

Brief report

Paradoxical secondary polycythemia in von Hippel-Lindau patients treated with anti-vascular endothelial growth factor receptor therapy

Stéphane Richard, Laure Croisille, Jeannine Yvart, Nicole Casadeval, Pascal Eschwège, Nozar Aghakhani, Philippe David, Alain Gaudric, Paul Scigalla, and Olivier Hermine

Von Hippel-Lindau (VHL) disease is a dominantly inherited familial cancer syndrome caused by germline mutations in the VHL tumor-suppressor gene. Central nervous system (CNS) and retinal hemangioblastomas are highly vascular tumors that are hallmarks of the disease. These tumors overexpress vascular endothelial growth factor (VEGF) and represent a potential target for anti-angiogenic drugs. We observed, after 3 to 4 months of

treatment, secondary paradoxical polycythemia in 3 VHL patients with CNS or retinal hemangioblastomas treated by the anti-VEGF receptor SU5416. Hematocrit was normal before the beginning of the trial, and no progression of hemangioblastomas was observed. Polycythemia vera and all known causes of secondary polycythemia were also ruled out. Polycythemia has never been reported in current SU5416 trials for advanced malignancies

and could express a specific action on red blood cell precursors occurring only in the absence of a functional VHL gene. These findings could also affect the inclusion of VHL patients with pre-existing polycythemia in future anti-VEGF receptor trials. (Blood. 2002;99:3851-3853)

© 2002 by The American Society of Hematology

Introduction

Von Hippel-Lindau (VHL) disease (OMIM 193.300) is a dominantly inherited familial cancer syndrome predisposing to various benign or malignant tumors: central nervous system (CNS) and retinal hemangioblastomas, renal cell carcinoma and renal cysts, pancreatic tumors and cysts, pheochromocytoma, and endolymphatic sac tumors. Renal cell carcinoma is becoming the main cause of death, but hemangioblastomas remain the most prototypic lesion of VHL disease.^{1,2} Hemangioblastomas are highly vascularized tumors consisting of blood-filled capillaries separated by intervascular stromal cells. CNS hemangioblastomas may cause life-threatening complications, and their treatment remains mainly neurosurgical. Retinal hemangioblastomas are a major cause of visual morbidity and sometimes blindness because treatment by cryotherapy or laser is not always effective.

VHL disease results from germline mutations in the VHL tumor-suppressor gene located on 3p25-26. An important function of the VHL protein (pVHL) is the inhibition of angiogenesis by negative regulation of hypoxia-inducible mRNAs, such as the mRNAs encoding the vascular endothelial growth factor (VEGF).² pVHL represents the adaptor unit of a multiprotein complex that targets specific proteinlike transcription factors HIF-1 α and HIF-2 α for degradation by the proteasome.²⁻⁵ Thus, the absence or inactivation of pVHL leads to constitutive expression of HIF by VHL tumors, resulting in the overproduction of VEGF and other hypoxia-inducible factors such erythropoietin (EPO), GLUT-1, and transforming growth factor- α . Indeed, the hallmark of all VHL tumors, and moreover of hemangioblastomas, is a high degree of vascularization.¹ pVHL has multiple other functions, including

cell-cycle regulation and fibronectin metabolism, and there is substantial evidence that pVHL may act as a gatekeeper protein.²

Because of the major role of pVHL in VEGF expression and the key pathway of VEGF in hemangioblastoma development, medical treatment with anti-angiogenic drugs was recently proposed in VHL patients affected by untreatable or multiple CNS or retinal hemangioblastomas.⁶ Among anti-angiogenic drugs, SU5416 (Sugen, San Francisco, CA) is a prototype of a novel class of promising cytostatic agents that function by selectively inhibiting the receptor kinase VEGFR-2 (Flk-1/KDR).^{7,8} SU5416 also targets the stem cell factor receptor c-kit.⁹ SU5416 inhibits the growth of colon cancer liver metastasis by inducing tumor and endothelial apoptosis, and it is being tested in several trials in various advanced malignancies and Kaposi sarcoma (see the NCI Database, www.cancertrials.nci.nih.gov).^{7,8} Finally, the first report of stable remission achieved after administration of the drug was recently reported in a patient with acute myeloid leukemia relapse.¹⁰

