty consecutive HHT patients were randomized and treated in the phase 2 study, with 4 parallel groups in a 1:1:1:1 ratio. One group received placebo (n = 21); the other 3 received bevacizumab nasal spray. Each bevacizumab group received a different dose of the drug (25 mg [n = 20], 50 mg [n = 20], or 75 mg [n = 19] per treatment) in 3 doses 14 days apart for a total treatment duration of 4 weeks, resulting in a total dose of 75 mg, 150 mg, and 225 mg in each treatment group. Main outcomes: mean monthly epistaxis duration for 3 consecutive months immediately after the end of the treatment.

Results: of the 80 patients who were randomized (mean age, 60.47 [SD, 10.61] years; 37 women [46.25%]), 75 completed the study. Mean monthly epistaxis duration measured at 3 months was not significantly different in the 59 patients receiving bevacizumab in comparison with the placebo group (P = .57) or between the bevacizumab groups. The mean monthly epistaxis duration was 259.2 minutes (95% CI, 82.1-436.3 minutes) in the 25-mg group, 244.0 minutes (95% CI, 81.8-406.2 minutes) in the 50-mg group, 215.0 minutes (95% CI, 102.8-327.2 minutes) in the 75-mg group, and 200.4 minutes (95% CI, 109.3-291.5 minutes) in the placebo group. Toxicity was low and no severe adverse events were reported. This study was terminated prior to phase 3 for treatment futility after interim analysis on the recommendations of an independent data monitoring committee.

Implications: Among patients with HHT, there were no significant between-group differences in the use of topical intranasal treatment with bevacizumab vs estriol vs tranexamic acid vs placebo and epistaxis frequency. This means the active treatments were not shown to be any more effective at reducing HHT nosebleeds than the saline (placebo) spray.

FOLLOW-UP OF THALIDOMIDE TREATMENT IN PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA

A.E. Hosman, C.J.J. Westermann, R. Snijder, F. Disch, C.L. Mummery, J.J. Mager Rhinology 2015; 53:340-344.

The authors of this article performed a long term follow up of HHT patients treated with Thalidomide.

Patients with a hereditary vascular disorder called Rendu-Osler-Weber syndrome (Hereditary Hemorrhagic Telangiectasia, HHT) hemorrhage easily due to weak-walled vessels. Hemorrhage in lungs or brain can be fatal but patients suffer most from chronic and prolonged nosebleeds (epistaxis), the frequency and intensity of which increases with age. Several years ago, it was discovered serendipitously that the drug Thalidomide had beneficial effects on the disease symptoms in several of a small group of HHT patients: epistaxis and the incidence of anemia were reduced and patients required fewer blood transfusions. In addition, they reported a better quality of life. However, Thalidomide has significant negative side effects, including neuropathy and fatigue.

The authors followed up all HHT patients in the Netherlands who had been taking Thalidomide at the time the original study was completed to find out (i) how many had continued taking Thalidomide and for how long (ii) the nature and severity of any side-effects and (iii) whether side-effects had influenced their decision to continue taking Thalidomide.

Results: Only a minority of patients had continued taking the drug despite its beneficial effects on their symptoms and that the side effects were the primary reason to stop.

Implications: Despite symptom reduction, side effects meant patients tended to stop using Thalidomide. As a result, a large-scale clinical trial is not justified, although individual trial of treatment in the most severely affected patients may be considered.

BEVACIZUMAB TO TREAT CHOLANGIOPATHY IN HEREDITARY HEMORRHAGIC TELANGIECTASIA: BE CAUTIOUS: A CASE REPORT.

Maestraggi Q, Bouattour M, Toquet S, Jaussaud R, Kianmanesh R, Durand F, Servettaz A. Medicine (Baltimore) 2015 Nov;94(46).

The authors of this article described what happened to one HHT patient.

Objective: To determine whether topical therapy with any of 3 drugs with differing mechanisms of action was effective in reducing HHT-related epistaxis.

