

## Anti-VEGF therapy in pituitary carcinoma

Leon D. Ortiz · Luis V. Syro · Bernd W. Scheithauer ·  
Ayca Ersen · Humberto Uribe · Camilo E. Fadul ·  
Fabio Rotondo · Eva Horvath · Kalman Kovacs

Published online: 15 September 2011  
© Springer Science+Business Media, LLC 2011

**Abstract** We report the case of a 44-year-old male patient with an aggressive silent corticotroph cell pituitary adenoma, subtype 2. In that it progressed to carcinoma despite temozolomide administration, anti-VEGF therapy was begun. MRI, PET scan and pathologic analysis were undertaken. After 10 months of anti-VEGF (bevacizumab) treatment no progression of the lesion was noted. The tumor was biopsied and morphological analysis showed severe cell injury, vascular abnormalities and fibrosis. Bevacizumab treatment has continued for additional 16 months to present with stabilization of disease as documented on serial MRI and PET

scans. This is the first case of a bevacizumab-treated pituitary carcinoma with long-term, now 26 months, control of disease. The present findings are promising in that anti-angiogenic therapy appears to represent a new option in the treatment of aggressive pituitary tumors.

**Keywords** Pituitary carcinoma · Angiogenesis inhibitors · Vascular endothelial growth factor-A · Bevacizumab · Temozolomide

---

L. D. Ortiz  
Division of Neuro-Oncology, Instituto de Cancerologia,  
Clinica Las Americas, Medellin, Colombia

L. V. Syro  
Department of Neurosurgery, Hospital Pablo Tobon Uribe  
and Clinica Medellin, Medellin, Colombia

B. W. Scheithauer (✉)  
Department of Laboratory Medicine and Pathology,  
Mayo Clinic, 200 First Street, SW, Rochester, MN 55905, USA  
e-mail: scheithauer.bernd@mayo.edu

A. Ersen  
Department of Pathology, Dokuz Eylul University,  
Izmir, Turkey

H. Uribe  
Department of Neurosurgery, Clinica SOMA, Medellin,  
Colombia

C. E. Fadul  
Section of Hematology/Oncology and Neurology,  
Dartmouth Hitchcock Medical Center, Lebanon, NH, USA

F. Rotondo · E. Horvath · K. Kovacs  
Department of Laboratory Medicine, St. Michael's Hospital,  
University of Toronto, Toronto, ON, Canada

