Supplementary Online Content

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Full inclusion and exclusion criteria and complete research protocol

This supplementary material has been provided by the authors to give readers additional information about their work.

North American Study of Epistaxis in HHT (NOSE)

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BACKGROUND AND SIGNIFICANCE

HHT Related Epistaxis

Hereditary hemorrhagic telangiectasia (HHT) is an hereditary vascular condition characterized by the development of abnormal connections between arteries and veins throughout the body (Guttmacher 1995). These abnormal blood vessels are referred to as arteriovenous malformations (AVM) if they are large and telangiectasias if they are small. Common manifestations of HHT include epistaxis (nosebleeds), anemia, stroke related to pulmonary AVM, hemorrhage related to cerebral AVM, and heart failure from liver AVM.

The prevalence of HHT has been reported to vary widely, from 1/39,216 (2.55/100,000) in Northern England (Porteous 1992), to as high as 1/1,331(75.1/100,000) in Bonaire and Curacao (Westermann 2003). One of the most detailed studies of prevalence, performed in the County of Fyn, Denmark found a prevalence of 1/6,410 (15.6/100,000) in 1995 (Kjeldsen 1999). The only US study determined a prevalence of 1/16,500 (6.06/100,000) in Vermont (Guttmacher 1994), which equates to a prevalence of approximately 18,000 based on the current US population of 300 million. The HHT Foundation International currently quotes the prevalence of HHT in the US at between 1/10,000 and 1/5,000, which falls roughly in the middle of most international estimates of prevalence. These latter estimates indicate a maximum US prevalence of between 30,000 and 60,000.

The most common manifestation of HHT is recurrent and spontaneous epistaxis, which is seen in 90-95% of patients by age 40-50 years and is due to the presence of telangiectasias on the nasal mucosa (Guttmacher 1995, Plauchu 1989). Although the mean age of onset is 12 years (Aassar 1991), epistaxis does not usually become troublesome until after age 20-30 years. In one study, the mean frequency was 18 episodes per month, and the mean duration was 7.5 minutes, with a trend for both of these to increase with advancing age (Aassar 1991). A retrospective study of 49 German patients found that 22% had monthly episodes, 46% had weekly episodes, and 18% had daily episodes (Folz 2005a). Episodes lasted 1-10 minutes in half of patients and 11-30 minutes in the other half. Episodes greater than 30 minutes were rare.

HHT-related epistaxis is associated with significant morbidity and has a significant impact on physical and psychological functioning in patients with HHT. In a retrospective study of 75 American patients, 72% of patients with epistaxis felt that it had a moderate to severe impact on their lifestyle (Aassar 1991). In a study of 77 German patients with definite or possible HHT, the scores for all domains of the Short Form-36 Health Survey (SF-36) except bodily pain were significantly reduced when compared to healthy Germans (Geisthoff 2007). The frequency of epistaxis and the subjective impediments from epistaxis correlated highly with several domains. Furthermore, epistaxis was the leading reason given for changes in profession, restrictions in athletic activity, and presence of psychological strain related to HHT. 46% of patients with epistaxis require transfusions at one point and 54% report a progressive course over time (Reilly 1984). Furthermore, mortality was greater in HHT than expected (36 observed versus 25.8 expected in Denmark) (Kjeldsen 1999). In this study, HHT was considered a contributory cause of death in 36% of patients who had died, and all had transfusion dependent bleeding from gastrointestinal and/or nasal sources.

The most common treatments for HHT-related epistaxis currently include a variety of substances applied to the nasal mucosa (e.g. petrolatum, estrogens, and saline), oral

estrogens, oral tranexamic acid or aminocaproic acid, electrocautery, laser photocoagulation of telangiectasias, and skin grafts (septal dermoplasty). None of these treatments is curative. There have only been four randomized treatment studies published so far: two have several methodologic weaknesses (Vase 1981, Yaniv 2009); one has only been published in abstract form; and the fourth evaluated medical treatment in combination with laser photocoagulation (Bergler 2002). Therefore, the optimal treatment for HHT-related epistaxis remains uncertain (Pagella 2006, Karapantzos 2005). The best studied treatments have included oral estrogens, intranasal estriol, oral tranexamic acid, and laser photocoagulation as detailed below.

A variety of photocoagulation techniques for the treatment of HHT-related epistaxis have been reported during the past decade. Folz et al reported in abstract form on 48 HHT patients who underwent Nd:YAG laser photocoagulation of intranasal telangiectasias (Folz 2003). 83% of patients showed improved epistaxis for an average duration of 1 year. However, most patients required repeat laser treatment during that period and general anesthesia was required in all. Karapantzos et al reported 27 HHT patients with mild to moderate epistaxis who underwent Nd:YAG laser photocoagulation (Karapantzos 2005). Although quality of life was improved after 24 months of follow up, 67% of patients required 2 or more treatments and general anesthesia was required in all. Most recently, Pagella et al performed argon plasma coagulation (APC) in 43 HHT patients with recurrent epistaxis and reported on 39 patients who had adequate follow up (Pagella 2006). While 92% of patients had a reduction in bleeding postoperatively, the benefit lasted 6 months in 38% and 2 years in only 21% (Pagella 2006, Pagella 2007). These and at least 8 other reports of photocoagulation indicate that the procedure is beneficial in a large percentage of patients; however, the disadvantages include its invasiveness, a need for local expertise, limited durability of response, a need for repeated procedures, some degree of pain, and the need for general anesthesia in most.

The above data indicate that epistaxis is a serious problem for many patients with HHT. It adversely affects quality of life and is associated with significant morbidity and mortality. Current treatments are typically of transient benefit and are associated with numerous shortcomings. A detailed analysis of telangiectasia distribution in HHT patients with a rigid endoscope showed that 80-90% of telangiectasias were in the anterior 2 cm of the vestibule (Folz 2005b). This suggests that most telangiectasias are within reach of where a nasal spray could be applied. We therefore propose to perform a randomized, placebo-controlled trial of four intranasal sprays in the treatment of HHT-related epistaxis. Based on the data below, we predict that this study will demonstrate efficacy for one or more active treatments. By showing that a placebo-controlled trial can be successfully performed in patients with HHT-related epistaxis, this trial will act as the cornerstone and stimulus for additional translational research, multicentered treatment studies, and FDA approval of the first safe and effective medication for this condition.

Treatment Rationale

Estrogen Treatment of HHT-Related Epistaxis

There are 3 major naturally occurring estrogens in women: estradiol, estrone, and estriol. Estradiol is the most potent, and is the main estrogen produced from menarche to menopause. As a group, the estrogens have protean clinical effects including stimulation of endometrial and uterine growth, decreased bone resorption, alterations in lipid metabolism, reduction in antithrombin III, increased platelet adhesion, sodium retention, and increased genital blood flow. Many studies have shown a small but significantly increased risk of breast cancer and endometrial cancer in certain populations. These clinical effects are most prominent with

estradiol, which has one hundred times the potency of estriol (Strauss 2004). Estriol is believed to have minimal carcinogenic potential and one study showed no increase in the risk of breast cancer compared with estradiol (Bergkvist 1976). The side effect profile of estrogens is likely affected by its route of ingestion. During oral estrogen administration the concentration of estradiol in the liver sinusoids is four to five times higher than that in the systemic circulation, which can modulate the expression of many hepatic-derived proteins and increase side effects. In contrast, transdermal estrogen delivers the hormone directly into the systemic circulation and avoids the first-pass hepatic effect, lessening the metabolic effects (Menon 2006).

The potential role of estrogens in HHT was first pointed out by Koch et al in 1952 when they noted that bleeding from telangiectasias was often worse at the end of the menstrual cycle, after oophorectomy, and at the menopause (Koch 1952). They further reported on 5 patients who had an excellent clinical response to 0.25 to 1 mg of oral ethinyl estradiol daily. Subsequent experiments showed that diethylstilbesterol on the nasal mucosa of guinea-pigs resulted in squamous metaplasia of the normally ciliated columnar epithelium of the nose (Harrison 1959).

