ELLIPSE Study: A Phase 1 study evaluating the tolerance of bevacizumab nasal spray in the treatment of epistaxis in hereditary hemorrhagic telangiectasia.

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ELLIPSE Study: A phase-1 study evaluating the tolerance of bevacizumab nasal spray to treat epistaxis in Hereditary Haemorrhagic Telangiectasia

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ABSTRACT

Background: Hereditary hemorrhagic telangiectasia is a dominantly inherited genetic vascular disorder in which epistaxis is the most frequent manifestation responsible for high morbidity. Management of this symptom has no standard and local treatments are often aggressive, their efficacy is variable and has not been proven. Antiangiogenic drugs, such as bevacizumab, are a new treatment strategy. Its systemic administration in patients with HHT improves liver damage-related symptoms and epistaxis. To limit the systemic adverse effects of bevacizumab and to ease administration, a local administration seems suitable.

Primary objective: to evaluate the tolerance of increasing doses of bevacizumab administered as a nasal spray in patients with HHT-related epistaxis. Secondary objectives were to study bevacizumab bioavalability and efficacy against epistaxis when given as nasal spray.

Methodology: Phase-1, randomized, double-blind, placebo-controlled, monocentric study carried out sequentially (dose escalation) on 5 groups of 8 patients. Each group was made up of 6 verum and 2 placebos. Five increasing doses of bevacizumab nasal spray (25 mg/mL) were evaluated: 12.5, 25, 50, 75 and 100 mg.

Results: A total of 41 patients were included between October 2011 and October 2012. Bevacizumab nasal spray was well tolerated in all patients and the drug was not detected in their serum. No dose limiting toxicity was observed. No efficacy was observed at any dose in this study.

Conclusion: Based on these results, bevacizumab nasal spray is a safe treatment of epistaxis in HHT. However, a randomized phase 2 study is needed to determine its efficacy.

Trial Registration: ClinicalTrials.gov Identifier #NCT01507480

Words: 237

INTRODUCTION

Hereditary hemorrhagic telangiectasia (HHT) (OMIM#187300) is a dominantly inherited genetic vascular disorder characterized by recurrent epistaxis, cutaneous telangiectasia and visceral arteriovenous malformations (AVM) that affect many organs, including the lungs, gastrointestinal tract, liver and brain. Diagnosis is based on the Curaçao criteria and is considered definite if at least three of the four following criteria are fulfilled [1]: 1) spontaneous and recurrent epistaxis, 2) telangiectasia, 3) family history and 4) visceral lesions.

The most apparent expression of the disorder is the occurrence of spontaneous, repeated epistaxis [2]. These epistaxis can be severe and life threatening; they are often the cause of chronic anaemia, and can require continuous martial treatment and multiple transfusions. The handling of this major symptom is badly coded and often demands local treatments or medication whose efficacy is not sufficiently documented [3], [4].

Two genes are associated with HHT: *ENG* coding for endoglin[5] and *ACRLV1* coding for the activin receptor-like kinase 1, ALK-1[6]. Mutations in either one of these two genes account for most clinical cases. In addition, mutations in *MADH4 (*encoding SMAD4), which are responsible for juvenile polyposis / HHT overlap syndrome, have been described[7]. *ENG* and *ACVRL1* encode endothelial cell transmembrane proteins that appear to be components of the receptor complexes for growth factors of the Transforming Growth Factor-beta superfamily (TGF-beta). It has thus been hypothesized that HHT is related to an imbalanced state between anti-angiogenic factors and pro-angiogenic factors (such as VEGF)[8].

Because of the molecular mechanisms involved in both angiogenesis and HHT, as well as the mechanisms of action of anti-VEGF such as bevacizumab, a prospective study has been performed using intravenous bevacizumab in severe hepatic forms of HHT and reported a significant improvement of liver consequences as well as epistaxis[9]. To limit the systemic

adverse effects of bevacizumab and to ease administration, a local administration seemed suitable. We therefore investigated bevacizumab transport through porcine nasal mucosa to determine antibody bioavailability and we evidenced absorption of bevacizumab into nasal mucosa[10]. Furthermore, several published cases reported a potential efficacy of bevacizumab nasal spray[11-16].

Our aim was to evaluate safety of bevacizumab nasal spray. The secondary objectives were (1) to study systemic passage and pharmacokinetic of bevacizumab in blood after nasal spray delivery, (2) to evaluate efficacy on epistaxis (duration and number), on anemia (hemoglobinemia and ferritinemia) and on number of blood transfusions.

