Case Reports in Gastroenterology

Case Rep Gastroenterol 2017;11:293–304

DOI: 10.1159/000475748
Published online: May 17, 2017

Single Case

Intravenous Bevacizumab Therapy in a Patient with Hereditary Hemorrhagic Telangiectasia, *ENG* E137K, Alcoholic Cirrhosis, and Portal Hypertension

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Keywords

Bevacizumab · *ENG* E137K · Hereditary hemorrhagic telangiectasia · Iron dextran · Transfusion · Vascular endothelial growth factor

Abstract

Intravenous bevacizumab decreased mucosal bleeding in some patients with hereditary hemorrhagic telangiectasia (HHT). We treated a 47-year-old male who had HHT, severe epistaxis, and gastrointestinal bleeding, alcoholic cirrhosis, and portal hypertension with intravenous bevacizumab 2.5 mg/kg every 2 weeks. We tabulated these measures weekly during weeks 1–33 (no bevacizumab); 34–57 (bevacizumab); and 58–97 (no bevacizumab): hemoglobin (Hb) levels; platelet counts; units of transfused packed erythrocytes (PRBC units); and quantities of iron infused as iron dextran to support erythropoiesis. We performed univariate and multivariable analyses. We sequenced his *ENG* and *ACVRL1* genes. Epistaxis and melena decreased markedly during bevacizumab treatment. He reported no adverse effects due to

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bevacizumab. Mean weekly Hb levels were significantly higher and mean weekly PRBC units and quantities of intravenous iron were significantly lower during bevacizumab treatment. We performed a multiple regression on weekly Hb levels using these independent variables: bevacizumab treatment (dichotomous); weekly platelet counts; weekly PRBC units; and weekly quantities of intravenous iron. There was 1 positive association: (bevacizumab treatment; p = 0.0046) and 1 negative association (PRBC units; p = 0.0004). This patient had the novel *ENG* mutation E137K (exon 4; c.409G→A). Intravenous bevacizumab treatment 2.5 mg/kg every 2 weeks for 24 weeks was well-tolerated by a patient with HHT due to *ENG* E137K and was associated with higher weekly Hb levels and fewer weekly PRBC units.

Introduction

Hereditary hemorrhagic telangiectasia (HHT; Osler-Weber-Rendu syndrome) is an autosomal dominant disorder characterized by telangiectases of skin and mucosae and arteriovenous malformations of the lungs, liver, and central nervous system. HHT occurs in 1 per 5,000–10,000 persons [1, 2]. The most common manifestation of HHT is blood loss from nasal and gastrointestinal telangiectases. Some patients develop anemia and iron deficiency [1–4]. Telangiectases and arteriovenous malformations progress with age in most patients. Penetrance of HHT phenotypes is almost 100% by the age of 40 years [1–4]. Plasma levels of vascular endothelial growth factor (VEGF) and transforming growth factor-β1 are increased in HHT [5]. Nasal mucosal levels of VEGF, transforming growth factor-β1, and the receptor activin receptor-like kinase 1 are also increased [6]. Mutations in genes that encode either endoglin (*ENG*, chromosome 9q34.1; OMIM #13119) [7] or activin receptor-like kinase 1 (*ACVRL1*, chromosome 12q11-q14; OMIM #601284) [8] account for most HHT cases.

Bevacizumab, a humanized anti-VEGF antibody, is an angiogenesis inhibitor used to treat several types of malignancies [9]. In an initial report, epistaxis and anemia due to HHT in a man with mesothelioma abated after he was treated with intravenous bevacizumab and pemetrexed [10]. Intravenous bevacizumab decreased severe nasal or sinus mucosal bleeding [11–20] or gastrointestinal bleeding [17, 19, 20] in other patients with HHT without diagnoses of malignancy.