In a thorough literature review of hormonal therapy for HHT-related epistaxis, Jameson concluded that "Anecdotal evidence with high-dose [oral] estrogen appeared to show benefit" (Jameson 2004). However, nearly all of these studies were uncontrolled and/or retrospective. The only placebo-controlled trial of estrogen for HHT-related epistaxis randomized 31 patients to oral estradiol valerate 2 mg twice daily versus placebo for a total of 3 months and concluded that there was no benefit (Vase 1981). However, the authors noted that there "was a weak tendency towards reduction in the frequency of bleeding," a rise in hemoglobin from 6.7 to 8.2 mmol/l, and a significant decrease in transferrin in the estrogen group relative to placebo. Potential weaknesses of this study included use of only an intermediate dose of estrogen and a small number of patients. Unfortunately, most studies of oral estrogens (mainly estradiol) in HHT have reported significant side effects including thromboembolic disease, nausea, breast cancer, ovarian tumors, intermenstrual bleeding, breast tenderness, and testicular atrophy, loss of libido and gynecomastia in men (Vase 1981, Harrison 1982, Flessa 1977, Harrison 1964).

In an effort to avoid the systemic side effects of high dose oral estrogens and to capitalize on the purported benefit of estrogen-induced squamous metaplasia of the nasal mucosa, recent studies have examined the effects of intranasal estriol ointment. Bergler et al randomized 26 consecutive HHT patients who were treated with APC of nasal telangiectasias to receive adjuvant therapy with either intranasal 0.1% estriol ointment 0.5 mg twice daily, or placebo ointment (Bergler 2002). After 12 months of treatment the estriol group had decreased frequency and intensity of epistaxis, and more satisfaction with treatment compared with the placebo group. Prolonged treatment of 52 HHT patients with 0.1% intranasal estriol ointment 0.5 mg twice daily, was not associated with any systemic side effects or change in serum estriol levels at 3 months, 6 months, and 18 months relative to baseline (Sadick 2003). A recent histologic study showed the development of squamous metaplasia of nasal mucosa within 6 months after starting intranasal 0.1% estriol ointment (Sadick 2005a).

Taken together, these data support the following conclusions: 1) high dose oral estradiol and low dose topical estriol may reduce the frequency and severity of HHT-related epistaxis, 2) oral estradiol is associated with a significant risk of serious complications, and 3) topical estriol is

well tolerated even during prolonged use. These conclusions form the basis of our hypothesis that treatment with estriol nasal spray will improve the frequency and duration of HHT-related epistaxis.

Tranexamic Acid Treatment of HHT-Related Epistaxis

Tranexamic acid (TA) is an antifibrinolytic agent that binds reversibly to plasminogen and thus inhibits the lysis of fibrin by plasmin. TA is currently FDA approved for the prevention of hemorrhage following dental extraction in patients with hemophilia. It is often used off-label and has been shown to decrease bleeding between 20 and 50% in a wide variety of clinical scenarios including primary menorrhagia, gastrointestinal bleeding from peptic ulcers, urinary tract bleeding, oral bleeding, and during cardiac surgery (Mannucci 1998). For example, in a randomized placebo-controlled study of 210 patients undergoing open heart surgery, TA 10 grams intravenously resulted in a 48% decrease in operative blood loss and a 69% decrease in the number of units of red blood cells transfused (Katsaros 1996). There was no difference in the incidence of postoperative complications such as stroke or deep venous thrombosis.

Several studies have looked at topical use of TA in patients without HHT who had various clinical conditions. One of the first reports of topical TA randomized 39 patients to receive 10 ml of 4.8% TA mouthwash (480 mg/day) versus placebo prior to oral surgery and then four times daily for 7 days afterwards (Sindet-Pederson 1989). Postoperative bleeding was seen in 5% of patients receiving TA versus 40% receiving placebo ($p=0.01$) and no systemic side effects were observed. Tibbelin et al randomized 68 patients with acute epistaxis to a single application of intranasal of 15 ml of 10% TA gel (1500 mg) or placebo gel (Tibbelin 1995). TA showed a trend toward improved cessation of bleeding within 30 minutes of application and a reduction in rebleeding over the next 10 days (44 versus 66%) compared with placebo. Although these effects are encouraging, it is difficult to extrapolate the results from a single application to possible efficacy in a chronic condition like HHT. In a rabbit model, topical ocular administration of 10% TA solution (5 mg every 8 hours) versus intravenous administration (25 mg/kg every 8 hours) for 3 days showed a higher aqueous concentration and a lower serum concentration in the topical group (Damji 1998). Other randomized placebo-controlled studies showed that topical TA reduced operative bleeding in 56 patients who underwent endoscopic sinus surgery (Jabalameli 2005) and 300 patients who underwent cardiac surgery (Baric 2007).

There are limited data on the use of TA in HHT-related epistaxis. The first report was a case of a 50 year old man with nearly daily epistaxis and severe anemia (hemoglobin 56 g/l)(Klepfish 2001). Daily intranasal administration of 10% TA nose drops (100 mg/ml; about 500 mg over 2-3 days) resulted in a marked decrease in epistaxis, an increase in hemoglobin to 134 g/l, and no adverse effects. Sabba et al reported on 3 HHT patients with epistaxis and anemia who experienced decreased epistaxis and improved hemoglobin while taking 4-4.5 g/d of oral TA (Sabba 2001). No adverse effects were noted. In the only randomized placebo controlled trial of TA (3 g daily by mouth) in HHT-related epistaxis, Geisthoff et al reported a 44% reduction in the incidence of epistaxis and a trend toward improved hemoglobin (Geisthoff 2003). One patient experienced thrombophlebitis but it was not mentioned whether this patient was taking TA or placebo. Most recently, 14 HHT patients with severe epistaxis were treated with oral TA 0.5-1 g three times daily and followed for a mean of 9.6 months (2-25 months). All patients experienced a decrease in frequency and/or severity of epistaxis, usually within 1 week. Epistaxis typically increased immediately after stopping TA. No patient reported adverse events. Out of the 30 patients presented in these studies, only 1 received a topical form of TA (Klepfish 2001).

Although there have been a few case reports of thrombotic complications following use of antifibrinolytic agents including TA, the placebo-controlled trials have not shown a significant increase in the risk of thrombosis (Mannucci 1998). Based on its mechanism of action, TA is unlikely to produce thrombotic complications unless the patient has an underlying thrombogenic stimulus such as disseminated intravascular coagulation (Hedner 2001). Other side effects are dose-dependent and include nausea, vomiting, abdominal discomfort, and diarrhea in up to 30%.

Taken together, these data support the following conclusions: 1) randomized studies show that oral and topical TA are effective in reducing bleeding in a variety of clinical scenarios, 2) mostly uncontrolled studies suggest that oral TA may reduce the frequency and severity, and perhaps the associated anemia, of HHT-related epistaxis, and 3) there is a very low rate of adverse events with the use of TA under these conditions. These conclusions form the basis of our hypothesis that treatment with TA nasal spray will improve the frequency and duration of HHT-related epistaxis.

Bevacizumab Treatment of HHT-Related Epistaxis

Bevacizumab (BEV) is a humanized antibody that binds to a protein called vascular endothelial growth factor (VEGF). VEGF is involved in the growth of both normal and abnormal blood vessels throughout the body. When bevacizumab binds to VEGF, it inhibits the actions of VEGF and theoretically decreases blood vessel growth. Single intravenous doses greater than 0.3 mg/kg were shown to completely inhibit free serum VEGF levels (Margolin 2001). When given intravenously at doses of 1-20 mg/kg it has a terminal half life of approximately 20 days (Lu 2008).

The initial clinical focus with bevacizumab was for the treatment of cancer by decreasing the blood vessel supply to growing tumors. It has been effective in the treatment of several cancers including lung and colon. When used to treat cancer, BEV has typically been infused intravenously at doses of 3-15 mg/kg every 1-3 weeks for a period of several months. At these doses, BEV has been associated with significant side effects in cancer patients. In an FDA summary of BEV in the treatment of nonsmall cell lung cancer, the most frequent side effects included weakness, pain, headache, systemic hypertension, nausea, vomiting, diarrhea, stomatitis, constipation, upper respiratory illness, epistaxis, dyspnea, exfoliative dermatitis, proteinuria, and neutropenia (Cohen 2007). The most serious side effects included gastrointestinal perforation, wound healing complications, bleeding, arterial thromboembolism, hypertensive crisis, nephrotic syndrome, and heart failure.