MATERIALS AND METHODS

This trial was registered with the ClinicalTrials.gov Identifier #NCT01507480. Enrolment began in October 2011.

Study design: Phase-1, randomized, double-blind, placebo-controlled, monocentric study carried out sequentially (dose escalation) on 5 groups of 8 patients. Each group was made up of 6 verum and 2 placebos. Five increasing doses of bevacizumab nasal spray (25 mg/mL) were evaluated: 12.5, 25, 50, 75 and 100 mg. To escalate to a higher dose level, at least sixth of the eight patients of each dose level should have completed 14 days of follow-up with no dose-limiting toxicity. At each dose level a safety assessment was carried out by a scientific committee. Adverse events (AE) were classified as certainly, probably, possibly or dubitable. Dose-limiting toxicity was defined by any grade three and four toxicity events on the National Cancer Institute's common toxicity criteria scale (NCI-CTC version 4.0) with the exception of rest dyspnea, epistaxis, anemia associated with epistaxis or the chronic digestive hemorrhages associated with HHT before treatment. The sample size was based on previous pilot studies and was pragmatic with regard to determining toxicity-based dose escalation[17]. Thus, it was planned to randomize a minimum of 16 and a maximum of 64 patients by including 5 groups of 8 patients and, if necessary 3 additional groups of 8 patients in case of dose-limiting toxicity, justifying a double sample for the dose.

Patients had a 3 months follow-up with visits at 14, 30 and 90 days after treatment including physical examination, laboratory testing (hemoglobinemia, ferritinemia), and assessment for adverse events.

Patients' selection

This study enrolled patients older than 18 years old, with clinically confirmed HHT (the presence of at least three of the Curaçao criteria) suffering from epistaxis (more than 30

minutes a month as a mean over a three month-period assessed using specific grids filled by the patients).

The main non-inclusion criteria were high blood pressure, presence of nasal septal perforation before treatment and previous bevacizumab treatment. Potentially eligible patients were identified during a 3 month-screening period. Compliance to treatment and ability to complete epistaxis grids were evaluated over this period. Patients were included chronologically by dose level. Within each dose level, randomization was performed for the allocation of verum or placebo.

This study was approved by the local research ethics committee and by the French Medical Products Agency (AFSSAPS/ANSM). Oral and written informed consent were obtained from all patients in accordance with national regulations.

Treatment

Patients received a one day treatment of bevacizumab or placebo intranasally. Bevacizumab (Avastin® 25 mg/mL, Roche, Basel, Switzerland: bevacizumab, trehalose dihydrate, sodium phosphate, polysorbate 20, and water for injections) was not diluted and packaged by a pharmaceutical department in a calibrated nasal spray bottle which delivered 0.05 or 0.1 ml per nebulization according to the dose. Each patient received 1 to 4 nasal nebulization, according to the dose, administered every 30 minutes into each nostril for 2 hours during a one-day hospitalization period in a phase I/II unit. The placebo used was 0.9% sodium chloride.

Outcome measures

The main criterion was to evaluate safety at each visit by physical and nose examination, as well as laboratory testing, and assessment for adverse events.

Secondary evaluation criteria were:

(1) Systemic passage and pharmacokinetic of bevacizumab. Its concentrations were measured in blood samples collected before treatment and, 2, 4, 6 and 24 hours after treatment. Bevacizumab serum concentrations were measured using a validated ELISA technique [18].

(2) Daily epistaxis report using a grid to record daily duration and number of episodes. Hemoglobinemia, ferritinemia (laboratory testing) and number of blood transfusions were systematically recorded at each visit.

Statistical analysis

The efficacy population was defined by all randomized patients and the safety population by all treated patients. Quantitative parameters at inclusion were presented as mean ± standard deviation and median (minimum and maximum) for two groups (placebo group and bevacizumab group considered as a whole) and were compared using Student t-test (or Mann-Whitney test in case of non-normality). Qualitative parameters at inclusion were presented in terms of number (percentage) and compared using chi-square test (or Fisher exact test where conditions for chi-square test were not fulfilled).