Study design

Case report

We recently started in our Department of Urology a phase 2 clinical trial in VHL disease with SU5416. After approval by our local Institutional Review Board of a specific protocol in accordance with the Helsinki recommendations, and after written informed consent of each patient, 3 French VHL patients with CNS or retinal hemangioblastomas were included in the study (Table 1). They were treated by intravenous administration of 145 mg/m²

From Génétique Oncologique EPHE, UPRES 1601, Service d'Urologie, Laboratoire d'Hématologie, Service de Biophysique et Médecine Nucléaire, and Service de Neurochirurgie, CHU, Le Kremlin-Bicêtre; Service de Néphrologie, Service d'Hématologie Clinique, and CNRS UMR 8603, Hôpital Necker, Paris; Laboratoire d'Hématologie, Hôtel-Dieu, Paris; Service d'Ophthalmologie, Hôpital Lariboisière, Paris, France; and Sugan Inc, San Francisco, CA.

Submitted November 2, 2001; accepted January 3, 2002.

Supported in part by grants from the Ligue Nationale contre le Cancer (Comité du Cher) and the Association Française de Recherche Génétique.

Reprints: Stéphane Richard, Génétique Oncologique EPHE, CHU, 94276 Le Kremlin-Bicêtre, France; e-mail: stephane.richard@kb.u-psud.fr.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

© 2002 by The American Society of Hematology

Table 1. Clinical and biologic data

	Patient 1	Patient 2	Patient 3
Age (y)	29	62	51
Sex	Female	Female	Male
Indication of SU5416 therapy	Retinal Hb	Cervical Hb	Cerebellar Hb
Other VHL manifestations	Quiescent cerebellar Hb Renal and pancreatic cysts	Quiescent cerebellar Hb Renal and pancreatic cysts	Small renal tumor Renal and pancreatic cysts
Hematocrit (%)/hemoglobin (g/L)			
Before SU5416 therapy	39/12.3	45/14.9	50/16.6
After SU5416 therapy*	51/16.3†	53/17.2†	56/18.4‡
Red blood cell mass (mL/kg)†	32.2	36.4	37.7
Variation of globular volume	+ 26%	+ 28%	+ 28%
Erythropoietin (N: 5-25 mU/mL)			
Before SU5416 therapy	2.0	2.0	10.0
After 12 weeks of drug	1.9	2.6	8.0
Assessment of VHL tumors after 12 weeks of drug	Stable	Stable	Stable
Interruption of drug	No	No	No
Treatment of polycythemia	Bloodletting (1 × 300 mL)	Bloodletting (3 × 300 mL)	Bloodletting (2 × 300 mL)
Recurrence of polycythemia	No	No	No

Hb, hemangioblastoma.

*Maximal value of hematocrit; †after 16 weeks of drug; ‡after 12 weeks of drug.

SU5416 twice a week after premedication by dexamethasone and histamine antagonists to prevent potential hypersensitivity reactions related to the drug or to excipients in the formulation, especially polyoxyethylated castor oil (Cremophor). The safety of SU5416 was assessed monthly through physical examinations, toxicity assessments of vital signs, and laboratory tests (hematology, coagulation, and clinical chemistry).

Methods

Serum EPO was measured by enzyme-linked immunosorbent assay with a commercial kit (R & D Systems, Abingdon, Oxon, United Kingdom).

Red blood cell volume was measured according to the recommendations of the International Committee for Standardization in Hematology (ICSH 1973) using chromium Cr 51 sodium chromate.¹¹ Plasma volume was estimated from red blood cell volume and corrected venous hematocrit. Normal volumes were estimated from the weights and heights of patients according to the Pearson correlation coefficient.¹²

To assess the possible formation of endogenous erythroid colonies, peripheral blood mononuclear cells from the 3 patients were plated in duplicate with and without the addition of 3 IU/mL recombinant human EPO in a methylcellulose medium containing 30% fetal calf serum and supplemented or not with interleukin-3. In parallel, one positive control (polycythemia vera) and 3 negative controls (2 healthy subjects and one with secondary polycythemia) were studied.