When used intravenously, side effects have generally been dose related. Gordon et al reported on 25 patients who received 0.1, 0.3, 1, 3, and 10 mg/kg IV of bevacizumab (5 patients at each dose level) at baseline, week 4, week 5, and week 6 (Gordon 2001). The infusions were generally well tolerated. No patient had grade 3 or 4 toxicity that was attributable to bevicizumab at doses of less than 1 mg/kg. Maximum plasma concentration was 4.8-8.9 mcg/ml in the 0.3 mg/kg group compared with 186-294 mcg/ml in the 10 mg/kg group. Yang et al reported on 116 patients with metastatic renal cell cancer who were randomized to 3 or 10 mg/kg IV of bevacizumab versus placebo every 2 weeks indefinitely (Yang 2003). Side effects were more common at the 10 mg/kg BEV, but were similar in frequency between the 3 mg/kg BEV and placebo (except for epistaxis). For example, hypertension was seen in 14, 1, and 2 patients receiving 10 mg/kg, 3 mg/kg, and placebo,

while epistaxis was seen in 8, 5, and 1 patient.

BEV has also been shown to be effective in the treatment of certain non-malignant diseases of the eyes that involve abnormal blood vessel growth (e.g. age-related macular degeneration or ARMD and corneal neovascularization or CNV). In an internet safety registry of 7,113 intravitreal injections of BEV (usually 1-1.25 mg per eye) in 5,228 patients, there was no increase in adverse systemic events over that which would be expected in an elderly population (Fung 2006). The most common adverse events were corneal abrasion 0.15%, mild ocular discomfort 0.14%, ocular inflammation 0.14%, and increased blood pressure 0.21%. In 2007, Lynch et al reported an extensive literature review that included 19 published series of 850 eyes treated with intravitreal BEV (usually 1.25 mg, 1-3 doses over several months) for various neovascular ocular conditions (Lynch 2007). A variety of adverse effects related to ocular injection were reported and included discomfort, inflammation, and retinal tears. Limitations of these studies included a lack of a randomized control group in all, retrospective design in some, and unclear methods of monitoring for side effects in most. Recently, Bashshur et al reported a prospective nonrandomized trial of intravitreal injection of 2.5 mg of BEV in 51 eyes of 51 patients (Bashshur 2009). Patients received a mean of 4.9 injections during 24 months and showed improvement in visual acuity. Side effects were monitored at each visit. There were no ocular side effects and no changes in BP. One patient required coronary bypass surgery but it was not possible to determine its relationship to BEV.

There is a modest literature on the topical use of BEV in human CNV. The first study in humans reported that topical ocular use of BEV, 2 mg/d for 30 days, resulted in decreased neovascularization and no local or systemic adverse events (DeStafeno 2007). Similarly, Dastjerdi et al reported on the daily topical ocular use of BEV, 1-2 mg/d for 3 weeks, in 10 patients with CNV (Dastjerdi 2009). They found a significant decrease in neovascularization and no adverse events including no changes in BP. Kim et al administered topical BEV, 1.25 mg/eye/d, to 10 eyes of 7 patients for 3 months (Kim 2008). They noted corneal epithelialization defects in 6 eyes, usually at around 1 month. No systemic adverse events were seen. Nomoto et al performed an extensive pharmacokinetic comparison of intravitreal and topical ocular BEV in 72 rabbits weighing 1.9-2.5 kg (Nomoto 2009). The topical dose was 1.25 mg in one eye 6 times daily for 1 week (roughly 3 mg/kg/d or 21 mg/kg/week) versus a single 1.25 mg intravitreal injection. Topical BEV resulted in a retinal tissue concentration of 18 ng/g and a peak plasma concentration of 14 ng/ml, versus 93,990 ng/g and 2087 ng/ml for the intravitreal route. These data show that while topical BEV does penetrate the eye to a minor degree, its systemic absorption is <1% that seen with intravitreal injection, despite a 42-fold larger dose over 1 week.

Vascular endothelial growth factor (VEGF) is increased in the plasma (Sadick 2005b) and serum (Cirulli 2003) of patients with HHT. Increased plasma VEGF levels have also been shown to correlate with the intensity of VEGF staining and microvessel density in nasal mucosa from HHT patients (Sadick 2005b). The first report of bevacizumab in patients with HHT was published in 2006 by Flieger and colleagues (Flieger 2006). This report described a patient with HHT and intestinal bleeding who was treated with bevacizumab for a malignant tumor. Treatment with bevacizumab stabilized his cancer and also markedly improved his anemia and reduced his need for blood transfusion. Mitchell et al reported a HHT patient who had improvement in liver AVM related heart failure after treatment with bevacizumab (Mitchell 2008). An additional two patients received bevacizumab for HHT related gastrointestinal bleeding and liver AVM and had improvement, but these have not been published in scientific

journals yet (personal communications from Dr. Murali Chakinala at Washington University in St Louis and Dr. Frank Miller at UCSD in San Diego). In a dose ranging pilot study, 6 patients with various complications of HHT received BEV at a dose of 1 mg/kg IV every 3 weeks for a total of 6 doses over 3 months. These infusions were tolerated well without any significant side effects (personal communication 3/9/10 from Carlo Sabba at Bari University in Spain).
There are published reports on 14 patients who have received BEV for HHT related epistaxis. Bose and colleagues reported a case of a patient with epistaxis and severe anemia who was refractory to iron therapy (Bose 2009). Four cycles of BEV, 5-10 mg/kg IV every 2 weeks, resulted in a significant decrease in epistaxis and anemia; no side effects were reported. Two additional patients with severe epistaxis received BEV 5-7.5 mg/kg IV every 2 weeks for 1 year and 3 months with improvement in epistaxis and anemia (Oosting 2009; Retornaz 2009). Simonds et al reported on 10 patients with HHT related epistaxis who received intranasal submucosal injection of 100 mg of BEV in conjunction with KTP laser treatment (Simonds 2009). When compared to a retrospective control group who had received only KTP laser by the same surgeon, the patients who received BEV plus laser showed a modest improvement in frequency of nosebleeds, need for blood transfusions, and quality of life. 40% of the patients who received BEV developed a nasal septal perforation. The authors have since modified their treatment by excluding the nasal septum from injection and have had no further perforations in over 40 patients (personal communication from Terry Davidson, UCSD, San Diego, CA). Most recently, a patient with daily epistaxis was treated with topical BEV nose spray (10 mg/ml) at a dose of 1 mg in each nostril twice daily for 2 weeks (Davidson 2010). Within 4 days his epistaxis ceased and remained well controlled for 3-4 months, at which time his epistaxis returned and again responded to repeat treatment. This patient's sister took the same treatment with some benefit (personal communication, Dr. Scott Olitsky, Kansas City, MO). Neither patient suffered any adverse events. Lastly, at the Medical College of Georgia we have used the same protocol of submucosal injection of 100 mg of BEV in conjunction with laser treatment as described by Simonds and colleagues (Simonds 2009) in 18 patients with HHT-related epistaxis since March 2010, and have encountered no overt adverse events related to the use of BEV (personal experience, Dr. James Gossage).