We report observations on a male patient with HHT, a novel *ENG* mutation, severe epistaxis and gastrointestinal bleeding, cirrhosis, and portal hypertension who was treated with intravenous bevacizumab for an interval of 24 weeks. We analyzed his mean weekly hemoglobin (Hb) levels, platelet counts, units of packed erythrocytes administered by transfusion (PRBC units), and quantities of intravenous iron dextran infused to support erythropoiesis during the 24-week bevacizumab treatment interval with corresponding measures in preceding and succeeding intervals (33 and 40 weeks, respectively) without bevacizumab therapy using univariate and multivariable methods. We discuss our observations in the context of reports of bevacizumab treatment of other patients with HHT and severe mucosal bleeding.

Case Presentation

Clinical Course

A 40-year-old white man presented with a life-long history of innumerable telangiectases of the skin and mucosae of nasal cavities and lips. At presentation, he reported having intermittent epistaxis and melena. His mother died of central nervous system hemorrhage and his brother died of pulmonary hemorrhage. Both had HHT. Physical examination confirmed the presence of many telangiectases of the lips, nasal mucosae, and finger pads and revealed that his liver was palpable 3 cm below the right costal margin (Table 1). He had severe anemia (Hb 4.8 g/dL) and elevated serum activities of alanine and aspartate aminotransferases (Table 1). Endoscopy of the upper gastrointestinal tract revealed telangiectases of the gastric antrum and proximal duodenum (Fig. 1). CT and MRI scans did not detect vascular lesions in lungs, liver, brain, or other organs. The patient met each of the 4 Curaçao criteria for diagnosis of HHT [1]. Thus, these manifestations were interpreted as HHT, chronic blood loss anemia, and probable steatohepatitis.

The patient developed alcoholism and drank ~750 mL of liquor each day. Liver biopsy at the age of 42 years revealed severe steatohepatitis and fibrosis grade 2 (Fig. 2). He subsequently developed cirrhosis with consequent portal hypertension and splenomegaly. By the age of 45 years, he had daily epistaxis and melena attributed to HHT but no apparent bleeding from esophagogastric varices. His Hb levels were as low as 2.8 g/dL. Treatments with estrogen, vasopressin, thalidomide, aminocaproic acid, and endoscopic laser cautery did not decrease his severe mucosal bleeding and thus we treated him with bevacizumab at the age of 47 years.

We tabulated baseline laboratory measures during weeks 1–33 (Table 1). No bevacizumab was administered during this interval. In weeks 34–57 (total 24 weeks), we infused bevacizumab, 2.5 mg/kg every 2 weeks. Soon after initiation of bevacizumab treatment, his epistaxis and melena decreased markedly. He reported having greater energy and well-being during bevacizumab treatment, although his Hb level was infrequently >7.0 g/dL. A second interval of observation without bevacizumab infusion extended from weeks 58–97 (Table 1). During the 97 weeks, he received 80 units of packed erythrocytes (PRBC) and 19.7 g of iron as intravenous iron dextran.

At the age of 49 years, he developed acute abdominal pain and fever and was admitted to hospital. A CT scan of the abdomen revealed marked distension of the small bowel without free air and large varices (Fig. 3). Review of selected laboratory measures over the course of his entire illness demonstrated persistent blood loss anemia, progressive decrements of serum ALT and AST activities, progressive increments of alkaline phosphatase activity, and the development of hypoalbuminemia and splenomegaly (Table 1). Except blood loss anemia, these findings were interpreted as consequences of advanced cirrhosis due to alcoholism. He died of sepsis and liver failure without evidence of a perforated viscus.

Treatment with Packed Erythrocytes and Intravenous Iron Dextran

PRBC units were transfused, as indicated. Iron as iron dextran was administered intravenously in 500 mg doses to support erythropoiesis, as indicated [21]. We tabulated quantities of iron infused weekly (mg).