For the analysis of safety, number of related and graded adverse event was counted for bevacizumab and placebo groups. For the analysis of secondary outcomes (efficacy criteria), monthly mean of number and duration of epistaxis (over a period of 3 months) and number of transfusions were compared before and after treatment using a Student's test for dependant sample (or Wilcoxon signed rank test in case of non-normality). Differences between monthly mean of number and duration of epistaxis before and after treatment were compared between placebo group and bevacizumab group with Student t-test (or Mann-Whitney test in case of non-normality). Trend over time on haemoglobinemia and ferritinemia was assessed using a

mixed model for repeated measures. All analyses were performed using SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA).

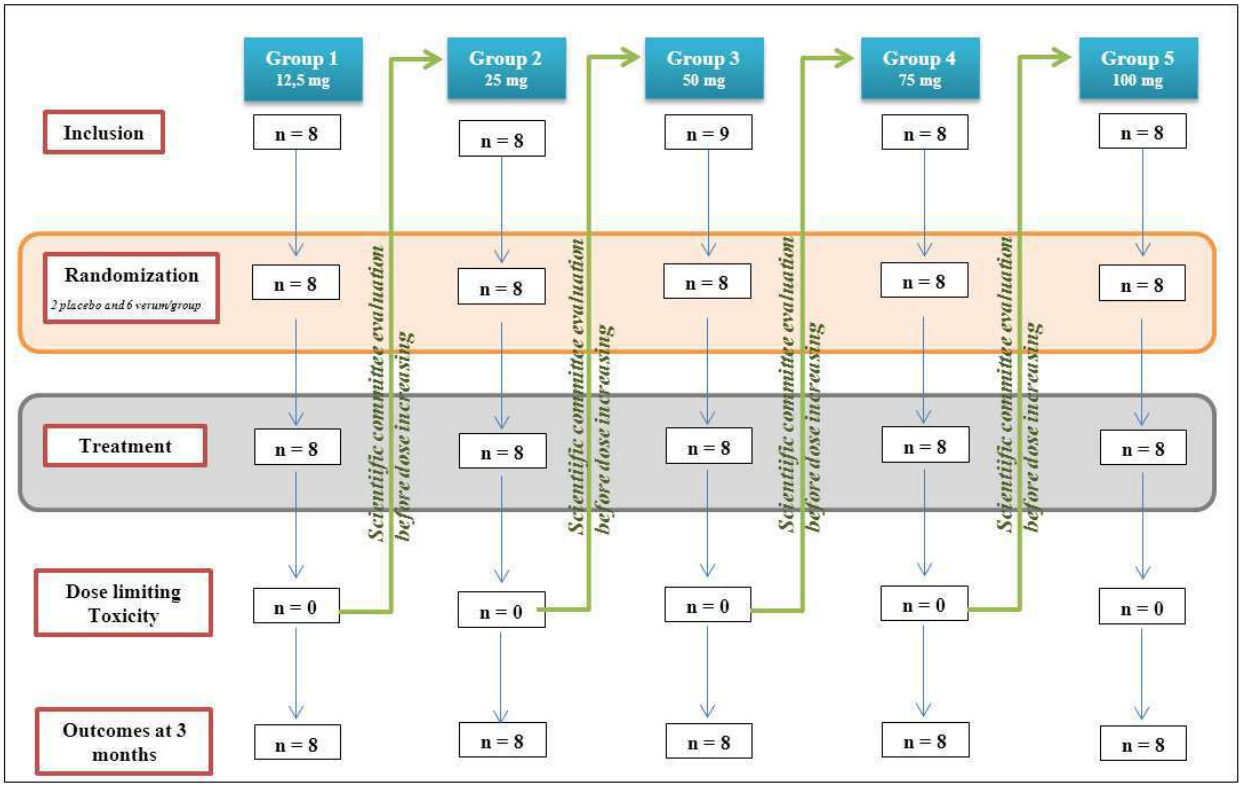
RESULTS

Forty-two inclusions corresponding to forty-one patients occurred between October 2011 and October 2012. Forty patients were randomized (one patient was excluded due to an intercurrent pathology). One patient was included and randomized twice since he was excluded due to fever before treatment visit (non-inclusion criterion). After disappearance of this criterion, he was finally again included and randomized. Two patients were included whereas they had an old perforation of the nasal septum which was not detected at inclusion but after treatment.

Finally, 40 patients (5 groups of 8 patients) received the treatment as indicated in the flowchart of the study (figure 1).