Hereditary Hemorrhagic Telangiectasia (HHT) is an inherited vascular dysplasia characterized by mucocutaneous telangiectasia and visceral arteriovenous malformations. Hepatic involvement with vascular malformations may lead to portal hypertension, biliary ischemia, and high-output cardiac failure. There is no curative treatment for the disease. Liver transplantation is indicated for life-threatening complications, but it carries significant risk due to surgery and immunosuppressive treatment. Some case reports or small open studies suggest that bevacizumab, a recombinant humanized anti-VEGF monoclonal antibody, should be efficient in limiting bleeding and in reducing liver disease in HHT. The authors report a case of a 63-year-old woman with HHT presenting with ischemic cholangiopathy. Liver transplant was indicated, but given a previous encouraging report showing a regression of biliary disease with bevacizumab in 3 patients with HHT this drug was proposed. No significant efficacy but a severe adverse effect was observed after 3 months: bilateral pulmonary embolisms, thrombosis in the right atrial cavity, and thrombosis of the right hepatic vein were evidenced. Bevacizumab was stopped; anticoagulant started. Four months later, the patient received a transplanted liver. She feels well 1 year later. This case report intends to provide the information for clinicians to consider the use of bevacizumab in HHT. Whereas several uncontrolled series and case reports have suggested the efficacy of this drug in reducing bleeding and liver disease, no severe side effects were mentioned to date. For the first time in HHT the authors report a life-threatening side effect of this drug and no efficacy. Moreover, systemic thrombosis, the observed complication, may preclude transplantation.

Implications: To date, caution is still essential when considering the use of systemic bevacizumab in HHT.

CAN IRON TREATMENTS AGGRAVATE EPISTAXIS IN SOME PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA?

Shovlin CL, Gilson C, Busbridge M, Patel D, Shi C, Dina R, Abdulla FN, Awan I. Laryngoscope 2016, Apr 23.

The authors of this article examined if there was evidence for iron treatments precipitating nosebleeds (epistaxis) in patients with Hereditary Hemorrhagic Telangiectasia (HHT), as suggested to them anecdotally within their clinical practice.

Design and Methods: Survey evaluation of HHT patients, and a randomized control trial in healthy volunteers. Nosebleed severity in response to iron treatments and standard investigations were evaluated by unbiased surveys in patients with HHT. Serial blood samples from a randomized controlled trial of 18 healthy volunteers were used to examine responses to a single iron tablet (ferrous sulfate, 200 mg).

Results: Iron tablet users were more likely to have daily nosebleeds than non-iron-users as adults, but there was no difference in the proportions reporting childhood or trauma-induced nosebleeds. Although iron and blood transfusions were commonly reported to improve nosebleeds, 35 of 732 (4.8%) iron tablet users, in addition to 17 of 261 (6.5%) iron infusion users, reported that their nosebleeds were exacerbated by the respective treatments. These rates were significantly higher than those reported for control investigations. Serum iron rose sharply in four of the volunteers ingesting ferrous sulfate (by 19.3-33.1 mol/L in 2 hours), but not in 12 dietary controls (2-hour iron increment ranged from -2.2 to +5.0 mol/L). High iron absorbers demonstrated greater increments in serum ferritin at 48 hours, but transient rises in circulating endothelial cells, an accepted marker of endothelial damage.

Implications: While iron supplementation is essential to treat or prevent iron deficiency, in a small subgroup of individuals, rapid changes in serum iron may provoke endothelial changes and hemorrhage. Similar results were obtained in a second survey (Patel et al, ERJ Open Res 2016), supported by laboratory data (Mollet et al, Pllos One 2016). The authors recommended possible recognition that iron treatments may exacerbate nosebleeds and identification of the "1 in 20" HHT patients to whom this applies as they may need modifications of iron treatments to reduce precipitating nosebleeds.

REPORTED CARDIAC PHENOTYPES IN HEREDITARY HEMORRHAGIC TELANGIECTASIA EMPHASIZE BURDENS FROM ARRHYTHMIAS, ANEMIA AND ITS TREATMENTS, BUT SUGGEST REDUCED RATES OF MYOCARDIAL INFARCTION.

Shovlin CL, Awan I, Cahilog Z, Abdulla FN, Guttmacher AE. Int J Cardiol 2016; 215:179-85.

The authors of this article examined if there was evidence for protection from myocardial infarction (heart attacks) in HHT, based on published good life-expectancy figures for treated HHT populations (Kjeldsen et al, J Intern Med 1999).

Cardiac phenotypes should be pronounced in HHT due to frequent systemic arteriovenous malformations (AVMs), iron deficiency anemia, hypoxemia, hyperdynamic circulations, venous thromboemboli, and paradoxical emboli through pulmonary AVMs.