Laboratory Methods

Complete blood counts were performed using a Cell-Dyn 1800® (Abbott Laboratories, Chicago, IL, USA). Reference ranges for Hb, leukocytes, and platelets are: 12.5–16.0 g/dL, 3.5–10.5 × 10^3/μL, and 140–440 × 10^3/μL, respectively.

The genes *ENG* (NG_009551) and *ACVRL1* (NG_009549) were examined by amplification and direct sequencing to detect mutations in their coding regions and splice sites. Genomic DNA was isolated using the DNeasy Blood and Tissue Kit® (Qiagen, Gaithersburg, MD, USA). DNA amplification was performed in a 50 μL reaction mixture using 20–50 ng of genomic DNA as template, PCR buffer containing 33.5 mM Tris HCl pH 8.8, 3.35 mM $MgCl_2$, 85 μg/mL BSA, 8.3 mM $(NH_4)_2SO_4$, 0.2 mM dNTPs, and 150 ng of each primer (Table 2), for 1 cycle at 96°C for 1 min, and 30 cycles at 95°C for 1 min, 62°C for 30 s, and 72°C for 1 min. PCR products were purified using QIAquick PCR Purification Kits® (Qiagen, Gaithersburg, MD, USA) and sequenced with an ABI3100 Genetic Analyzer.

Statistics

The analytic dataset consisted of observations for each of 97 consecutive weeks. We compared means of weekly data in 3 intervals using Student *t* tests (two-tailed). We performed multiple regression on weekly Hb using these independent variables: bevacizumab treatment (dichotomous variable); weekly platelet counts; weekly PRBC units; and weekly intravenous iron (mg). We used Excel 2000 (Microsoft Corp., Redmond, WA, USA) and GB-Stat (v. 8.0 2000; Dynamic Microsystems, Inc., Silver Spring, MD, USA). Values of $p < 0.05$ were defined as significant.

Results

Intervals with and without Bevacizumab

Mean weekly Hb levels were significantly higher during bevacizumab treatment. Mean PRBC transfused and quantities of intravenous iron were significantly lower during bevacizumab treatment (Table 3).

Regression on Hb

We used the following independent variables: bevacizumab treatment (dichotomous); weekly platelet counts; weekly PRBC units; and weekly quantities of intravenous iron. Regression on weekly Hb revealed 1 positive association (bevacizumab treatment; $p = 0.0046$); and 1 negative association (PRBC units; $p = 0.0004$). This regression explained 28.6% of the variance in Hb levels (ANOVA p of regression <0.0001).

ENG and ACVRL1 Sequencing

We identified a novel mutation E137K in exon 4 of the endoglin gene (*ENG* c.409G→A). No *ACVRL1* mutation was identified.

Discussion

Intravenous bevacizumab treatment of the present patient with HHT and severe mucosal bleeding was associated with significantly higher Hb levels, fewer units of PRBC transfusions, and lower quantities of intravenous iron in univariate comparisons. There was a significant positive association of Hb levels with bevacizumab treatment, after adjustment for other variables. PRBC units were negatively associated with bevacizumab treatment, after adjustment for other variables. Despite these favorable outcomes associated with bevacizumab treatment, he had persistent mucosal bleeding and anemia.

Objective measures of the efficacy of intravenous bevacizumab in controlling mucosal bleeding are not standardized. Hb levels and PRBC units were significant indicators in the present patient. Efficacy measures reported in other patients with HHT and mucosal bleeding include Hb levels [10, 19, 22], transfusion requirements [17, 23, 24], serum ferritin concentrations [17], and frequency or severity of epistaxis [10, 23, 25, 26].