Results and discussion

Eight to 10 weeks after the initiation of SU5416 treatment, we surprisingly observed a progressive increase of hematocrit in all 3 enrolled VHL patients. Polycythemia was maximal between 12 and 16 weeks (Table 1; Figure 1), and patients were symptom free except for slight breathlessness in one. Polycythemia was confirmed by isotopic measurement of a red blood cell mass that increased in each patient. Polycythemia vera was eliminated first because there was no evidence of splenomegaly, thrombocytosis, or neutrophil leukocytosis in any patient. Reticulocyte counts and serum B12 levels were also normal. In addition, no erythroid colonies from peripheral blood mononuclear cells were observed in the absence of EPO. Magnetic resonance imaging of the CNS showed stability of the size of CNS hemangioblastomas, and no other tumor cause of polycythemia was present. None of the patients had pheochromocytoma, and

only one had a small renal tumor that remained stable for years, as evidenced by computed tomography. Other causes of secondary polycythemia were also ruled out (normal arterial O₂ saturation levels, no smoking, no family histories of erythrocytosis).¹² Lastly, determination of blood EPO levels demonstrated no change in comparison with the initial measurements performed before the beginning of the SU5416 trial. The administration of SU5416 was not interrupted, and polycythemia was treated by bloodletting with good outcome and without recurrence up to the sixth month of treatment (Figure 1).

The occurrence of a secondary polycythemia in the 3 VHL patients treated by SU5416 was surprising; it has never been observed in the more than 700 patients with Kaposi sarcoma, advanced cancer, or acute leukemias included in the current SU5416 trials. On the contrary, some of these patients experienced anemia consistent with the negative action of SU5416 on c-kit.⁹ Thus, the occurrence of polycythemia at virtually the same time after the beginning of the treatment in the 3 VHL patients is highly suggestive of a direct action of the drug on this condition. It is well

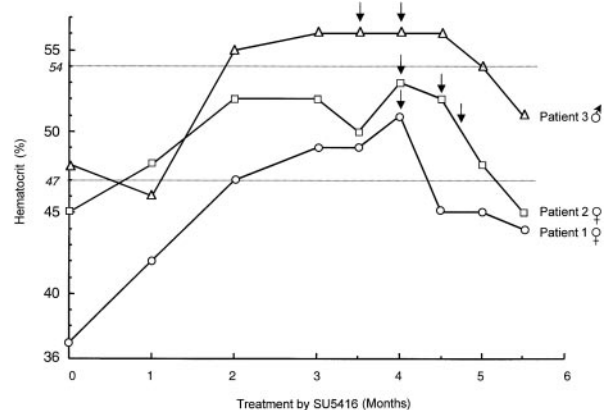


Figure 1. Evolution of hematocrit during treatment by SU5416. The evolution of hematocrit in the 3 enrolled VHL patients is shown: patient 1 (female; open circle), patient 2 (female; open square), patient 3 (male; open triangle). SU5416 administration was not interrupted, and polycythemia was treated by bloodletting (arrows) with good outcome and without recurrence up to the sixth month of treatment. Normal maximal values of hematocrit in males and females, respectively, are indicated by gray horizontal lines.

known that polycythemia may occur in approximately 15% to 20% of patients with large cerebellar hemangioblastomas as a consequence of high EPO production by neoplastic stromal cells, as demonstrated by *in situ* hybridization.^{1,13-16} In our study only one patient had a large enough cerebellar hemangioblastoma, but he had no initial polycythemia and no change in tumor size was observed after 3 months of treatment.

A trivial hypothesis could be that CNS hemangioblastoma reacted to the privation of VEGF induced by SU5416 in producing increased amounts of hypoxia-inducible peptides. Overexpression of EPO could have been responsible for polycythemia, but, at least in 2 patients, there were only small, quiescent cerebellar hemangioblastomas. In addition, there was no change in serum EPO levels, though this finding does not rule out increased EPO production. Another possibility could be an increased susceptibility of red blood cell precursors to EPO in VHL patients treated with SU5416, and we cannot exclude that SU5416 interacts with other receptors critical for erythropoiesis.