Taken together, these data support the following conclusions: 1) intravenous BEV is associated with significant side effects in cancer patients and these side effects are dose related, 2) intravenous BEV at doses of less than 1 mg/kg are well tolerated, 3) intravitreal injection of BEV, 1-25-2.5 mg, is not associated with systemic side effects, 4) topical ocular BEV at doses of 1-2 mg/day for up to 3 months is not associated with systemic side effects, but prolonged use may result in local ocular adverse events, 5) topical ocular BEV at doses of 21 mg/kg/week in rabbits is associated with trivial systemic absorption, 6) case control studies, case reports, and personal experience in 42 patients with HHT suggest that intravenous, submucosal, and topical BEV are well tolerated and may be effective in treatment of a wide variety of HHT related complications including epistaxis, and 7) serious cases of hemorrhage related to BEV have always occurred with high dose intravenous administration and have usually occurred at a site of known pathology such as lung hemorrhage into a lung tumor. These conclusions form the basis of our hypothesis that treatment with BEV nasal spray will improve the frequency of HHT-related epistaxis. The dose we have chosen is 4 mg/day for 7 days. From an efficacy standpoint, this dose is based on the finding that a similar topical dose was beneficial in patients with CNV and 2 patients with HHT related epistaxis. From a safety standpoint, a similar dose has not been associated with significant systemic adverse events, even when used daily for up to 3 months, and much higher topical doses have been associated with trivial systemic absorption.

Biochemical Mediators in HHT

There are at least 5 genetic types of HHT (Gossage 2007). HHT-1 is the most common type in North America, and is due to a mutation in the gene coding for endoglin (Bossler 2006). HHT-2 is more common in Europe, and is due to a mutation in the gene coding for ALK-1 (Lesca 2006). Tissue specimens from patients with HHT-1 and HHT-2, and from knockout animal models are consistent with a haploinsufficiency mechanism in which endothelial expression of these proteins is decreased by approximately 50% (Bourdeau 2000, Driesche 2003). The exact mechanisms by which decreased levels of endoglin and ALK-1 result in altered angiogenesis and formation of AVM is unclear; however, both proteins are involved in the TGF-beta signaling pathway which is believed to be integrally involved in vascular remodeling. TA has recently been shown to markedly upregulate the expression of both endoglin and ALK-1 in human endothelial cells in vitro (Fernandez 2007). Upregulation occurred at both the mRNA and protein levels. It is possible that upregulation of these deficient proteins in the endothelial cells of HHT patients may improve vascular integrity, suggesting a possible second mechanism by which TA could decrease HHT-related epistaxis. Although there are no reports of the effects of estrogen on regulation of the endoglin or ALK-1 genes, at least 3 estrogen-responsive elements have been identified in the 5 prime flanking region of the endoglin gene (Rius 1998).

Vascular endothelial growth factor (VEGF) is a potent angiogenic factor that is increased in the plasma (Sadick 2005b) and serum (Cirulli 2003) of patients with HHT. Recently, increased plasma VEGF levels have been shown to correlate with the intensity of VEGF staining and microvessel density in nasal mucosa from HHT patients (Sadick 2005b). Bevacizumab directly inhibits the action of VEGF (Cohen 2007). The effects of estrogen and TA on regulation of VEGF in HHT patients are unknown.

It is unlikely that the local effects of the nasal sprays in this study will affect systemic mediators enough to allow measurable changes in plasma levels during treatment. However, it is possible that baseline levels of VEGF and other mediators might predict the response to one or more of the active drugs in this study. Similarly, the HHT causative mutation and baseline levels of various RNA may also predict response. We postulate that if one or more active drugs is successful in reducing the frequency of epistatxis, this response may be predicted by retrospective analysis of the HHT causative mutation and baseline levels of RNA and other biochemical mediators such as VEGF.

RESEARCH DESIGN AND METHODS

Specific Aim 1. To determine if intranasal estriol spray will decrease the frequency of epistaxis (nosebleeds) in patients with hereditary hemorrhagic telangiectasia (HHT).

Specific Aim 2. To determine if intranasal tranexamic acid spray will decrease the frequency of epistaxis (nosebleeds) in patients with hereditary hemorrhagic telangiectasia (HHT).

Specific Aim 3. To determine if intranasal bevacizumab spray will decrease the frequency of epistaxis (nosebleeds) in patients with hereditary hemorrhagic telangiectasia (HHT).

Specific Aim 4. To determine if treatment response to one or more agents correlates with underlying HHT causative mutation, baseline RNA levels, or baseline levels of various mediators such as VEGF.

Study Hypotheses

1. Treatment with intranasal estriol spray will improve epistaxis frequency in patients with HHT.
2. Treatment with intranasal tranexamic acid spray will improve epistaxis frequency in patients with HHT.
3. Treatment with intranasal bevacizumab spray will improve epistaxis frequency in patients with HHT.
4. Treatment response to one or more agents may correlate with underlying HHT causative mutation, baseline RNA levels, or baseline levels of various mediators such as VEGF (exploratory hypothesis)..

Basic Design and Rationale

140 patients with moderate to severe epistaxis secondary to HHT will be randomized to receive one of four intranasal sprays for a period of 12 weeks and then followed for an additional 12 weeks off therapy. Enrollment will occur over a period of 18-36 months. The primary endpoint will be the frequency of epistaxis. Secondary endpoints will include duration of epistaxis, the Hoag Epistaxis Severity Score (ESS), a quality of life survey, satisfaction with treatment, hemoglobin and ferritin levels, transfusion requirements, and treatment failure. The sprays will be: saline spray (Placebo); estriol 0.1% in methylcellulose suspension (EST); tranexamic acid 10% in saline (TA), and bevacizumab 1% in saline (BEV). All sprays will be applied to the nasal mucosa by an identical spray bottle at a dose of 0.1 ml per nostril twice daily (total dose of 0.4 ml daily). Thus, the delivered doses will be: EST, 0.4 mg/day; TA, 40 mg/day; BEV, 4 mg/day.

A placebo arm has been included to avoid unblinded treatment effect biases that could potentially affect the primary outcome and several of the secondary outcomes. A placebo arm is also necessary to address the shortcomings of most previous studies in this area. Selected doses have been based on the data presented in the section on Background and Significance. HHT-related epistaxis is associated with significant morbidity and some long term mortality. We therefore believe that the potential benefits of this study outweigh the hypothesized minimal risks.

Inclusion Criteria (all must be present)

1. A diagnosis of definite or possible HHT by the Curacao criteria (Shovlin 2000) or a positive DNA test for HHT (as characterized by a disease causing mutation in the gene coding for endoglin, ALK-1, or SMAD-4). According to the Curacao criteria, a definite diagnosis of HHT is defined as having at least 3 of the following criteria while a possible diagnosis is defined as 2 criteria:
   a. Spontaneous and recurrent epistaxis.
   b. Multiple telangiectasias at characteristic sites (lips, oral cavity, fingers, nose).
   c. Visceral lesions such as gastrointestinal telangiectasias and arteriovenous malformations (AVM) in lung, brain, spine and liver.
   d. A history of definite HHT in a first degree relative using these same criteria.
2. Epistaxis of at least 1 minute (on average) and which occurs at least once weekly when averaged during the preceding 8 weeks.
3. Epistaxis severity score (ESS) of at least 3.0.
4. Age of at least 18 years.
5. Written and informed consent obtained prior to study entry.
6. Subject is able and willing to return for outpatient visits.
7. The epistaxis is considered to be clinically stable during the past 8 weeks in the clinical judgment of the investigator (i.e. no major changes in frequency or duration of epistaxis or in transfusion requirements).
8. Negative pregnancy test at enrollment for women of child-bearing potential.

Exclusion Criteria (all must be absent)

1. Allergy to any of the active treatment agents or their spray additives.
2. Estimated life expectancy less than 1 year.
3. A psychiatric or substance abuse problem that is expected to interfere with study compliance.
4. History of deep venous thrombosis (DVT), pulmonary embolism (PE), acute myocardial infarction (MI), arterial thromboembolism, or ischemic stroke in the past 6 months.
5. History of estrogen receptor positive breast cancer.
6. History of receiving more than 12 units of red blood cells in the past 12 weeks.
7. Presence of an untreated coagulopathy that is felt to be contributing to the epistaxis.
8. Presence of active disseminated intravascular coagulation.
9. Uncontrolled hypertension (SBP >160 and/or DBP >100).
10. Presence of untreated brain AVM.
11. Presence of active malignancy in the brain, lung, or colon.
12. Presence of symptomatic heart failure.
13. Use of estrogens, epsilon aminocaproic acid, tranexamic acid, or thalidomide by any route for more than 1 week in the past 12 weeks. Any use of a VEGF inhibitor by any route in the past 24 weeks.
14. Baseline use of the following anticoagulants is not allowed: warfarin or other vitamin K antagonists at any dose; unfractionated or low molecular weight heparins at standard doses for treatment of venous thromboembolism (VTE); or aspirin at >325 mg/day. Baseline use of the following anticoagulants is allowed: heparins at standard doses for VTE prophylaxis; clopidogrel; or aspirin at ≤325 mg/day.
15. Addition of new treatments for epistaxis in the past 12 weeks (including laser ablation of nasal telangiectasias and over the counter medications).