Further studies are needed to establish optimal intravenous bevacizumab doses and treatment schedules for patients with HHT. In 1 patient, bevacizumab 2.0 mg/kg every 2 weeks was beneficial [27]. In the present case and in another patient [15], there was objective benefit of bevacizumab 2.5 mg/kg every 2 weeks. The dose of 5 mg/kg every 2 weeks was effective in other case reports [10–13, 28]. In a prospective, noncomparative trial of intravenous bevacizumab therapy involving 25 patients with HHT, different maintenance regimens were evaluated using simulation based on transit compartments and direct inhibition pharmacokinetic-pharmacodynamic models [26]. The simulations predicted that monthly 5 mg/kg infusions of bevacizumab should control both cardiac index and epistaxis [26]. Objective benefits persisted weeks or months after bevacizumab therapy was discontinued in some patients [11, 15, 19, 28].

Adverse events have occurred in some patients with HHT treated with intravenous bevacizumab. Of 25 patients treated with bevacizumab 5 mg/kg every 2 weeks for management of severe hepatic vascular malformations and high cardiac output, 20 patients developed headache, nausea and vomiting, asthenia, diarrhea, or pain, and 2 patients developed grade 3 hypertension [29]. A woman treated with bevacizumab 5 mg/kg every 2 weeks developed bilateral pulmonary embolism and thromboses of the right atrium and right hepatic vein [30]. A man treated with bevacizumab 5 mg/kg every 2 weeks developed tachycardia with each infusion [23]. Headache, change in taste, diarrhea, dizziness, and delayed wound healing occurred in ≥1 of 5 patients treated with bevacizumab 0.125 mg/kg every 4 weeks [25]. These adverse events are consistent with those observed in patients with malignancy treated with intravenous bevacizumab [9]. The development of high-titer anti-bevacizumab antibodies is rare [9, 31] and has not been reported in patients with HHT.

The present patient and 2 other men treated with intravenous bevacizumab [12, 15] had different pathogenic *ENG* alleles. Although genotype/phenotype correlations differ in HHT [2, 32], plasma VEGF levels and VEGF tissue expression in HHT patients with *ENG* mutations did not differ significantly from those of HHT patients with either *ACVRL1* mutations or both *ENG* and *ACVRL1* mutations [33].

Conclusions

Intravenous bevacizumab treatment 2.5 mg/kg every 2 weeks for 24 weeks was well-tolerated by a male patient with HHT due to *ENG* E137K and was associated with higher weekly Hb levels and fewer weekly PRBC units.

Acknowledgements

This work was supported in part by the Brookwood Biomedical and Southern Iron Disorders Center. P.L.L. is supported by NIH 5R01DK53505-12.

Statement of Ethics

This work was performed in accordance with the guidelines of the Declaration of Helsinki. This work was approved by the Institutional Review Board of Brookwood Medical Center as a study protocol entitled "To treat patients in our clinic who have bleeding from hereditary hemorrhagic telangiectasia using a monoclonal antibody that is FDA approved for treatment of certain cancers." The Institutional Review Board of Brookwood Medical Center does not assign numbers to approved protocols. The patient described herein gave written informed consent for his participation and treatment as specified in the study protocol. This patient's sister and legal next-of-kin provided written consent for the present authors to publish this work after the death of her brother.

Disclosure Statement

None of the authors has a competing interest to report.

Author Contributions

L.F.B. conceived the study, treated the patient, and tabulated data. P.L.L. performed *ENG* and *ACVRL1* mutation analyses. L.L. tabulated data. J.C.B. treated the patient, performed statistical analyses, and drafted the manuscript. All authors edited and approved the manuscript in its final form.