Results: In an international survey, 1025 respondents (median age 55years) met HHT diagnostic criteria: 942 (91.9%) reported nose-bleeds, 452 (44.1%) at least daily. AVMs were commonly reported in pulmonary (544, 53%), hepatic (194, 18.9%) and/or cerebral (92, 9.0%) circulations. 770/1025 (75%) had used iron tablets, 256 (25.0%) intravenous iron, and 374 (36.5%) received blood trans-fusions. Arrhythmias were reported by 113/1025 (11%, including 44 (4.3%) with atrial fibrillation), angina by 36 (3.5%), and cardiac failure by 26 (2.5%). In multivariate logistic regression, these phenotypes were associated with hepatic AVMs/pulmonary hypertension (relatively interchangeable variables), blood transfusions, and intravenous iron. Cardiac insufficiency/failure often provokes intensive anemia treatments, but associations with arrhythmias, particularly with a greater transfusion burden, were less easy to explain.
Myocardial infarction (23/1025; 2.2%), and abnormal coronary angiogram (≤31/76, ≤54%) rates appeared low. Provocative preliminary data were obtained including HHT-affected respondents' parents and grandparents in whom HHT could be confidently assigned, or excluded based on autosomal dominant inheritance patterns: in crude and survival analyses, myocardial infarctions were reported less frequently for individuals with HHT, particularly for males (p=0.001).

Implications: Arrhythmias are the most common cardiac phenotype in HHT, and likely to be aggravated by iron deficiency anemia, its treatments, and/or high output states due to AVMs. Myocardial infarction rates may be reduced in this apparently high risk population.

LIFE EXPECTANCY OF PARENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA

de Gussem EM, Edwards CP, Hosman AE, Westermann CJ, Snijder RJ, Faughnan ME, Mager JJ. Orphanet J Rare Dis. 2016; 11:46

The authors of this article investigated life-expectancies of parents of HHT patients compared with their non-HHT partners.

The study used self- or telephone-administered questionnaires sent to patients extracted from the databases of 2 participating HHT Centres: the Toronto HHT Database (Toronto, Canada) and the St. Antonius Hospital HHT Database (Nieuwegein, The Netherlands). The study examined the life expectancies of their parents.

Results: 225/407 (55%) of respondents were included creating parental HHT- (n = 225) and control groups (n = 225) of equal size. Two hundred thirteen/225 (95%) of the HHT group (parents) had not been screened for organ involvement of the disease prior to death. The life expectancy in parents with HHT was slightly lower compared to parents without (median age at death 73.3 years in patients versus 76.6 years in controls, p0.018), comparable with the findings of Donaldson et al (Neurology May 5, 2015). Parents with ACVRL 1 mutations had normal life expectancies, whereas parents with Endoglin mutations died 7.1 years earlier than controls (p = 0.024). Women with Endoglin mutations lived a median of 9.3 years shorter than those without (p = 0.04). Seven/123 (5%) of deaths were HHT related with a median age at death of 61.5 years (IQ range 54.4-67.7 years

Implications: Life expectancy in a largely unscreened population with HHT is worse than in their non-HHT partners, more specifically for patients with Endoglin mutations and especially women. Because patients with ACVRL1 mutations have a normal life expectancy, the reduction in life expectancy in patients with an Endoglin mutation is probably related to complications of untreated pulmonary AVMs and cerebral VMs. The authors propose that life expectancy in HHT can be normal if patients are screened and both pulmonary AVMs and cerebral VMs are properly treated in a timely way. To prevent complications of HHT, referral of patients with (suspected) HHT to an HHT Centre of Excellence for screening, and if necessary treatment, is highly recommended.

LOWER RISK OF INTRACRANIAL ARTERIOVENOUS MALFORMATION HEMORRHAGE IN PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA.

Yang W, Liu A, Hung AL, Braileanu M, Wang JY, Caplan JM, Colby GP, Coon AL, Tamargo RJ, Ahn ES, Huang J. Neurosurgery 2016; 78(5):684-93.

Patients diagnosed with Hereditary Hemorrhagic Telangiectasia (HHT) are at risk of developing intracranial arteriovenous malformations (AVM). However, the clinical manifestations and natural history of HHT-related AVMs remain unclear due to the rarity of these lesions.