No dose limiting toxicity was observed. Therefore, the the following dose levels were tested: 12.5 mg, 25 mg, 50 mg, 75 mg and 100 mg.

Figure 1: Flowchart of the study



Patients' characteristics before treatment (n=40) are summarized in table 1. Placebo and bevacizumab groups were similar except for monthly number of epistaxis. The mutated gene was *ACVRL1* in 25 cases, *ENG* in 14 cases and not known in 1 cases.

Table 1: Patients' characteristics before treatment

Variable	Expression	Entire group n = 40	Placebo group n = 10	Treatment group n = 30	p-value
Age (years)	Mean ± SD	56.8 ± 10.2	57.9 ± 8.9	56.5 ± 10.7	0.93
	Median	57.9	59.3	57.1	
	(Min - Max)	(38.7 - 75.5)	(40.6 - 72.7)	(38.7 - 75.5)	
Females (%)	n (%)	21 (52.5)	6 (60.0)	15 (50.0)	0.91
Body mass index (kg/m²)	Mean ± SD	25.7 ± 4.6	24.9 ± 3.3	26.0 ± 5.0	0.94
	Median	24.5	24.8	24.5	
	(Min - Max)	(19.0 - 37.5)	(20.3 - 30.7)	(19.0 - 37.5)	
ENT surgery-before treatment	n (%)	15 (37.5)	4 (40.0)	11 (36.7)	1.00
Laser	n (%)	10 (25.0)	3 (30.0)	7 (23.3)	0.88
Biological glue	n (%)	2 (5.0)	1 (10.0)	1 (3.3)	0.59
aetoxysclerol	n (%)	2 (5.0)	1 (10.0)	1 (3.3)	0.59
Arterial embolisation	n (%)	1 (2.5)	0 (0.0)	1 (3.3)	1.00
Arterial surgery	n (%)	1 (2.5)	0 (0.0)	1 (3.3)	1.00
Other treatment	n (%)	6 (15.0)	0 (0.0)	6 (20.0)	0.42
Epistaxis/month					
Duration	Mean ± SD	165.9 ± 110.5	155.8 ± 112.9	169.23± 111.4	
	Median	128.5	121.5	158.5	
	(Min - Max)	(26 - 490)	(52 - 451)	(26 - 490)	0.94
Number	Mean ± SD	23.1 ± 14.5	30.7 ± 15.2	20.5 ± 13.5	0.07
	Median	20	26.5	18.5	
	(Min - Max)	(5 - 62)	(13 - 58)	(5 - 62)	
Hemoglobinemia (g/L)	Mean ± SD	122.0 ± 23.5	122.0 ± 26.0	122.0 ± 23.1	0.98
	Median	125.5	132	124	
	(Min - Max)	(72 - 161)	(75 - 155)	(72 - 161)	
Ferritinemia (µg/l)	Mean ± SD	34.7 ± 32.1	23.8 ± 22.3	38.4 ± 34.2	0.24
	Median	26.5	13.5	29	
	(Min - Max)	(1 - 171)	(1 - 72)	(1 - 171)	

Adverse effects

No grade 3 and 4 certainly or probably related adverse events (AE) were recorded. Three grade 2 AE were considered as dubitable or possibly related (rhinopharyngitis (n=1), cephalgia (n=1), moderate blood hypertension (n=1)). Lastly, 8 grade 1 AE (nausea (n=1), vomiting (n=1), asthenia (n=1), erythemia (n=1), headache (n=4)) were registered among 30 patients treated with bevacizumab .Three AE were observed among 10 patients from placebo group (headache (n=2) and skin rash (n = 1)). No related AE was observed among the 2 patients with nasal septum perforation.

Two unrelated serious AE were observed (anemia (n=1) and uterine surgery (n=1)) among patients treated with bevacizumab *versus* one unrelated serious AE among placebo group (retinal detachment (n = 1)).

Bevacizumab pharmacokinetics

Bevacizumab has not been detected in any blood samples whatever the sampling time.

Efficacy on epistaxis is summarized in table 2 and figure 2. No significant difference was observed before and after treatment on epistaxis (number and duration) or blood transfusions between bevacizumab and placebo groups. Duration of epistaxis after treatment was also not significant between bevacizumab and placebo groups (p=0.628).

There was no significant trend over time on ferritinemia and haemoglobinemia whatever the dose level (figure 2).