16. Presence of another overt cause (e.g. overt gastrointestinal bleeding) that is felt to be significantly contributing to anemia.
17. Lactating women.

Visit Schedule and Required Evaluations

Note that the evaluations for Visit 1 may occur over the span of 3 weeks to allow scheduling of all evaluations. The first dose of study medication should be taken within 3 weeks of written informed consent. Visits 1 and 6 will be conducted on site at the research facility. Visits 2, 3, 4, 5 and 7 may occur by phone. The Visit Schedule is shown in tabular form in Table 1. The time window for completion of Visits 2-5 will be ±4 days and for Visits 6-7 will be ±1 week.

1. Visit 1 (baseline)
    a. Review inclusion and exclusion criteria.
    b. Written informed consent process.
    c. Medical history including concomitant medications and transfusion history.
    d. Safety questions.
    e. Physical examination including inspection of nasal septum.
    f. Review frequency and duration of epistaxis during the past 12 weeks.
    g. Hoag epistaxis severity score (ESS).
    h. Short Form-36 Health Survey (SF-36).
    i. Supplemental Epistaxis Questionnaire.
    j. Blood collection for CBC, platelets, ferritin, PT, PTT, pregnancy test, DNA analysis, and biomarkers.
    k. Nasal endoscopy.
2. Visit 2 (1 week)
    a. This visit may be conducted by phone.
    b. Confirm willingness to continue in the study.
    c. Medical history including concomitant medications and transfusion history.
    d. Safety questions.
    e. Review Nosebleed Diary.
3. Visit 3 (2 weeks)
    a. This visit may be conducted by phone.
    b. Confirm willingness to continue in the study.
    c. Medical history including concomitant medications and transfusion history.
    d. Safety questions.
    e. Review Nosebleed Diary.
4. Visit 4 (4 weeks)
    a. This visit may be conducted by phone.
    b. Confirm willingness to continue in the study.
    c. Medical history including concomitant medications and transfusion history.
    d. Safety questions.
    e. Review Nosebleed Diary.
5. Visit 5 (8 weeks)
    a. This visit may be conducted by phone.
    b. Confirm willingness to continue in the study.
    c. Medical history including concomitant medications and transfusion history.
    d. Safety questions.
    e. Review Nosebleed Diary.

6. Visit 6 (12 weeks)
    a. Confirm willingness to continue in the study.
    b. Medical history including concomitant medications and transfusion history.
    c. Safety questions.
    d. Physical examination including inspection of nasal septum.
    e. Review Nosebleed Diary.
    f. Hoag epistaxis severity score (ESS).
    g. Short Form-36 Health Survey (SF-36).
    h. Supplemental Epistaxis Questionnaire.
    i. Blood collection for CBC, platelets, and ferritin level.
    j. Collect all unused drug.
7. Visit 7 (24 weeks)
    a. This visit may be conducted by phone.
    b. Medical history including concomitant medications and transfusion history.
    c. Safety questions.
    d. Review Nosebleed Diary.
    e. Hoag epistaxis severity score (ESS).

Table 1. Visit Schedule

Evaluation	Base-line	1 wk	2 wk	4 wk	8 wk	12 wk	24 wk
Review inclusion and exclusion criteria	X						
Written informed consent process	X						
Confirm willingness to continue in the study		X	X	X	X	X	
Medical history	X	X	X	X	X	X	X
Transfusion history	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X
Safety questions	X	X	X	X	X	X	X
Physical examination	X					X	
Inspection of nasal septum	X					X	
Review frequency and duration of epistaxis during the past 8 weeks	X						
Review Nosebleed Diary		X	X	X	X	X	X
Hoag epistaxis severity score (ESS)	X					X	X
Short Form-36 Health Survey (SF-36)	X					X	X
Supplemental Epistaxis Questionnaire	X					X	X
CBC, platelets, ferritin level	X					X	
PT, PTT	X						
Pregnancy test	X						
Blood collection for plasma biomarkers	X						
Saliva collection for DNA/RNA analysis	X						
Nasal endoscopy	X						
Stratification and randomization	X						
Ship drug to patient (2^{nd} day air)	X						
Collect unused drug						X	

Withdrawal Criteria
Patients will be withdrawn from the study for any of the following reasons: repeated lack of compliance, acute DVT, acute PE, acute ischemic stroke, acute myocardial infarction, the need for transfusion of more than 12 units of blood cells over a 12 week period, any nasal surgery (including laser cautery, electrocautery, chemical cautery, and septal dermoplasty; but not nasal packing), pregnancy, investigator judgment that the patient needs more substantial treatment, or by patient request. See also the section on Concomitant Medications. Study medication will be stopped immediately at the time of study withdrawal.

Treatments
The optimal properties of a spray vehicle for this study would maximize nasal mucosal contact and hydration, the ability to easily mix with water soluble drugs to optimize consistency and drug release, and relative inertness to maximize shelf life. Benzalkonium chloride 0.013% will be used as a preservative. No other additives will be used except as noted below. Opaque polyethylene metered dose spray bottles (0.1 ml per spray) of 30 ml capacity, will be obtained from Letco Medical (Decatur, AL) and will be used for all treatment preparations. Our testing on 10 bottles has determined an average spray volume of 0.104 ml/spray and an average residual volume of 0.5 ml when spray volume becomes inconsistent.

<u>Estriol Spray</u>
1. Preparation. Estriol spray will be prepared at O'Brien Pharmacy in Mission, Kansas. Estriol Micronized USP will be obtained from PCCA USA (Houston, TX). A recent Certificate of Analysis states that purity is 98.3%. A 0.1% spray (100 mg estriol suspension in 100 ml 0.5% carboxymethylcellulose sodium suspension) will be mixed according to USP 795 standards. All mixing will occur under a hood and in a closed system when possible.
2. Packaging and storage. 4 or 15 ml of spray will be packaged into individual spray bottles and labeled with lot number, preparation date, volume, and expiration date. Each lot will be stored at room temperature at O'Brien Pharmacy. An expiration date of 2 weeks will be indicated on the 4 ml bottles and 141 days will be indicated on the 15 ml bottles.
3. Potency over time testing. A small lot of estriol spray was prepared as above and a sample was sent to Eagle Analytical (Sugarland, TX) on 12/18/09 to determine serial potency by HPLC. Potency testing showed: baseline, 99.7%; 86 days, 96.7%; 141 days, 97.3%.

<u>Tranexamic Acid Spray</u>
1. Preparation. TA spray will be prepared at O'Brien Pharmacy in Mission, Kansas. TA BP will be obtained from PCCA USA (Houston, TX). A recent Certificate of Analysis states that purity is 99.4%. A 10% spray (10 g TA in 100 ml 0.9% sodium chloride) will be mixed according to USP 795 standards. All mixing will occur under a hood and in a closed system when possible.
2. Packaging and storage. 4 or 15 ml of spray will be packaged into individual spray bottles and labeled with lot number, preparation date, volume, and expiration date. Each lot will be stored at room temperature at O'Brien Pharmacy. An expiration date of 2 weeks will be indicated on the 4 ml bottles and 135 days will be indicated on the 15 ml bottles.
3. Potency over time testing. A small lot of tranexamic acid spray was prepared as above and a sample was sent to Eagle Analytical (Sugarland, TX) on 12/18/09 to determine serial potency by HPLC. Potency testing showed: baseline, 105%; 50 days, 105%; 98 days, 106%; 135 days, 105%.