Patients diagnosed with hereditary hemorrhagic telangiectasia (HHT) are at risk of developing intracranial arteriovenous malformations (AVM). However, the clinical manifestations and natural history of HHT-related AVMs remain unclear due to the rarity of these lesions. The objective of the study was to clarify the clinical characteristics and hemorrhagic risk in HHT-related AVMs. The authors performed a retrospective review of all patients diagnosed with both HHT and intracranial AVMs who were evaluated at their institution from 1990 to 2013. Patients with missing data or lost to follow-up were excluded. Baseline characteristics and subsequent hemorrhagic risk were evaluated.

Results: in an AVM database of 531 patients with 542 AVMs, a total of 12 HHT patients (2.3%) with 23 AVMs were found. Mean age at diagnosis was 36.5 years, with 41.7% male. Compared to patients with sporadic AVMs, patients with HHT were less likely to present with ruptured AVM (P = .04), headaches (P = .02), and seizures (P = .02), and presented with better modified Rankin scores (P , .01). HHT-related AVMs were smaller in size (P , .01), of lower Spetzler-Martin grade (P = .01), and had less temporal lobe involvement (P = .02) compared to sporadic AVMs. Six HHT patients (50.0%) were found with multiple intracranial AVMs. One hemorrhage was found during an observation period of 149.6 patient-years and 297.5 lesion-years, translating to 1.3% per patient per year or 0.7% per AVM per year.

Implications: HHT-related AVMs are smaller in size with lower Spetzler-Martin grade and less temporal lobe involvement than sporadic AVMs. Patients with HHT frequentlypresent with multiple intracranial AVMs. Conservative management is generally recommended due to lesion multiplicity and relatively low hemorrhagic risk.

EFFECT OF CENTER VOLUME ON OUTCOMES IN HOSPITALIZED PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA

Vivek N. Iyer, MD, MPH; Waleed Brinjikji, MD; Bibek S. Pannu, MBBS; Dinesh R. Apala, MBBS; Giuseppe Lanzino, MD; Harry J. Cloft, MD, PhD; Sanjay Misra, MD; Michael J. Krowka, MD; Christopher P. Wood, MD; and Karen L. Swanson, MD Mayo Clin Proc 2016 Dec; 91(12):1753-1760 Epub 2016 Nov 01

Objective: To determine whether hospitalized patients with hereditary hemorrhagic telangiectasia (HHT) had better outcomes at high-volume treatment centers (HVCs).

Patients and Methods: The Nationwide Inpatient Sample (2000-2011) was used to identify HHT-related hospitalizations. Hospitals were classified based on quartiles of annual HHT discharge volume. The 75th percentile cutoff value (third quartile) was used to classify hospitals as low-volume centers (1-7 HHT discharges per year) or as HVCs (!8 discharges per year. Demographic features, complication rates, and outcomes were compared between the 2 groups.

Results: We identified 9440 hospital discharges in patients with HHT. Of these patients, 6856 (72.6%) were admitted to low-volume centers and 2584 (27.4%) to HVCs. The former were more likely to be of white race, older, and with higher income levels (P<.001 for each). The HVCs had higher rates of anemia, epistaxis, congestive heart failure, pulmonary hypertension, and cerebral and pulmonary arteriovenous malformations and lower rates of ischemic stroke and myocardial infarction. After adjusting for baseline differences in a multivariate model, patients treated at HVCs were more likely to be discharged home (odds ratio [OR]1/41.35; 95% CI, 1.21-1.52; P<.001) and less likely to be discharged to short-term rehabilitation facilities (OR1/40.45; 95% CI, 0.31-0.64; P<.001). Patients treated at HVCs also had a significantly lower risk of in-hospital mortality (OR1/40.51; 95% CI, 0.34-0.74; P<.001).

Implications: HHT patients treated at high volume centers (those seeing large numbers of HHT patients) experienced better outcomes with lower in-hospital mortality and higher rates of discharge to home (rather than short term rehabilitation facilities). These improved outcomes occurred despite the fact that HHT patients treated at high volume centers were sicker with higher rates of co-morbidities. Data from this study strongly supports the expansion of HHT centers of excellence worldwide.