Table 2: Efficacy on epistaxis

Variable		Group 1 12.5 mg n=6	Group 2 25 mg n=6	Group 3 50 mg n=6	Group 4 75 mg n=6	Group 5 100 mg n=6	Placebo group n=10
Epistaxis duration before treatment (min /month)	Mean ± SD	79.22 ± 57.90	143.52 ± 93.55	214.12 ± 102.53	244.58 ± 157.09	177.82 ± 73.54	164.68 ± 123.73
	Median (Min - Max)	65.1 (26.8 - 175.1)	120.8 (55 - 307.5)	202.2 (98.7 - 370.2)	193.75 (57.4 - 500.4)	170.95 (85 - 301.4)	126.7 (48.3 - 490.4)
Epistaxis duration after treatment (min /month)	Mean ± SD	77.12 ± 57.12	131.88 ± 136.77	274.68 ± 273.91	179.45 ± 117.02	187.70 ± 95.15	127.02 ± 68.10
	Median (Min - Max)	52 (28 - 167.9)	107.8 (18.9 - 393.5)	177.25 (102.9 - 830.3)	189 (39.6 - 372.5)	214.2 (19.9 - 276.3)	151.15 (13.3 - 202.4)
p-value		0.89	0.64	0.68	0.10	0.76	0.11
Epistaxis number before treatment (n /month)	Mean ± SD	24.53 ± 15.95	19.67 ± 10.11	10.98 ± 6.02	23.45 ± 20.49	29.90 ± 17.95	29.99 ± 15.26
	Median (Min - Max)	20.35 (10.8 - 55.4)	18.15 (7.7 - 33.4)	9.8 (5.1 - 21.1)	16.15 (9 - 64.7)	27.55 (8.4 - 59.9)	27.8 (12.5 - 66.2)
Epistaxis number after treatment (n /month)	Mean ± SD	20.77 ± 10.20	17.45 ± 18.15	11.37 ± 6.02	24.82 ± 20.38	30.28 ± 17.98	26.75 ± 12.82
	Median (Min - Max)	16.7 (10.1 - 37)	11.55 (3.7 - 52.8)	9.9 (6 - 21.9)	17.35 (10.4 - 64.1)	24.75 (10.1 - 58.2)	25.8 (3.3 - 43.5)
p-value		0.29	0.74	0.11	0.68	0.91	0.45

Figure 2: Efficacy on biological parameters (2a: Hemoglobine level before and after treatment, 2b: Ferritinemia before and after treatment)