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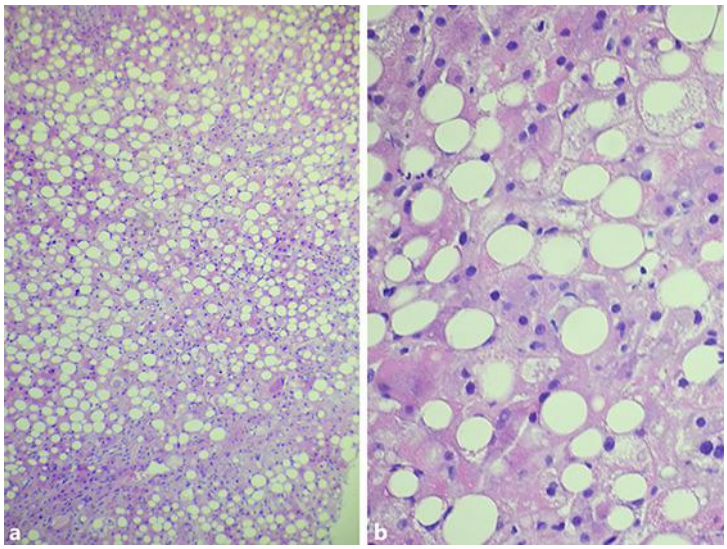

Fig. 1. Photomicrographs of the liver of a 42-year-old man with HHT and alcoholism. **a** Severe macrovesicular steatohepatitis (original magnification, 100×). **b**, Severe macrovesicular steatohepatitis (original magnification, 400×). Grade 2 fibrosis was detected with Mallory trichrome staining (not shown). Mallory bodies were not observed.

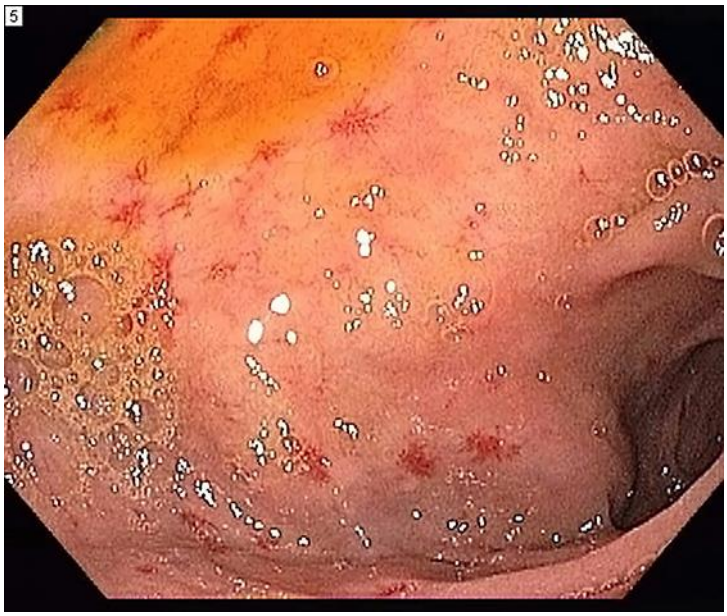

Fig. 2. Multiple spider-like mucosal telangiectases of the gastric antrum and proximal duodenum in a 40-year-old man at the diagnosis of HHT.