Bevacizumab Spray

1. Preparation. Bevacizumab spray will be prepared at O'Brien Pharmacy in Mission, Kansas. BEV will be obtained from Genetech Co. A 1% spray (1.6 ml of a 25 mg/ml BEV solution in 2.4 ml 0.9% sodium chloride) will be mixed according to USP 795 standards. All mixing will occur under a hood and in a closed system when possible.
2. Packaging and storage. 4 ml of spray will be packaged into individual spray bottles and labeled with lot number, preparation date, volume, and expiration date. Each lot will be stored refrigerated at O'Brien Pharmacy. An expiration date of 2 wk (per USP 795 standards) will be assumed.
3. Potency over time testing. A small lot of bevacizumab spray was prepared as above and a sample was sent to Dr. Peter Koulen, PhD, at the University of Missouri Kansas City to determine potency by native PAGE and VEGF ELISA assays. There was no significant loss of potency between day 0 and day 14 (refrigerated) by these assays (data on file with the FDA).

Placebo Spray

Preparation, packaging and storage. Placebo spray will be prepared at O'Brien Pharmacy in Mission, Kansas. 4 or 15 ml of normal saline will be packaged into individual spray bottles and labeled with lot number, preparation date, volume, and expiration date. Each lot will be stored at room temperature at O'Brien Pharmacy. An expiration date of 2 weeks will be indicated on the 4 ml bottles and 180 days will be indicated on the 15 ml bottles. based on a 2 year shelf life from the manufacturer.

Treatment Assignment

The proposed study is a parallel four-group design where one group will receive a placebo spray and the other three groups will receive active treatment sprays, EST or TA or BEV. Patients will be randomized into one of the four groups based on stratification for initial epistaxis frequency (at least once a week but less than 7 times per week versus at least 7 times per week) and study site. The randomization will be issued in blocks of 4 for each site. Treatment assignment will be known only to O'Brien Pharmacy and the statistician.

Treatment Dispensing

All treatments will be dispensed from O'Brien Pharmacy in 12 week allotments (4 bottles, numbered 1-4) and shipped 2nd day air on ice directly to the patient. Bottle 1 will be stored refrigerated by the patient, while the remaining bottles will be kept at room temperature. Preliminary experience with BEV indicates that a topical nasal exposure of 1-2 weeks or a single submucosal nasal injection results in a treatment response of at least 3-4 months. Accordingly, patients randomized to BEV will only receive active drug during week 1, and all other groups will receive active treatment for 12 weeks. This treatment schema is shown in Table 2. All bottles will be consecutively numbered and patients will have a printed schedule indicating specific dates during which to use each bottle. This treatment schedule will be reinforced by telephone call or email on a regular basis.

Contraception

Bevacizumab carries a class C warning for Pregnancy (teratogenic in animals but no human data). Sexually active females of child-bearing potential will be required to use contraception during this study and through the 24 week visit (23 weeks after the last bevacizumab treatment). Women of child bearing potential will be defined as non-menopausal women who

have not undergone hysterectomy or bilateral oophorectomy. Appropriate methods of contraception will include tubal ligation, barrier with contraceptive jelly, non-hormonal IUD, and any standard hormonal method that does not include an estrogen (e.g. progestin only pills and progestin only IUD are acceptable).

Table 2. Treatment dispensing schema.

Treatment group	Wk 1 (bottle 1)*	Wk 2-4 (bottle 2)	Wk 5-8 (bottle 3)	Wk 9-12 (bottle 4)
Placebo	P	P	P	P
EST	EST	EST	EST	EST
TA	TA	TA	TA	TA
BEV	BEV	P	P	P

*Week 1 is dispensed in 4 ml bottles; all other treatments are dispensed in 15 ml bottles.
Definitions: Wk=Week; P=Placebo; EST=Estriol; TA=Tranexamic acid; BEV=bevacizumab.

Adherence Evaluation

Adherence with spray use will be assessed at each visit to the research facility. Patients will be asked to save the spray bottles and turn them in at each visit. Residual volume in each bottle will be measured with a 10 ml graduated cylinder at each treatment center to assess adherence with treatment. Patients will also be asked to make an assessment of how many doses of spray they used each week, and this will be recorded in the Nosebleed Diary.

Concomitant Medications and Nasal Irrigation

Information about concomitant medications will be collected at each phone or facility visit and recorded in the case report form (CRF). The patient will be allowed to use nasal sprays and ointments (e.g. petrolatum or saline) if they are already taking them at baseline and as long as they are not listed under the exclusion criteria. Any such spray or ointment is to be applied at least 30 minutes following the use of the treatment spray. The following medications are forbidden to be initiated after enrollment in this study and will be grounds for withdrawal from the study if taken for more than 1 week: any estrogen, tranexamic acid, or epsilon aminocaproic acid by any route; nasal ointments or creams that are not part of this study. Any use of VEGF inhibitors outside of this study will be grounds for withdrawal. If any of the above medications were initiated for the treatment of epistaxis, these patients will be recorded as treatment failures. The following medications are forbidden to be initiated after enrollment in this study and will be grounds for withdrawal from the study (but not treatment failure) if taken for more than 4 days: warfarin or other vitamin K antagonists at any dose; unfractionated or low molecular weight heparins at standard doses for treatment of venous thromboembolism (VTE); clopidogrel; or aspirin at >325 mg/day. Initiation of short term treatment with heparins at standard doses for VTE prophylaxis or aspirin at ≤325 mg/day are allowed for up to 2 weeks.

If some form of nasal irrigation is used by the patient at baseline, it should be continued during the study in the same fashion. Nasal irrigation should be performed before using the treatment spray.

For patients using concomitant sprays, ointments, and/or nasal irrigation at baseline, the appropriate sequence of treatments would therefore be: 1) nasal irrigation, 2) treatment spray, and then 3) concomitant spray or ointment 30 minutes later.

Observations

Primary Endpoints

The primary endpoint will be the frequency of epistaxis when averaged over weeks 5-12 of the treatment period when compared to placebo spray. This data will be gleaned from the Nosebleed Diary (see Appendix) which the patient will be encouraged to fill out on a daily basis. If the patient does not fill it out on a daily basis, he or she will be asked to estimate the total number of epistaxes each week and this will be entered into the last column of the frequency section of the diary. An episode of epistaxis will be defined as red blood dripping from the nose or down the back of the throat, but will not include discharge of bloody mucus during nose blowing or episodes that are triggered by routine nasal irrigation.

Secondary Endpoints

1. Duration of epistaxis. This data will be gleaned from the Nosebleed Diary (see Appendix) which the patient will be encouraged to fill out on a daily basis. If the patient does not fill it out on a daily basis, he or she will be asked to estimate the average duration of epistaxis each week and this will be entered into the last column of the duration section of the diary. As the duration of epistaxis can be difficult to define, patients will be allowed to define duration as they have done in the past. If they do not already have a sense for how to determine duration, they will be encouraged to determine it from the beginning of an episode (see above) until red blood stops dripping from their nose or down the back of their throat.
2. Hoag Epistaxis Severity Score (see Appendix). This index categorizes the severity of epistaxis on a continuous 10 point scale based on the patient's answers to 6 questions about the frequency of epistaxis, the duration of epistaxis, the intensity of epistaxis, previously seeking medical attention for epistaxis, the presence of anemia, and previously receiving a blood transfusion for epistaxis. It will be scored by the Principal Investigator or study coordinator at baseline, 12 weeks, and 24 weeks based on the medical history and the results of the hemoglobin measurement at the time of each visit.
3. Short Form-36 Health Survey (SF-36)(see Appendix). This is a health related quality of life survey that has been in use for over 12 years. On average, it can be completed in less than 10 minutes. The SF-36 instrument is divided into 36 items that assesses a patient's quality of life along eight domains (physical function, role physical, bodily pain, general health, vitality, social functioning, role emotion, and mental health) and two summary scales (physical health and mental health). Higher scores suggest less impairment of health-related quality of life. The questionnaire has good test-retest reliability and cross-sectional validity for disease-specific and physical dimensions of SF-36 but not always for the mental health dimension of the SF-36 (Ware 1992). This survey will be administered to all patients at baseline, 12 weeks, and 24 weeks.
4. Supplemental Epistaxis Questionnaire. This short survey will be administered to all patients at baseline 12 weeks, and 24 weeks. It will include 6 questions focused on HHT to supplement health related quality of life information gathered from the SF-36. These questions are partially based on the landmark study by Geisthoff et al (9):
    a. If you have changed your profession in the last 12 weeks for health related reasons related to HHT, please list the two most important reasons.
    b. If you have limited your athletic activities in the last 12 weeks for health related reasons related to HHT, please list the two most important reasons.