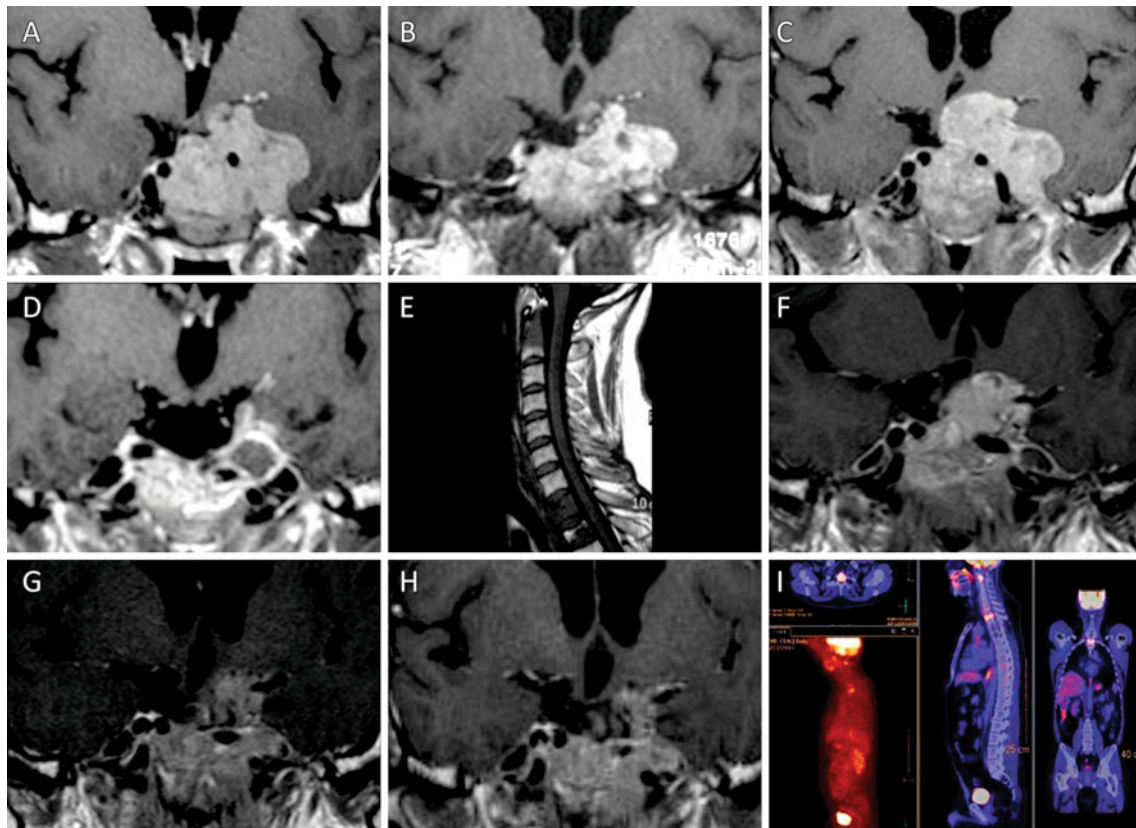
### Introduction

The term angiogenesis denotes the neoformation of blood vessels [1]. Oxygen plays an important role in its regulation through Hypoxia-Inducible Factor (HIF) [2]. Recognition that control of the process has therapeutic implications inspired extensive research [3]. Stimulation of angiogenesis is of therapeutic importance in ischemic heart disease, peripheral vascular disease, and wound healing [4]. Inhibition of angiogenesis may be of therapeutic value in various types of cancer in and age-related macular degeneration [5–7]. Increased vascularity leading to accelerated tumor growth was reported in several tumor types. Folkman suggested that anti-angiogenesis could be an effective strategy in the treatment of tumors [8].

### Subject and methods

#### Case report

A 38-year-old male patient with an aggressive silent corticotroph cell adenoma, subtype 2 presented in 2005. After four prior surgeries and radiotherapy, tumor persisted and grew



**Fig. 1** Magnetic resonance imaging (MRI) and positron emission tomography (PET) scans documenting the evolution of the patient. **a** Preoperative MRI scan. **b** Postoperative MRI scan demonstrating subtotal resection. **c** Tumor regrowth after 8 months of standard-regimen temozolomide treatment. **d** Postoperative MRI after subtotal re-excision. **e** Spinal metastases at C2 and T1 after 16 months of

standard-regimen temozolomide treatment. **f** Sellar tumor recurrence after 8 months of temozolomide treatment at metronomic dose. **g** Postoperative MRI. **h** MRI after 24 months of bevacizumab therapy showing stabilization of the sellar lesion. **i** PET scan with no new metastatic lesions

(Fig. 1a). A subtotal resection was undertaken (Fig. 1b). Temozolomide (TMZ) was administered at a standard-dose and regimen (200 mg/m<sup>2</sup>/day, 5/28) for 8 months (Fig. 1c) but without response. He was again operated (Fig. 1d). TMZ was restarted at the same dose and continued for 16 months, during which time he developed two vertebral metastases at levels C2 and T1. Both were confirmed by surgery and pathology (Fig. 1e). Since O<sup>6</sup>-methylguanine-DNA methyltransferase (MGMT) immunoexpression was high, TMZ was administered in a metronomic dose (75 mg/m<sup>2</sup>/day continuously, 28/28) in order to deplete MGMT. Focal radiotherapy to the spine was also undertaken. Eight months later, a sellar tumor regrowth was noted (Fig. 1f), which prompted reoperation (Fig. 1g). Histologically, as documented in a prior publication, the tumor showed no morphological change [9]. Conclusive VEGF immunoreactivity was found in the cytoplasm of tumor cells. Intravenous bevacizumab therapy was started at 10 mg/kg every 2 weeks. At 10 months, no progression was noted and the tumor was biopsied. Bevacizumab treatment has continued for additional 16 months. Presently, after 26 months of treatment, resultant stabilization of the

disease is documented on serial MRI and PET scans (Fig. 1h, i). No new metastases were noted.

