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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Abbreviations: HHT, hereditary hemorrhagic telangiectasia; AVM, arteriovenous malformations; ENG, Endoglin; ACRLV1 or ALK-1, activin receptor-like kinase 1; MADH4 or SMAD4, mothers against decapentaplegic homolog 4; VEGF, vascular endothelial growth factor; PHRC, National Research Program; AE, adverse events; NCI-CTC, National Cancer Institute’s common toxicity criteria scale

Introduction

Hereditary hemorrhagic telangiectasia (HHT; Online Mendelian Inheritance in Man® #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 anemia, and can require continuous management including iron
supplementation and multiple blood transfusions. The handling of this major symptom is not well established and often demands local treatments or medication whose efficacy is not sufficiently documented.\(^3,4\)

Two genes are associated with HHT: \textit{ENG} coding for endoglin\(^5\) and \textit{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 \textit{MADH4} (encoding SMAD4), which are responsible for juvenile polyposis / HHT overlap syndrome, have been described.\(^7\) It has been hypothesized that HHT is related to an imbalanced state between anti-angiogenic factors and pro-angiogenic factors, such as vascular endothelial growth factor (VEGF).\(^8\)

Because of the molecular mechanisms involved in both angiogenesis and HHT, as well as the mechanisms of action of VEGF-targeting agents such as bevacizumab, a prospective study was performed using intravenous bevacizumab in severe hepatic forms of HHT and significant improvement of liver consequences as well as epistaxis were reported.\(^9\) To limit the systemic adverse effects of bevacizumab and to ease administration, local administration seemed suitable. We therefore investigated bevacizumab transport through porcine nasal mucosa to determine antibody bioavailability and we observed evidence of absorption of bevacizumab into nasal mucosa.\(^10\) Furthermore, several published cases reported a potential efficacy of bevacizumab nasal spray.\(^11-15\)

Our primary aim in the study reported here was to evaluate the safety in humans of a bevacizumab nasal spray. The secondary objectives were 2-fold: (1) to study systemic passage and the pharmacokinetics of bevacizumab in blood after nasal spray delivery; and (2) to evaluate efficacy on epistaxis (duration and number), on anemia (hemoglobinemia and ferritinemia) and on the number of blood transfusions.

\section*{Results}

Forty-two inclusions occurred between October 2011 and October 2012. One patient was excluded due to an intercurrent pathology, and one was included and randomized twice because he was excluded due to fever before a treatment visit (non-inclusion criterion). After the disappearance of this criterion, he was finally included again and randomized. Two patients were included who were found to have an old perforation of the nasal septum, which was not detected at inclusion but after treatment. In these two cases, the study was temporarily suspended after each of the observations and each case was discussed by the independent committee and ear/nose/throat specialists who looked at the initial nasal endoscopy and compared it to the endoscopy after treatment. In both cases, it was clear that nasal perforation was hidden by nasal crusts and has not been viewed at the time of inclusion.

In total, 40 patients (5 groups of 8 patients) received the treatment as indicated in the flowchart of the study (Fig. 1). No dose limiting toxicity was observed. Therefore, the following dose levels were tested: 12.5 mg, 25 mg, 50 mg, 75 mg and 100 mg.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{flowchart.png}
\caption{Flowchart of the study}
\end{figure}
No significant difference was observed before and after treatment. The placebo and bevacizumab groups were similar except for monthly number of epistaxis. The mutated gene quantification of 0.033 mg/L. Bevacizumab was not detected in any blood samples whatever the sampling time using a validated ELISA method and a limit of detection (n = 1)).

There was no significant trend over time on ferritinemia and hemoglobinemia regardless of the dose level (Fig. 2). This was the first prospective Phase 1 clinical study on a bevacizumab nasal spray in HHT patients. Our results show that intranasal bevacizumab was very well tolerated immediately, 30 and 90 d after a one-day nasal spray administration, regardless of the dose. Preclinical studies have previously suggested that bevacizumab was well tolerated by the mucosa, as have retrospective studies. For safety reasons, however, it was deemed appropriate to begin with a single dose to test for tolerance. In the study presented here, no treatment-related adverse events were observed. Patients were carefully followed for nasal cartilaginous septum perforation.