DOI: 10.1159/000475748

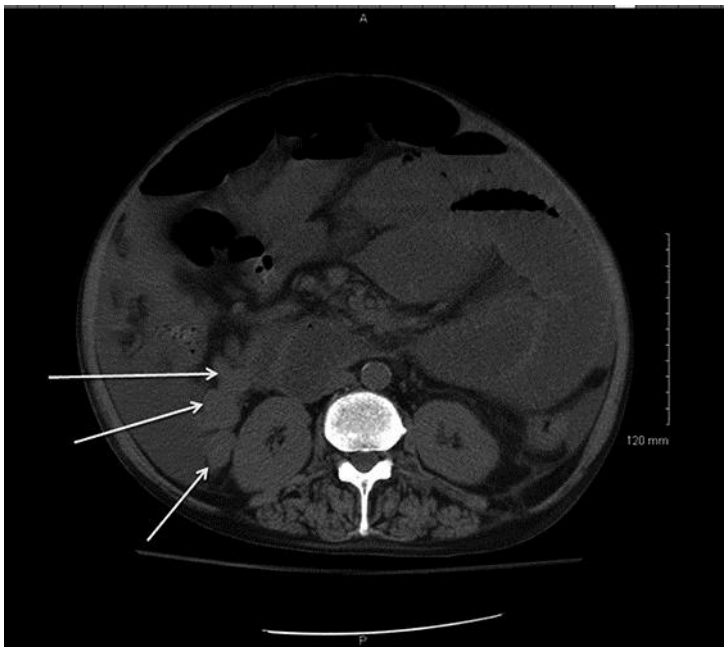

Fig. 3. Noncontrast CT scan transverse image of the abdomen in a 46-year-old man with HHT and alcoholic cirrhosis hospitalized with abdominal pain and fever. This image reveals marked distention of the small intestine, air-fluid levels in the small intestine, absence of free air in the peritoneum, and multiple large varices adjacent to the right kidney (arrows).

Table 1. Laboratory values in a man with HHT and alcoholism

Age, years/months	40/6	45/8	46/2	46/8	49/3
Clinical events	Diagnosis	Week 1[a]	Week 34[a]	Week 58[a]	Final hospitalization
Hb, g/dL	4.8	5.4	2.8	6.6	5.6
Leukocytes, $\times 10^3/\mu L$	3.0	2.4	4.4	4.0	2.6
Platelets, $\times 10^3/\mu L$	265	37	199	92	93
Bilirubin, total, mg/dL	0.7	0.6	0.4	0.9	1.1
Bilirubin, unconjugated, mg/dL	0.32	0.28	0.16	0.48	0.56
Albumin, g/dL	3.1	2.5	2.6	3.1	1.6
ALT, IU/L	65	29	35	28	15
AST, IU/L	104	23	37	40	17
Alkaline phosphatase, IU/L	93	206	156	208	800
Liver/spleen palpable, cm	3/0	6/0	–	–	3/2

ALT, alanine aminotransferase; AST, aspartate aminotransferase. Reference ranges: Hb 12.0–18.0 g/dL; leukocytes 4.1–10.9 × $10^3/\mu L$; platelets 140–440 × $10^3/\mu L$; total bilirubin 0.1–1.2 mg/dL; direct bilirubin 0.00–0.40 mg/dL; ALT 0–40 IU/L; AST 0–55 IU/L; and alkaline phosphatase 25–150 IU/L.

[a] We tabulated baseline laboratory measures for the present study during weeks 1–33. No bevacizumab was administered during this interval. In weeks 34–57 (total 24 weeks), we infused bevacizumab, 2.5 mg/kg every 2 weeks. During weeks 58–97, no bevacizumab was administered.