### Pathology

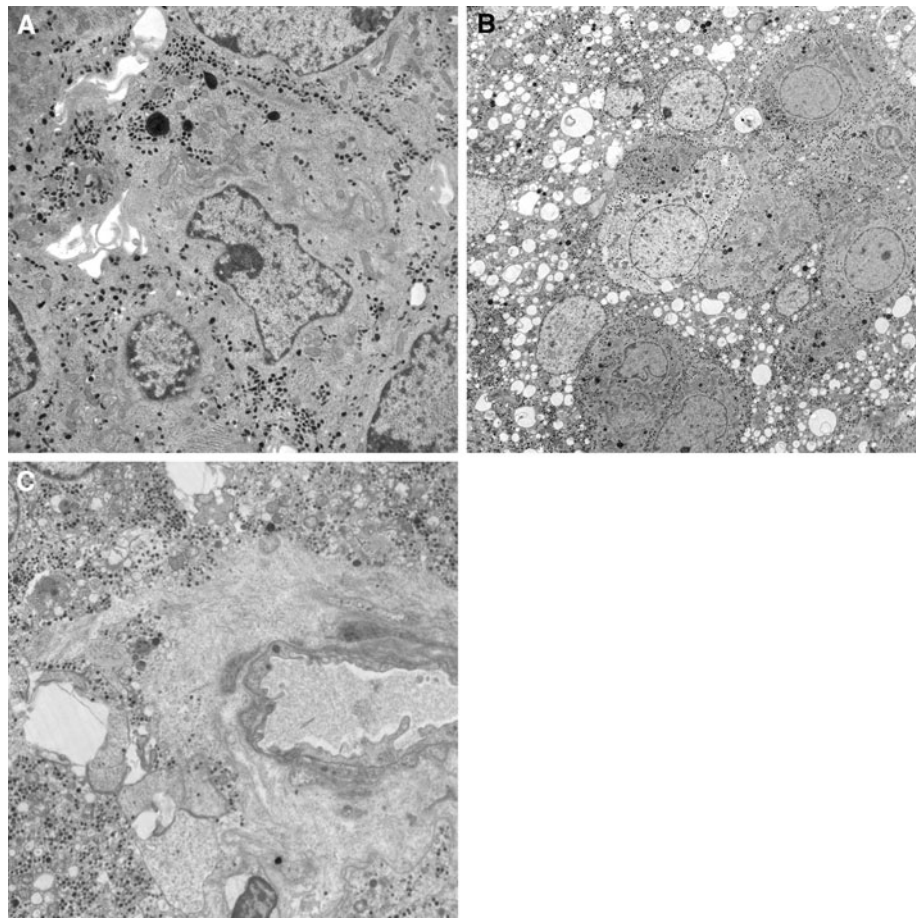
The surgically removed tumor was formalin-fixed, paraffin-embedded, sectioned at 5 microns and stained with hematoxylin-eosin and the periodic acid-Schiff (PAS) method. Immunohistochemistry employed the streptavidin biotin peroxidase complex method. For transmission electron microscopy, glutaraldehyde-fixed, osmicated, Epon-embedded tissue was studied on a Hitachi 7650 transmission electron microscope.

## Results

### Pathology

The specimen resected at the 10th month of bevacizumab therapy showed severe cellular injury and fibrosis. When