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

### Table 1. Patients’ characteristics before treatment

| Variable | Modality | 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 |
|----------|----------|-----------------------|---------------------|---------------------|---------------------|----------------------|----------------------|
| Age (years) | Median (Min - Max) | 52.0 ± 10.0 | 62.4 ± 10.8 | 60.3 ± 10.4 | 54.3 ± 8.2 | 53.4 ± 12.7 | 57.9 ± 8.9 |
| Females (%) | n (%) | 1 (16.7) | 2 (33.3) | 4 (66.7) | 5 (83.3) | 3 (50.0) | 6 (60.0) |
| Body mass index (kg/m²) | Median (Min - Max) | 27.0 ± 4.6 | 26.7 ± 5.4 | 27.1 ± 7.2 | 24.2 ± 3.0 | 24.9 ± 3.3 | 24.9 ± 3.3 |
| Nasal surgery | Laser | n (%) | 1 (16.7) | 2 (33.3) | 4 (66.7) | 2 (33.3) | 2 (33.3) | 4 (40.0) |
| Biological glue | n (%) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Etoxysclerol | n (%) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Arterial embolization | n (%) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Arterial surgery | n (%) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Other treatment | n (%) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Hemoglobinemia (g/L) | Mean ± SD | 139.5 ± 18.9 | 131.0 ± 23.3 | 112.2 ± 28.3 | 120.8 ± 13.8 | 106.3 ± 17.6 | 122.0 ± 26.0 |
| Ferritinemia (µg/l) | Mean ± SD | 62.8 ± 60.0 | 36.3 ± 10.3 | 38.3 ± 32.9 | 20.3 ± 8.6 | 34.2 ± 28.4 | 23.8 ± 22.3 |

Values are n (%) unless otherwise specified.

The patients’ characteristics before treatment (n = 40) are summarized in Table 1. The placebo and bevacizumab groups were similar except for monthly number of epistaxis. The mutated gene was ACVR1I in 25 cases, ENG in 14 cases and unknown in 1 case.

**Adverse effects**

No grade 3 and 4 certainly or probably related adverse events (AE) were recorded. Three AE grade 2 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 the placebo group (headache (n = 2) and skin rash (n = 1)).

No related AE were observed in 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 vs. one unrelated serious AE in the placebo group (retinal detachment (n = 1)).

**Bevacizumab pharmacokinetics**

Bevacizumab was not detected in any blood samples whatever the sampling time using a validated ELISA method and a limit of quantification of 0.033 mg/L.

**Efficacy on epistaxis**

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 the bevacizumab and placebo groups. Moreover, the dose level did not modify epistaxis (duration: P = 0.40; number: P = 0.88).

This was the first prospective Phase 1 clinical study on a bevacizumab nasal spray in HHT patients. Our results show that intranasal bevacizumab was very well tolerated immediately, 30 and 90 d after a one-day nasal spray administration, regardless of the dose. Preclinical studies have previously suggested that bevacizumab was well tolerated by the mucosa, as have retrospective studies. For safety reasons, however, it was deemed appropriate to begin with a single dose to test for tolerance. In the study presented here, no treatment-related adverse events were observed. Patients were carefully followed for nasal cartilaginous septum perforations which have been described as a side effect of intravenous bevacizumab in cancer patients. This complication was also described after bevacizumab submucosal injections or laser treatments but never with topical treatments. 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 that should limit transport through biological membranes. However, FcRn, a receptor expressed on many epithelial surfaces including bronchial cells,23 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 detection limit of the ELISA technique used to measure bevacizumab serum concentrations was 0.033 mg/L.17 Bevacizumab was used without dilution and it has been shown that the drug is very stable, even after storage, making possible use in a nasal spray.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 HHT patients. In future studies, moistening of the nasal mucosa before treatment may be considered.

Nose bleeds are a major life-threatening complication in HHT. A significant improvement in epistaxis after intravenous bevacizumab has been shown previously, 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 hemoglobinemia was observed after a one-day administration, regardless of 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.13,14,22,25 However, the 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 and that a single drug administration does not expose nasal mucosal tissue to bevacizumab in levels sufficient to improve epistaxis. Furthermore, the calculation of the sample size was not designed for a Phase 2 study and perhaps more patients are needed to prove a significant effect on epistaxis duration. To date, two Phase 2 studies on bevacizumab nasal spray in HHT have been registered in clinicaltrials.gov (NCT01397695, NCT01408030).

The study reported here has limitations related to the choice of a single dosing schedule. No preclinical or clinical data on intranasal bevacizumab clearance are available, and a single drug administration is not comparable to repeated nasal spray. Additional safety studies with a multiple administration schedule could be discussed prior to initiation of a Phase 2 trial. In conclusion, bevacizumab given by nasal spray as a 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 the most appropriate daily dose of bevacizumab that should be used in the nasal spray.

**Patients 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 performed 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 six of the eight patients at each dose level had to have completed 14 d of follow-up with no dose-limiting toxicity. At each dose level a safety assessment was performed by a scientific committee. Adverse events (AE) were classified as certain, probable, possible or dubitable. Dose-limiting toxicity was defined by any grade three or 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.16 It was thus 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 3-mo follow-up with visits at 14, 30 and 90 d after treatment including a physical examination, laboratory testing (hemoglobinemia, ferritinemia), and assessment for adverse events. Patients received a one-day treatment of bevacizumab or placebo intranasally. Undiluted bevacizumab (Avastin® 25 mg/mL, Roche: bevacizumab, trehalose dihydrate, sodium phosphate, polysorbate 20, and water for injections) was packaged by a pharmaceutical department in a calibrated nasal spray bottle that delivered 0.05 ml per nebulization depending on the dose. Each patient received 1 to 4 nasal nebulizations, depending on the total dose (12.5, 25, 50, 75 and 100 mg), administered every 30 min into each nostril for 2 h during a one-day hospitalization period in a Phase 1/2 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 examinations, as well as laboratory testing, and assessment for adverse events.