PI3 KINASE INHIBITION IMPROVES VASCULAR MALFORMATIONS IN MOUSE MODELS OF HEREDITARY HEMORRHAGIC TELANGIECTASIA TYPE 2

Roxana Ola, Alexandre Dubrac, Jinah Han, Jennifer S Fang, Feng Zhang, Bruno, Larrivée, Monica Lee, Ana Angulo Urarte, Gael Genet, Karen K Hirschi, William C.Sessa, Francesc Vinals Canals, Mariona Graupera, Minhong Yan, Lawrence H Young, S. Paul Oh, Anne Eichmann Nature Communications 2016 nov 29; Article no. 13650

Activin-receptor like kinase 1 (ALK1) is an endothelial serine-threonine kinase receptor for bone morphogenetic proteins (BMP) 9 and 10. Inactivating mutations in the ALK1 gene cause hereditary hemorrhagic telangiectasia type 2 (HHT2), a disabling inherited disease characterized by excessive angiogenesis with arterial-venous malformations (AVMs) that are prone to bleeding. Here we show that inducible, endothelial specific homozygous Alk1 inactivation and BMP9/10 ligand blockade both lead to excessive angiogenesis and AVM formation in postnatal retinal vessels in mice. VEGF and PI3K/AKT signaling were increased after Alk1 deletion. Genetic deletion of the signal transducing Vegfr2 receptor prevented angiogenesis but did not revert AVM formation. In contrast, pharmacological PI3K inhibition efficiently prevented AVM formation, and reverted established AVMs. Thus, Alk1 deletion leads to increased endothelial PI3K pathway activation that may be a novel target for the treatment of vascular lesions in HHT2.

Implications: PI3K/AKT signaling were increased after Alk1 deletion. They also showed that pharmacological PI3K inhibition efficiently prevented AVM formation and reverted established AVMs, indicating that PI3K/AKT inhibition could be a novel therapy for HHT. Many PI3K/AKT/mTOR inhibitors have been developed for treating cancers. Some of these can be tested for HHT patients.

DEFECTIVE FLUID SHEAR STRESS MECHANOTRANSDUCTION MEDIATES HEREDITARY HEMORRHAGIC TELANGIECTASIA.

Baeyens N, Larrivée B, Ola R, Hayward-Piatkowskyi B, Dubrac A, Huang B, Ross TD, Coon BG, Min E, Tsarfati M, Tong H, Eichmann A, Schwartz MA.
J Cell Biol. 2016 Sep 26;214(7):807-16

Morphogenesis of the vascular system is strongly modulated by mechanical forces from blood flow. Hereditary hemorrhagic telangiectasia (HHT) is an inherited autosomal-dominant disease in which arteriovenous malformations and telangiectasias accumulate with age. Most cases are linked to heterozygous mutations in Alk1 or Endoglin, receptors for bone morphogenetic proteins (BMPs) 9 and 10. Evidence suggests that a second hit resultsin clonal expansion of endothelial cells to form lesions with poor mural cell coverage that spontaneously rupture and bleed. We now report that fluid shear stress potentiates BMPs to activate Alk1 signaling, which correlates with enhanced association of Alk1 and endoglin. Alk1 is required for BMP9 and flow responses, whereas endoglin is only required for enhancement by flow. This pathway mediates both inhibition of endothelial proliferation and recruitment of mural cells; thus, its loss blocks flow-induced vascular stabilization. Identification of Alk1 signaling as a convergence point for flow and soluble ligands provides a molecular mechanism for development of HHT lesions.

Implications: Fluid shear stress makes BMP9 signal more effective in activating ALK1 signaling. BMP9 signaling inhibits proliferation of endothelial cells and stabilizes blood vessels. When ALK1 is deficient, this flow-induced BMP9-ALK1 signaling is impaired, and causes more proliferation of endothelial cells and destabilization of blood vessels, which may be associated with AVM formations.

ALK1 CONTROLS ARTERIAL ENDOTHELIAL CELL MIGRATION IN LUMENIZED VESSELS.

Rochon ER, Menon PG, Roman BL
Development. 2016 Jul 15;143(14):2593-602.

Heterozygous loss of the arterial-specific TGF type I receptor, activin receptor-like kinase 1 (ALK1; ACVRL1), causes hereditary hemorrhagic telangiectasia (HHT). HHT is characterized by development of fragile, direct connections between arteries and veins, or arteriovenous malformations (AVMs). However, how decreased ALK1 signaling leads to AVMs is unknown. To understand the cellular mis-steps that cause AVMs, we assessed endothelial cell behavior in alk1-deficient zebrafish embryos, which develop cranial AVMs.