Table 2. Primers for *ENG* and *ACVRL1* sequencing

Forward primer	Sequence	Reverse primer	Sequence
ENG Ex 1F	CCCAGTGACAAAGCCCGTGGCACT	ENG Ex 1R	CAAGGATGGCTCTGCTGGGCGTGAG
ENG Ex 2F	GATATCCACCTCATAAGGTGGCTG	ENG Ex 2R	ATGCCCACATCACTCTCTTGGCAG
ENG Ex 3F	GTGGAAGCATCCAAATCATCACTG	ENG Ex 3R	AGGACCCTGGTGAATAATGTCAAG
ENG Ex 4F	GCTGACTCCACAAATTACTTCCTG	ENG Ex 4R	TGCCCAAGTTTGAGGTGTGGGCCAG
ENG Ex 5F	CCCTCTGCAGCACCGTCCTGCCTG	ENG Ex 5R	GAGAAAGCGACTGTGCTCTCACAG
ENG Ex 6F	CTGTGAGAGCACAGTCGCTTTCTCCT	ENG Ex 6R	CTGCTGTGTCCCCACTCCTGCTGCG
ENG Ex 7F	GCAGCAGGAGTGGGGACACAGCAG	ENG Ex 7R	ATCTTGGCTCACTGCAACCTCCAT
ENG Ex 8F	GAGGTTGCAGTGAGCCAAGATCGT	ENG Ex 8R	AGGCTTGCAGAGGGACGTGACTTG
ENG Ex 9F	GGTTGTGGTCAGTCCTTGGTGCTG	ENG Ex 9R	CTGCAGCCTGCTCTCCCAAACACA
ENG Ex 10F	TGTGTTTGGGAGAGCAGGCTGCAG	ENG Ex 10R	ACCGAGGCATTCCAGACACACATGG
ENG Ex 11F	CTCCACAGGGCCATGATGCCTGTT	ENG Ex 11R	TCTCTCCCTCTCCCGTGCACCCAG
ENG Ex 12F	TTTCCACTGTGAGGACTCAGGGGT	ENG Ex 12R	TGCCAGGCCACATGCCTGATTAAG
ENG Ex 13F	CAGAGGCATCCAGCTACGAAGCGGT	ENG Ex 13R	TGCCCAGGCCGTTTCTCAGGGCTG
ENG Ex 14R	AGAGCTGGCACCAAAGCCCACATG	ENG Ex 14R	ACCGCCACCACGGGCTCCCGCTTG
ACVRL1 Ex 1F	TACAGTCTCGGCTCTGTCTCCCACG	ACVRL1 Ex 1R	CAGGAGCAGCTTGCCTTTCTAATG
ACVRL1 Ex 2F	AACTCTGTGATTTCCTCTGGGCAG	ACVRL1 Ex 2R	TTCTCCCCAGCTTCTCAAGTTCAG
ACVRL1 Ex 3F	TCAGACGAGAGGGACAGTAGGACAG	ACVRL1 Ex 3R	CTTTATTGGCCAGAGCATGAGAGG
ACVRL1 Ex 4F	GGACTCTGGGATCTAACTGGCAGAG	ACVRL1 Ex 4R	TGGGTCACTGCAAGCTCCTCACTCG
ACVRL1 Ex 5F	GGAGCTTGCAGTGACCCAGCAGGT	ACVRL1 Ex 5R	CACCGCCTGTGATTCCAGTAGCCAA
ACVRL1 Ex 6F	AACCTAAGGGTCTGGGGTTCTGTG	ACVRL1 Ex 6R	GTTCTGTTAATGTCTGGAGGTCTG
ACVRL1 Ex 7F	CTAGCTTAGCAGTGACCCAGTCCAT	ACVRL1 Ex 7R	ATCATGGTCACCGCCCACAGGCAAAG
ACVRL1 Ex 8F	CTCTCTGTCCCACTGTTTCTCTCAGT	ACVRL1 Ex 8R	GGCCATGGGCACTGGCCATGGCTG
ACVRL1 Ex 9F	ATTGCATTATACTGTCCCTCTCAGG	ACVRL1 Ex 9R	GAGGCCTCAGACACAAGTTCCTGG
ACVRL1 Ex 10F	CATCCTTTCTCTCCTGCTTATGTCT	ACVRL1 Ex 10R	CGCTTTGAGCAGGCCAGACAGCAG

Table 3. Observations in a man with HHT and alcoholism

Interval, weeks	1–33	34–57	58–97
Bevacizumab, 2.5 mg/kg every 2 weeks	no	yes	no
Hb, g/dL	5.4±1.0 (p = 0.0101)	6.5±1.7	4.6±1.4 (p < 0.0001)
Platelets, $\times 10^3/\mu L$	203±60 (p = 0.0454)	160±65	184±44 (0.1685)
Packed erythrocytes, units	2.2±1.1 (p = 0.0001)	1.0±0.9	2.3±1.5 (p = 0.0001)
Iron dextran, mg	200±240 (p = 0.0209)	60±160	160±200 (p = 0.0300)

Variables are displayed as means of weekly values ± 1 standard deviation. p values in interval columns 1–33 and 58–97 represent comparisons with observations in bevacizumab treatment interval 34–57 weeks.