c. If you have experienced psychological strain related to HHT in the last 12 weeks , please list the two most important reasons.
d. If you could fix one aspect of your HHT, what would it be?
e. During the past 12 weeks, what has been the most challenging aspect of living with HHT-related nosebleeds?
f. How satisfied are you with the results of the treatment during the past 12 weeks? (A 10-point Likard scale will be used where:
    i. 1 = Very unsatisfied
    ii. 5 = Neither satisfied or unsatisfied
    iii. 10 = Very satisfied

5. Hemoglobin level and ferritin level. CBC and ferritin level will be measured at baseline and 12 weeks.
6. Transfusion history. The number of units of red blood cells transfused in the interval since the last visit will be assessed at each visit.
7. Treatment failure. Treatment failure will be defined as occurrence of any of the following during the treatment period: electrocautery, chemical cautery, or laser ablation of nasal telangiectasias; septal dermoplasty; Young's nasal closure surgery; initiation of a new treatment modality for epistaxis; withdrawal from the study due to treatment side effects; death related to epistaxis or the study protocol. The following will not be listed as treatment failure: 1) seeking medical attention in a doctor's office or emergency room, or 2) nasal packing. Study drug will be stopped immediately at the time of treatment failure.

DNA, RNA, and Plasma Biomarkers

A baseline blood sample (10 ml) will be collected and processed into four to ten 0.5 ml aliquots of plasma which will be frozen and stored at -70 F. DNA and RNA samples will be collected using the Oragene DNA (OG-500) and RNA (RE-100) saliva kits (Genotek, Ontario, Canada) and then frozen and stored at -70 F for later extraction. Before storage, samples will be de-identified and labeled with an ID number. The code list linking the patient's data to the ID numbers will be kept only at the recruiting center in a locked office or password protected spreadsheet. All samples will be sent to a central lab for long term storage. If the samples are not used within 20 years, they will be destroyed.

These samples will have two potential purposes. If one or more of the active study drugs is shown to be beneficial, these samples can be examined to look for baseline predictors of treatment response. A second purpose will be to examine correlates between baseline clinical features (epistaxis severity, other HHT manifestations such as lung AVM, and telangiectasia scoring) and DNA, RNA, or biochemical mediators at baseline. Use and analysis of these samples will not be part of the scope of this protocol and grant – only their collection. Any future use or analysis will stem from a future protocol and/or grant.

Other Observations

Agreeable patients will undergo nasal endoscopy at baseline in order to stage their telangiectasias using the Shapshay Classification. Endoscopy will be performed with patients awake and seated. Immediately prior to the procedure, oxymetazoline or phenylephrine nose drops will be instilled to decongest the nasal mucosa. The nose will then be examined with a nasolaryngoscope. This observation will be used to retrospectively analyze the relationship between appearance at baseline nasal endoscopy and the following: ultimate response to treatment; HHT causative DNA mutation; baseline biomarkers; and baseline clinical aspects related to epistaxis (e.g. epistaxis frequency). This assessment is considered to be standard

of care for HHT patients with nosebleeds and therefore patients may still enroll in the study if they choose not to undergo nasal endoscopy.

Safety Analysis and Adverse Event Reporting

Safety endpoints in this study will include adverse events (AE) and discontinuation of study treatment due to AE. An AE will be defined as any untoward medical occurrence, including an unexpected exacerbation of a pre-existing condition, that occurs during the 24 week study period. The baseline history, physical examination, and laboratory data will be used as the reference point for determining the presence of an AE. Patients will be specifically queried about the following symptoms and occurrences (safety questions) at each visit: nausea, vomiting, diarrhea, abdominal pain, visual changes (acuity, field, color), breast tenderness and swelling, hot flashes, edema, headaches, muscle cramps, vaginal bleeding in postmenopausal women, thromboembolic events, cough, chest pain, gastrointestinal and lung bleeding, nasal symptoms, change in cutaneous telangiectasias, and unexpected benefits from treatment. The nasal septum will be inspected at baseline and again at week 12 for septal perforation. Any significant AE should be further evaluated according to standard medical guidelines.

The intensity of each AE will be categorized as mild (easily tolerated), moderate (interferes with daily activities) or severe (incapacitating or causing inability to work or perform usual activities). Medical judgment will be used to determine the causal relationship of study treatments or procedures to the AE:

- YES, there is a reasonable possibility (i.e. there is evidence to suggest a causal relationship) that the study drug or procedure caused the AE, or
- NO, there is no reasonable possibility that the study drug or procedure caused the AE.

A serious adverse event (SAE) will be defined as any AE which:

- Results in death.
- Is immediately life-threatening.
- Results in persistent or significant disability.
- Requires or prolongs patient hospitalization.
- Is a congenital anomaly/birth defect.
- Based on medical judgment, requires intensive medical treatment or surgical treatment to prevent one of the above outcomes.

All AE will be documented in the CRF and reported to the DSMB intermittently in tabular form. All SAE should be reported to the Sponsor (James Gossage) as soon as possible, but no later than 72 hours after the Site confirms that the event is an SAE. For all SAE, the Sponsor will determine (with the assistance of the submitting Site), whether that SAE is expected or unexpected, and whether that SAE is causally related to the drug or study procedure. All SAE that are both unexpected and causally related to the study drug or procedure will be reported to the FDA and other Sites as soon as possible, but no later than 15 calendar days after the Sponsor determines that the event qualifies for reporting. In case of a fatal or life-threatening SAE that is both unexpected and causally related to the study drug or procedure, the FDA and other Sites will be notified by telephone or facsimile transmission no later than 7 calendar days after the Sponsor determines that the event qualifies for reporting. Any severe AE (expected or unexpected) that is judged to be causally related to study drug (by either the investigator or DSMB) will be grounds for potential cessation of study drug. SAE will be reported to the DSMB as outlined in the DSMB section. In the case where causality is uncertain AND the

patient has experienced a dramatic clinical benefit from the study drug, the DSMB may request unblinding of the treatment to assist in the determination of causality. If the patient is found to be on active drug and causality is reasonable, the decision to stop the study drug will be made based on whether the perceived benefit is outweighed by the perceived adversity as determined by the DSMB. All AE and SAE at each participating Site should also be reported to that Site's local IRB according to that IRB's guidelines.

DSMB

An independent data safety monitoring board will include 4 persons: an otolaryngologist who is expert in HHT, Dr. Terrence Davidson from UCSD University; a hematologist/oncologist, Dr. Abdullah Kutlar from Georgia Health Sciences University; a reproductive endocrinologist, Dr. Lawrence Layman from Georgia Health Sciences University; a pulmonologist, Dr. Jeffery Hoag, from Drexel University. The DSMB will confer by conference call on at least the following occasions (a quorum of at least 2 members will be necessary): following enrollment of the first 10 patients; following 12 weeks of treatment of the first 10 patients; following 12 weeks of treatment of the first 50 patients; following 12 weeks of treatment of the first 90 patients; following 24 weeks after enrollment of the final patient; and within 1 week of any serious adverse event (SAE) that is judged as potentially treatment related. In case of an SAE that is judged as not treatment related, the details will be communicated to the DSMB by email or phone within 72 hours of its occurrence, and a conference call will be convened at their request. Due to the predicted low risk of adverse events, no stopping rules have been developed, per recommendation of our Biostatistician.