Our data demonstrate that alk1 loss has no effect on arterial endothelial cell proliferation but alters arterial endothelial cell migration within lumenized vessels. In wild-type embryos, alk1-positive cranial arterial endothelial cells generally migrate towards the heart, against the direction of blood flow, with some cells incorporating into endocardium. In alk1-deficient embryos, migration against flow is dampened and migration in the direction of flow is enhanced. Altered migration results in decreased endothelial cell number in arterial segments proximal to the heart and increased endothelial cell number in arterial segments distal to the heart. We speculate that the consequent increase in distal arterial caliber and hemodynamic load precipitates the flow-dependent development of downstream AVMs.

Implications: The authors used zebrafish HHT model. In normal condition, endothelial cells in arteries migrate against the direction of blood flow. However when ALK1 is deficient, this migration of endothelial cells against the flow is impaired. Migration of ALK1-deficient endothelial cells in the direction of flow results in increased endothelial cell number and caliber at the distal segments of arteries. The authors

speculate this migration defect is a key event in AVM formation. If we understand how ALK1 signaling is involved in migration of endothelial cells against the flow, then it would bring new therapeutic targets for HHT.

INTERACTION BETWEEN ALK1 SIGNALING AND CONNEXIN40 IN THE DEVELOPMENT OF ARTERIOVENOUS MALFORMATIONS.

Gkatzis K, Thalgott J, Dos-Santos-Luis D, Martin S, Lamandé N, Carette MF, Disch F, Snijder RJ, Westermann CJ, Mager JJ, Oh SP, Miquerol L, Arthur HM, Mummery CL, Lebrin F. Arterioscler Thromb Vasc Biol. 2016 Apr;36(4):707-17.

Objective: To determine the role of Gja5 that encodes for the gap junction protein connexin40 in the generation of arteriovenous malformations in the hereditary hemorrhagic telangiectasia type 2 (HHT2) mouse model.

Approach and Results: We identified GJA5 as a target gene of the bone morphogenetic protein-9/activin receptor-like kinase 1 signaling pathway in human aortic endothelial cells and importantly found that connexin40 levels were particularly low in a small group of patients with HHT2. We next took advantage of the Acvrl1(+/-) mutant mice that develop lesions similar to those in patients with HHT2 and generated Acvrl1(+/-); Gja5(EGFP/+) mice. Gja5 haploinsufficiency led to vasodilation of the arteries and rarefaction of the capillary bed in Acvrl1(+/-) mice. At the molecular level, we found that reduced Gja5 in Acvrl1(+/-) mice stimulated the production of reactive oxygen species, an important mediator of vessel remodeling. To normalize the altered hemodynamic forces in Acvrl1(+/-); Gja5(EGFP/+) mice, capillaries formed transient arteriovenous shunts that could develop into large malformations when exposed to environmental insults.

Conclusion: We identified GJA5 as a potential modifier gene for HHT2. Our findings demonstrate that Acvrl1 haploinsufficiency combined with the effects of modifier genes that regulate vessel caliber is responsible for the heterogeneity and severity of the disease. The mouse models of HHT have led to the proposal that 3 events heterozygosity, loss of heterozygosity, and angiogenic stimulation-are necessary for arteriovenous malformation formation. Here, we present a novel 3-step model in which pathological vessel caliber and consequent altered blood flow are necessary events for arteriovenous malformation development.

Implications: The authors found expression level of a gap junction protein (a protein allow communications between cells) called connexin40 (GJA5) was lower in mice having low ALK1 expression (e.g. Alk1+/- mutants) compared to normal mice. When they created mice having low expression of both ALK1 and GJA5, these mice exhibited dilated vessels and reduced number of capillaries. These changes in blood vessels change hemodynamic condition, which may contribute to arteriovenous malformations.