Further *in vivo* and *in vitro* investigations are needed to elucidate this unexpected effect. Resolving this problem probably should be of great interest for understanding the action of SU5416 and improvement of anti-angiogenic therapy in VHL patients. Lastly, from a clinical point of view, initial polycythemia could be an exclusion criterion for enrollment of new patients in anti-VEGF receptor therapy trials.

Acknowledgments

We thank M. G. Allègre and Mrs. P. Rincé for technical assistance. We thank Mrs D. Broneer, Dr C. Bérout and Prof J. P. Grünfeld for their help in the preparation of the manuscript. We also thank Prof G. Tchernia, Prof C. Buffet, Prof G. Benoît, Prof D. Doyon, Prof M. Bléry, Dr J. Langlois, Prof A. Jardin, and the nursing staff of the Department of Urology.

References

- Richard S, David P, Marsot-Dupuch K, et al. Central nervous system hemangioblastomas, endolymphatic sac tumors and von Hippel-Lindau disease. *Neurosurg Rev*. 2000;23:1-22.
- Clifford SC, Maher ER. Von Hippel-Lindau disease: clinical and molecular perspectives. *Adv Cancer Res*. 2001;82:85-105.
- Maxwell PH, Wiesener MS, Chang GW, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature*. 1999;399:271-275.
- Ivan M, Kondo K, Yang H, et al. HIF α targeted for VHL-mediated destruction by proline hydroxylation: implications for O₂ sensing. *Science*. 2001;292:464-468.
- Jaakola P, Mole D, Tian YM, et al. Targeting of HIF- α to the von Hippel-Lindau ubiquitylation complex by O₂-regulated prolyl hydroxylation. *Science*. 2001;292:468-472.
- Harris A. Von Hippel-Lindau syndrome: target for anti-vascular endothelial growth factor (VEGF) receptor therapy. *Oncologist*. 2000;5(suppl 1):32-36.
- Fong TAT, Shawver LK, Sun L, et al. SU5416 is a potent selective inhibitor of the vascular endothelial growth factor (Flk-1/KDR) that inhibits tyrosine kinase catalysis, tumor vascularization, and growth of multiple tumor types. *Cancer Res*. 1999;59:99-106.
- Shaheen RM, Davis DW, Liu W, et al. Antiangiogenic therapy targeting the tyrosine kinase receptor for vascular endothelial growth factor receptor inhibits the growth of colon cancer liver metastasis and induces tumor and endothelial cell apoptosis. *Cancer Res*. 1999;59:5412-5416.
- Smolich BD, Yuen HA, West K, et al. The antiangiogenic protein kinase inhibitors SU5416 and SU6668 inhibit the SCF receptor (c-kit) in a human myeloid leukemia cell line and in acute myeloid leukemia blasts. *Blood*. 2001;97:1413-1421.
- Mesters RM, Padró T, Bieker B, et al. Stable remission after administration of the receptor tyrosine kinase inhibitor SU5416 in a patient with refractory acute myeloid leukemia. *Blood*. 2001;98:241-243.
- Pearson TC, Guthrie DL, Simpons J, et al. Interpretation of measured red cell mass and plasma volume in adults: expert panel on radionuclides of the International Council of Standardization in Haematology. *Br J Haematol*. 1995;89:748-756.
- Pearson TC. Diagnosis and classification of erythrocytoses and thrombocytoses. *Baillieres Clin Haematol*. 1998;11:695-720.
- Waldmann TA, Levin, Baldwin M. The association of polycythemia with cerebellar hemangioblastoma. *Am J Med*. 1961;31:318-324.
- Trimble M, Caro J, Talalla A, Brain M. Secondary erythrocytosis is due to a cerebellar hemangioblastoma: demonstration of erythropoietin mRNA in the tumor. *Blood*. 1991;78:599-601.
- Krieg M, Marti HH, Plate KH. Coexpression of erythropoietin and vascular endothelial growth factor in nervous system tumors associated with von Hippel-Lindau tumor suppressor gene loss of function. *Blood*. 1998;92:3388-3393.
- Vortmeyer AO, Gnarr JR, Emmert-Buck MR, et al. Von Hippel-Lindau gene deletion detected in the stromal cell component of a cerebellar hemangioblastoma associated with von Hippel-Lindau disease. *Hum Pathol*. 1997;28:540-543.