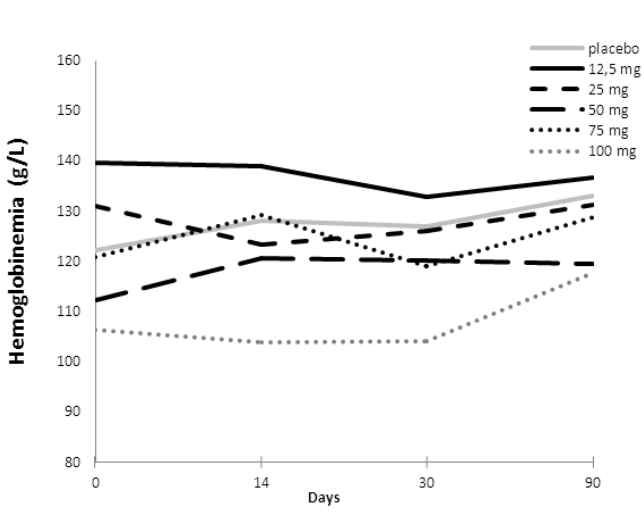


Fig 2a

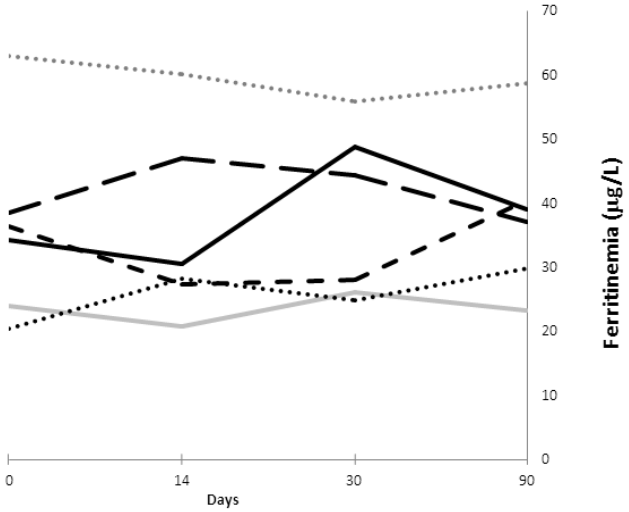


Fig 2b

DISCUSSION

This is the first prospective phase 1 clinical trial on bevacizumab nasal spray in HHT patients. Our results show that intranasal bevacizumab was very well tolerated immediately, 30 and 90 days after a one day nasal spray administration, whatever the dose. Preclinical study previously suggested that bevacizumab was well tolerated by mucosa[10], as did retrospective studies[16]. In the present study, no treatment-related adverse event was observed. Patients were carefully followed for nasal cartilaginous septum perforations which have been described as a side effect of intravenous bevacizumab in cancer patients[19-22]. This complication was also described after bevacizumab submucosal injection[14,23] or laser treatment but never with topical treatment. In this study, nasal cartilaginous septum perforation was not observed following treatment administration.

No systemic absorption was evidenced. However, we cannot exclude that bevacizumab may be measured in serum after repeated nasal administration. Bevacizumab has a high molecular weight (149 kDa), a characteristic which should limit transport through biological membranes. However, FcRn, a receptor expressed on many epithelial surfaces including bronchial cells, may allow the transcellular transfer of IgG through the mucosa, although its presence in the nasal mucosa has not been reported. *ex vivo* studies showed that a large amount of the antibody was able to penetrate and cross the porcine nasal cavity mucosa[10]. The limit of detection of the ELISA technique used to measure bevacizumab serum concentrations was 0.033 mg/L[18]. Bevacizumab was used without dilution and it has been shown that the drug is very stable, even after storage, allowing a nasal spray use[24]. However, the nasal mucosa of HHT patients is very often damaged and we can hypothesize that nose bleeds, nasal crusts and dry nose modified local absorption of bevacizumab. In future studies, a moistening of nasal mucosa before treatment may be discussed.

Nose bleeds are a major life threatening complication in HHT. A significant improvement of epistaxis after intravenous bevacizumab has been previously shown[9], but, to decrease the risk of systemic adverse effects of the drug, intranasal administration was developed. In the present study, no significant improvement on epistaxis and on hemoglobinia was observed after a one-day administration whatever the dose. In the literature, several case reports and pilot studies reported a potential effect of bevacizumab nasal spray on epistaxis in HHT, with different doses and frequency of administration[13-15,23,25]. However, frequency of epistaxis is highly variable in a given patient and between patients and it is currently not possible to conclude on efficacy based on those reports. We can hypothesize that a one-day treatment is not enough to act on epistaxis. Furthermore, the calculation of the sample size was not designed for a phase 2 study and maybe more patients are needed to prove a significant effect on epistaxis duration. To date, 2 phase-II studies on bevacizumab nasal spray in HHT are registered in clinicaltrials.gov and are still recruiting (NCT01397695, NCT01408030).

In conclusion, bevacizumab given by nasal spray as single dose is safe in HHT. A randomized phase 2 study is needed to assess the efficacy of this route of administration on epistaxis. Since we observed no difference between the doses tested, we cannot conclude on the most appropriate daily dose of bevacizumab which should be used in nasal spray.