Statistics

The proposed study is a parallel four-group design where one group will receive a placebo spray and the other three groups will receive active treatment sprays, EST, TA or BEV. Patients will be randomized into one of the four groups based on stratification for initial epistaxis frequency (at least once a week but less than 7 times per week versus at least 7 times per week) and study site. The randomization will be issued in blocks of 4. The primary outcome is the number of epistaxes per week averaged over weeks 5-12. Secondary outcomes will be: nosebleed duration (averaged over weeks 5-12), the Hoag Epistaxis Severity Score at week 12; quality of life measured using the SF-36 at week 12; patient satisfaction with treatment, hemoglobin and ferritin levels at week 12; units of red blood cells transfused (total during the 8 weeks prior to baseline versus the total during weeks 5-12); and the incidence of treatment failure. Rate of study drop-out or non-compliance will be compared between the groups to assess possible group differences.

The data will be assessed for normality, as well as for other assumptions of analysis of variance (ANOVA), and appropriate transformations will be used when necessary. The differences between the four groups for the primary outcome, patient satisfaction, and number of transfusions at the end of the treatment period (12 weeks) will be analyzed using a one-way ANOVA. Changes (post-study minus pre-study) in quality of life, CBC, platelets and ferritin levels, and ESS will be analyzed using analysis of covariance (ANCOVA) adjusting for pre-study values as a covariate before assessing group differences. The effect of the end of treatment will be assessed by calculating the difference in the average frequency of nosebleeds from 5-12 weeks (the final 8 weeks of the study period) and the average frequency from 13 to 24 weeks (the 12 week follow up period). ANCOVA will be used to adjust for nosebleed frequency during treatment before assessing group differences for a possible rebound effect after treatment cessation. When there are significant ANOVA or ANCOVA

results the adjusted group means will be compared using a Tukey's adjustment for the multiple comparisons. Contrasts will also be used to test for the difference between P vs. each of the treatment nasal sprays. Chi-square or Fisher's exact test will be used to assess differences in the rate of complications for each group. SAS 9.2 (SAS Institute, Inc, Cary, NC) will be used for all analyses.

Power Analysis

This is a four group placebo controlled design. The sample size for this study is based on frequency data from the Hoag ESS survey and hypothesized results for the primary outcome of epistaxis frequency shown in Table 3.

Table 3. Power Analysis

Group	Epistaxis frequency (per week)*
Placebo	7 ± 5
EST	3 ± 5
TA	3 ± 5
BEV	3 ± 5

* Reported as mean ± SD.

Patients who have at least one nose bleed per week will be recruited into the study. Based on preliminary data the average number of nose bleeds per week for these patients is 7.4±5.1. We expect a minimal placebo effect such that the average number of nose bleeds in the placebo group will be seven per week with a standard deviation of five per week. We hypothesize that treatment nasal sprays will reduce the average frequency by 50-60% (i.e. 3 per week). A sample size of 30 patients in each group (total N=120) will provide 89% power at alpha=0.05 to detect an overall significant ANOVA effect and at least 85% power to detect significant placebo vs. treatment contrasts. To account for an anticipated 15% drop out and/or noncompliance rate we will recruit 35 patients for each arm of the study (total N=140).

Record Keeping

Source documents will include the patient's hospital medical record (both electronic and paper) and documents collected only for research purposes (e.g. the Nosebleed Diary or SF-36). Information from the source documents will be transferred to specific CRFs. If data are collected onto the CRF for research purposes only, that CRF will serve as both the source document and CRF. CRFs will be stored in the patient's research binder. All research related records will be kept in a locked cabinet in the locked office of the Research Coordinator for this study or in the locked office of the Principal Investigator at each recruiting center. The code list linking the patient's data to the ID numbers will be kept only at the recruiting center in a locked office or password protected spreadsheet. For the purpose of analysis, de-identified data will ultimately be entered into a computer spreadsheet which will be password-protected and stored on the GHSU network (which is backed up off site on a daily basis).

Study Limitations

It is possible that 12 weeks is too short of a time for optimum response to the study medications, especially for the estriol spray. A prior study of estriol ointment in 12 patients evaluated nasal biopsies at 3, 6, and 12 months (Sadick 2005a). Although conversion of the nasal mucosa to a stratified squamous epithelium was not complete until 12 months, significant changes had occurred by 6 months, including development of a cobblestone pattern and loss of cilia. Historically, patients in Dr. Gossage's practice have reported benefit within 2-

12 weeks. Furthermore, Harrison and colleagues reported that oral estradiol at a relatively high dose was frequently effective at reducing transfusion requirements within 4-6 weeks of initiating treatment (Harrison 1982). We believe that for a treatment to be clinically acceptable for patients, it will have to be effective within 3 months.

The concentration of active drug in the TA and BEV groups may be inadequate. Since there are limited data with the use of these agents in HHT-related epistaxis, the proper concentration or dose is uncertain. However, multiple studies have previously demonstrated control of surgical bleeding using TA solution at a concentration of 100 mg/ml (10%). There are also several studies showing efficacy of a TA mouthwash at a concentration of 4.8% to treat dental bleeding following dental extractions. The concentration used for BEV is based on a case report using BEV nasal spray at a 1% concentration, and multiple papers studying the use of 1% BEV eye drops in the treatment of various ocular diseases.

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EPISTAXIS SEVERITY SCORE (ESS) FOR HHT (Version 06/07/11)

The purpose of these questions is to calculate a severity score of epistaxis (nose bleeding) for patients with Heredity Hemorrhagic Telangiectasia who are taking part in the HHT NOSE study.

Please answer each of the following questions as they pertain to your TYPICAL (usual or most common) symptoms during the past 4 weeks.

1. How often did you TYPICALLY have nose bleeding during the past 4 weeks?
   - ☐ Less than monthly [0]
   - ☐ Once per month [1]
   - ☐ Once per week [2]
   - ☐ Several per week [3]
   - ☐ Once per day [4]
   - ☐ Several per day [5]

2. How long did your nose bleeding episodes TYPICALLY last during the past 4 weeks?
   - ☐ Less than 1 minute [0]
   - ☐ 1 to 5 minutes [1]
   - ☐ 6 to 15 minutes [2]
   - ☐ 16 to 30 minutes [3]
   - ☐ More than 30 minutes [4]

3. How would you describe your TYPICAL nose bleeding intensity during the past 4 weeks?
   - ☐ Not Typically Gushing or Pouring [0]
   - ☐ Typically Gushing or Pouring [1]

4. Have you sought medical attention for your nose bleeding during the past 4 weeks?
   - ☐ No [0]
   - ☐ Yes [1]

5. Are you anemic (low blood counts) currently?
   - ☐ No [0]
   - ☐ Yes [1]
   - ☐ I don't know [2]

6. Have you received a red blood cell transfusion SPECIFICALLY for nose bleeding during the past 4 weeks?
   - ☐ No [0]
   - ☐ Yes [1]

This is the form used to document and calculate the ESS. The bracketed numbers in blue after each response show how data were documented and analyzed for Table S1 (these numbers were not present on the actual form that the patient would fill out). For example, most patients answered option 3, 4, or 5 for Question1, and the means for that Question averaged around 4.

Nosebleed Diary FRIDAY (weeks 1-12)

Initials / ID: _____ / _____

Directions: Fill in the number of separate nosebleeds that you had each day. Try to do this each day just before you go to bed. This will be easiest if you keep your diary in the same place as your study spray. The date in the second column represents the first day of each week. At the end of each week, estimate the total number of times that you used the spray and record that in the "Doses" column (the "goal" each week should be 14). Feel free to write down any comments in the last column.

Week	Date	Fri	Sat	Sun	Mon	Tue	Wed	Thu	Doses	Comments
1	mm/dd/yy									
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										

Directions: Fill in the total number of minutes that your nosebleeds lasted each day. For example, if a bleed at 9am lasted 5 minutes, one at 12pm lasted 2 minutes, and one at 7pm lasted 23 minutes, you would record 30 minutes (5 + 2 + 23 = 30). Try to do this each day just before you go to bed.

Week	Date	Fri	Sat	Sun	Mon	Tue	Wed	Thu	Comments
1	mm/dd/yy								
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									

Version 03/13/12