MICE LACKING ENDOGLIN IN MACROPHAGES SHOW AN IMPAIRED IMMUNE RESPONSE

Ojeda-Fernández L, Recio-Poveda L, Aristorena M, Lastres P, Blanco FJ, Sanz-Rodríguez F, Gallardo-Vara E, de las Casas-Engel M, Corbí Á, Arthur HM, Bernabeu C, Botella LM. PLoS Genet. 2016 Mar 24;12(3):e1005935

Endoglin is an auxiliary receptor for members of the TGF-superfamily and plays an important role in the homeostasis of the vessel wall. Mutations in endoglin gene (ENG) or in the closely related TGF- receptor type I ACVRL1/ALK1 are responsible for a rare dominant vascular dysplasia, the Hereditary Hemorrhagic Telangiectasia (HHT), or Rendu-Osler-Weber syndrome. Endoglin is also expressed in human macrophages, but its role in macrophage function remains unknown. In this work, we show that endoglin expression is triggered during the monocyte-macrophage differentiation process, both in vitro and during the in vivo differentiation of blood monocytes recruited to foci of inflammation in wild-type C57BL/6 mice. To analyze the role of endoglin in macrophages in vivo, an endoglin myeloid lineage specific knock-out mouse line (Eng(fl/fl)LysMCre) was generated. These mice show a predisposition to develop s pontaneous infections by opportunistic bacteria. Eng(fl/fl)LysMCre mice also display increased survival following LPS-induced peritonitis, suggesting a delayed immune response. Phagocytic activity is impaired in peritoneal macrophages, altering one of theβmain functions of macrophages which contributes to the initiation of the immune response. We also observed altered expression of TGF- 1 target genes in endoglin deficient peritoneal macrophages. Overall, the altered immune activity of endoglin deficient macrophages could help to explain the higher rate of infectious diseases seen in HHT1 patients.

Implications: The authors demonstrated macrophages express endoglin. Mice lacking endoglin-expression in macrophages are more susceptible to bacterial infections due to delayed initial immune response, which may be due to impaired phagocytic (engulfing) activity. They postulated this delayed immune response in endoglin-deficient macrophage may explain the higher rate of infectious diseases seen in HHT1 patients.

PERSISTENT INFILTRATION AND PRO-INFLAMMATORY DIFFERENTIATION OF MONOCYTES CAUSE UNRESOLVED INFLAMMATION IN BRAIN ARTERIOVENOUS MALFORMATION.

Zhang R, Han Z, Degos V, Shen F, Choi EJ, Sun Z, Kang S, Wong M, Zhu W, Zhan L, Arthur HM, Oh SP, Faughnan ME, Su H. Angiogenesis. 2016 Oct;19(4):451-61.

An abnormally high number of macrophages are present in human brain arteriovenous malformations (bAVM) with or without evidence of prior hemorrhage, causing unresolved inflammation that may enhance abnormal vascular remodeling and exacerbate the bAVM pheno-type. The reasons for macrophage accumulation at the bAVM sites are not known. We tested the hypothesis that persistent infiltration and pro-inflammatory differentiation of monocytes in angiogenic tissues increase the macrophage burden in bAVM using two mouse models and human monocytes. Mouse bAVM was i nduced through deletion of AVM causative genes, Endoglin (Eng) globally or Alk1 focally, plus brain focal angiogenic stimulation.

An endothelial cell and vascular smooth muscle cell co-culture system was used to analyze monocyte differentiation in the angiogenic niche.

After angiogenic stimulation, the Eng-deleted mice had fewer CD68(+) cells at 2 weeks (P = 0.02), similar numbers at 4 weeks (P = 0.97), and more at 8 weeks (P = 0.01) in the brain angiogenic region compared with wild-type (WT) mice. Alk1-deficient mice also had a trend toward more macrophages/ microglia 8 weeks (P = 0.064) after angiogenic stimulation and more RFP(+) bone marrow-derived macrophages than WT mice (P = 0.01). More CD34(+) cells isolated from peripheral blood of patients with ENG or ALK1 gene mutation differentiated into macrophages than those from healthy controls (P < 0.001). These data indicate that persistent infiltration and pro-inflammatory differentiation of monocytes might contribute to macrophage accumulation in bAVM. Blocking macrophage homing to bAVM lesions should be tested as a strategy to reduce the severity of bAVM.

The authors used mouse brain AVM models and showed more macrophages were recruited and accumulated to angiogenic (formation of new blood vessels) sites when Alk1 or Eng was deleted in mice. They postulated that macrophage accumulation might contribute to formation of AVMs, and if so blocking macrophage homing to bAVM lesions can be a strategy to reduce the severity of brain AVMs