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REFERENCES

1. Shovlin CL, Guttmacher AE, Buscarini E, et al. Diagnostic criteria for hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber syndrome). Am J Med Genet 2000;91:66-7.
2. Haitjema T, Balder W, Disch FJ, Westermann CJ. Epistaxis in hereditary haemorrhagic telangiectasia. Rhinology 1996;34:176-8.
3. Karapantzos I, Tsimpiris N, Goulis DG, Van Hoecke H, Van Cauwenberge P, Danielides V. Management of epistaxis in hereditary hemorrhagic telangiectasia by Nd:YAG laser and quality of life assessment using the HR-QoL questionnaire. Eur Arch Otorhinolaryngol 2005;262:830-3.
4. Folz BJ, Tennie J, Lippert BM, Werner JA. Natural history and control of epistaxis in a group of German patients with Rendu-Osler-Weber disease. Rhinology 2005;43:40-6.
5. McAllister KA, Grogg KM, Johnson DW, et al. Endoglin, a TGF-beta binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1. Nat Genet 1994;8:345-51.
6. Johnson DW, Berg JN, Baldwin MA, et al. Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2. Nat Genet 1996;13:189-95.
7. Gallione CJ, Repetto GM, Legius E, et al. A combined syndrome of juvenile polyposis and hereditary haemorrhagic telangiectasia associated with mutations in MADH4 (SMAD4). Lancet 2004;363:852-9.
8. David L, Mallet C, Mazerbourg S, Feige JJ, Bailly S. Identification of BMP9 and BMP10 as functional activators of the orphan activin receptor-like kinase 1 (ALK1) in endothelial cells. Blood 2007;109:1953-61.
9. Dupuis-Girod S, Ginon I, Saurin JC, et al. Bevacizumab in patients with hereditary hemorrhagic telangiectasia and severe hepatic vascular malformations and high cardiac output. JAMA : the journal of the American Medical Association 2012;307:948-55.
10. Samson G, Garcia de la Calera A, Dupuis-Girod S, et al. Ex vivo study of bevacizumab transport through porcine nasal mucosa. European journal of pharmaceutics and biopharmaceutics. eV 2012;80:465-9.
11. Simonds J, Miller F, Mandel J, Davidson TM. The effect of bevacizumab (Avastin) treatment on epistaxis in hereditary hemorrhagic telangiectasia. Laryngoscope 2009;119:988-92.
12. Rohrmeier C, Sachs HG, Kuehnel TS. A retrospective analysis of low dose, intranasal injected bevacizumab (Avastin) in hereditary haemorrhagic telangiectasia. Eur Arch Otorhinolaryngol 2012;269:531-6.
13. Davidson TM, Olitsky SE, Wei JL. Hereditary hemorrhagic telangiectasia/avastin. Laryngoscope 2010;120:432-5.
14. Karnezis TT, Davidson TM. Efficacy of intranasal Bevacizumab (Avastin) treatment in patients with hereditary hemorrhagic telangiectasia-associated epistaxis. Laryngoscope 2011;121:636-8.
15. Karnezis TT, Davidson TM. Efficacy of intranasal bevacizumab (Avastin) treatment in patients with hereditary hemorrhagic telangiectasia-associated epistaxis. Laryngoscope 2010.
16. Chen St, Karnezis T, Davidson TM. Safety of intranasal Bevacizumab (avastin) treatment in patients with hereditary hemorrhagic telangiectasia-associated epistaxis. Laryngoscope 2011;121:644-6.
17. Ivy SP, Siu LL, Garrett-Mayer E, Rubinstein L. Approaches to phase 1 clinical trial design focused on safety, efficiency, and selected patient populations: a report from the clinical trial design task force of the national cancer institute investigational drug steering committee. Clinical cancer research : an official journal of the American Association for Cancer Research 2010;16:1726-36.

18. Ternant D, Ceze N, Lecomte T, et al. An enzyme-linked immunosorbent assay to study bevacizumab pharmacokinetics. Ther Drug Monit 2010;32:647-52.
19. Saif MW, Mehra R. Incidence and management of bevacizumab-related toxicities in colorectal cancer. Expert Opin Drug Saf 2006;5:553-66.
20. Traina TA, Norton L, Drucker K, Singh B. Nasal septum perforation in a bevacizumab-treated patient with metastatic breast cancer. Oncologist 2006;11:1070-1.
21. Fakih MG, Lombardo JC. Bevacizumab-induced nasal septum perforation. Oncologist 2006;11:85-6.
22. Ruiz N, Fernandez-Martos C, Romero I, et al. Invasive fungal infection and nasal septum perforation with bevacizumab-based therapy in advanced colon cancer. J Clin Oncol 2007;25:3376-7.
23. Karnezis TT, Davidson TM. Treatment of hereditary hemorrhagic telangiectasia with submucosal and topical bevacizumab therapy. Laryngoscope 2012;122:495-7.
24. Kaja S, Hilgenberg JD, Everett E, Olitsky SE, Gossage J, Koulen P. Effects of dilution and prolonged storage with preservative in a polyethylene container on Bevacizumab (Avastin) for topical delivery as a nasal spray in anti-hereditary hemorrhagic telangiectasia and related therapies. Human antibodies 2011;20:95-101.
25. Guldmann R, Dupret A, Nivoix Y, Schultz P, Debry C. Bevacizumab nasal spray: Noninvasive treatment of epistaxis in patients with Rendu-Osler disease. Laryngoscope 2012;122:953-5.