Secondary evaluation criteria were:
(1) Systemic passage and the pharmacokinetics of bevacizumab. Its concentrations were measured in blood samples collected before treatment and 2, 4, 6 and 24 h after treatment. Bevacizumab serum concentrations were measured using a validated ELISA technique.17 The limit of quantification of this method was 0.033 mg/L.
(2) A daily epistaxis report using a grid to record daily duration (hemoglobinemia, ferritinemia), and assessment for adverse events.

**Table 2. Efficacy of bevacizumab nasal spray on epistaxis duration**

| 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 79.2 ± 57.9 | 143.5 ± 93.6         | 214.1 ± 102.5        | 244.6 ± 157.1        | 177.8 ± 73.5         | 164.7 ± 123.7        |
| Median (Min - Max)                         | 65.1 (26.8 - 175.1)   | 120.8 (55.0 - 307.5)| 202.2 (98.7 - 370.2)| 193.8 (57.4 - 500.4)| 171.0 (85.0 - 301.4)| 126.7 (48.3 - 490.4) |
| Epistaxis duration after treatment (min /month) | Mean ± SD 77.1 ± 57.1 | 131.9 ± 136.8        | 274.7 ± 273.9        | 179.5 ± 117.0        | 187.7 ± 95.2         | 127.0 ± 68.1         |
| Median (Min - Max)                         | 52.0 (28.0 - 167.9)   | 107.8 (18.9 - 393.5)| 177.3 (102.9 - 830.3)| 189.0 (39.6 - 372.5)| 214.2 (19.9 - 276.3)| 151.2 (13.3 - 202.4) |
| p-value*                                   | 0.89                  | 0.64                | 0.84l                | 0.10                | 0.76                | 0.28l                |
| Epistaxis number before treatment (n /month) | Mean ± SD 24.5 ± 15.9 | 19.7 ± 10.1          | 11.0 ± 6.0           | 23.5 ± 20.5          | 29.9 ± 17.9          | 30.0 ± 15.3          |
| Median (Min - Max)                         | 20.4 (10.8 - 55.4)    | 18.2 (7.7 - 33.4)    | 9.8 (5.1 - 21.1)     | 16.2 (9.0 - 64.7)    | 27.6 (8.4 - 59.9)    | 27.8 (12.5 - 66.2)   |
| Epistaxis number after treatment (n /month) | Mean ± SD 20.8 ± 10.2 | 17.5 ± 18.1          | 14.4 ± 6.0           | 24.8 ± 20.4          | 30.3 ± 18.0          | 26.8 ± 12.8          |
| Median (Min - Max)                         | 16.7 (10.1 - 37.0)    | 11.6 (3.7 - 52.8)    | 9.9 (6.0 - 21.9)     | 17.34 (10.4 - 64.1)  | 24.8 (10.1 - 58.2)   | 25.8 (3.3 - 43.5)    |
| p-value*                                   | 0.29                  | 0.74                | 0.11                 | 0.68                | 0.91                | 0.45                 |

**Table 2.**

Efficacy of bevacizumab nasal spray on epistaxis duration

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. Undiluted bevacizumab (Avastin® 25 mg/mL, Roche: bevacizumab, trehalose dihydrate, sodium phosphate, polysorbate 20, and water for injections) was packaged by a pharmaceutical department in a calibrated nasal spray bottle that delivered 0.05 or 0.1 ml per nebulization depending on the dose. Each patient received 1 to 4 nasal nebulizations, depending on the total dose (12.5, 25, 50, 75 and 100 mg), administered every 30 min into each nostril for 2 h during a one-day hospitalization period in a Phase 1/2 unit. The placebo used was 0.9% sodium chloride.
standard deviation and median (minimum and maximum) for two groups (placebo group and bevacizumab group considered as a whole) and were compared using the Student t test (or Mann-Whitney test in case of non-normality). Qualitative parameters at inclusion were presented in terms of percentage and compared using the chi-square test (or Fisher exact test where conditions for the chi-square test were not fulfilled).

For the safety analysis, the number of related and graded adverse events was counted for the bevacizumab and placebo groups. To analyze the secondary outcomes (efficacy criteria), the monthly mean of number and duration of epistaxis (over a period of 3 mo) and number of transfusions were compared before and after treatment using a Student’s t test for a dependent 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 the placebo and bevacizumab groups with a Student t (or Mann-Whitney test in case of non-normality). To compare the different dose levels, analyses of covariance (ANCOVA) on duration and number of epistaxis after treatment were performed for 6 groups adjusted on baseline values. Trend over time on hemoglobinemia and ferritinemia was assessed using a mixed model for repeated measures. All analyses were performed using SAS software version 9.2 (SAS Institute Inc.).

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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