**Fig. 2** **a** The pre-bevacizumab specimen demonstrated typical features of pituitary adenoma unassociated with degenerative changes. **b** The post-treatment specimen differed markedly. Although 30% of the specimen showed no significant cellular abnormality, the remainder featured nuclear irregularity, massive cytoplasmic vacuolization, breakage of cell membranes and the accumulation of membranous whorls. **c** Vascular collagen deposition was conspicuous (2A  $\times$  10,000, 2B  $\times$  3,000, 2C  $\times$  8,000)



compared with the previous specimens (Fig. 2a), both the ACTH and  $\beta$ -endorphin immunopositive tumor cells were ultrastructurally smaller, many but not all having undergone cytoplasmic vacuolization and rupture of cell membranes (Fig. 2b). Fibrosis was extensive, mainly affecting vessels in perivascular zones (Fig. 2c). Large, irregular vessels with thick walls as were many thin walled, newly formed vessels were highlighted on endoglin immunostaining. Whether the vascular changes and the cellular damage more dramatically noted ultrastructurally are related to bevacizumab therapy is unclear. The patient had twice undergone radiotherapy before the latest surgery. This may have contributed to the severe fibrosis observed, but the specimen obtained 1 year after radiotherapy revealed none. Thus, our sequential morphologic analyses suggest that the cell injury, vascular abnormalities, and fibrosis are the effects of bevacizumab therapy.

## Discussion

The vascular endothelial growth factor (VEGF) family consists of five glycoproteins, VEGFA through D as well

as placental growth factor (PGF). Of these, VEGFA is best characterized and commonly referred as VEGF [1]. It binds to three transmembrane tyrosine kinase receptors and is predominantly found on endothelial cells. VEGF affects endothelial cell proliferation and survival, chemotaxis of bone marrow-derived progenitor cells, vasodilatation and vascular permeability. VEGF and its receptors are key regulators of angiogenesis, and are regarded as prime therapeutic targets.

Targeting VEGF is intended to prevent the formation of new vasculature, blocking new vessel growth thus inhibiting tumor growth. Anti-angiogenic therapy can sensitize tumor stem cells to radio- and chemotherapy approaches to inhibit VEGF signaling include antibody neutralization of the ligand or receptor and blocking VEGF activation and signaling using tyrosine kinase inhibitors [10]. Bevacizumab, a recombinant, humanized, anti-VEGF monoclonal antibody, prevents the binding of VEGF to endothelial surface receptors [11, 12]. As an angiogenesis inhibitor delays tumor growth and it is used in the treatment of colorectal non-small cell pulmonary, renal and breast carcinoma, as well as recurrent anaplastic astrocytoma and glioblastoma [13–17]. Recently, it has been

proved useful in the treatment of cerebral radiation necrosis [18]. Having a circulating half-life of ~20 days, its optimal dose is 5–10 mg/kg every 2 weeks.

Pituitary carcinomas present a major therapeutic challenge. The spectrum of adjuvant therapies has proven palliative at best. Since 2006, temozolomide (TMZ) has been found efficacious in aggressive pituitary adenomas and carcinomas, the response rate being approximately 60% [19, 20].

This is the first case of a bevacizumab-treated pituitary carcinoma with long-term control of disease, now 26 months. Understandably, the present findings prompt enthusiasm in that anti VEGF therapy appears to represent a new option in the treatment of pituitary carcinoma. Nonetheless, more cases must be investigated before recommending the treatment. Caution is in order given the significant side effects of bevacizumab, including hypertension, hemorrhage, thrombosis, embolism, cerebrovascular ischemia, gastrointestinal perforation and impaired wound healing [21, 22].

Another important problem stems from the well known occurrence of tumor cell heterogeneity. Since several mutations may occur during neoplastic progression, tumor cells may respond differently. For example, some cells do not need much oxygen to thrive. Although some may die under hypoxic conditions, others survive, especially perivascular tumoral cells, successfully multiplying, invading and even giving rise to metastases [23–26]. Thus, it may be advisable to combine bevacizumab with chemotherapeutic agents, perhaps irinotecan or temozolomide [27], because bevacizumab does not seem to have direct cytolytic effect but besides its anti-angiogenic action it could sensitize tumoral cells to chemotherapy.

**Acknowledgments** Authors are grateful to the Jarislowsky and Lloyd Carr Harris Foundations for their continuous support. The authors thank Mrs. Denise Chase of Mayo Clinic for excellent assistance and secretarial support.

## References

1. Ellis LM, Hicklin DJ (2008) VEGF-targeted therapy: mechanisms of anti-tumour activity. *Nat Rev Cancer* 8(8):579–591. doi:10.1038/nrc2403
2. Zagzag D, Lukyanov Y, Lan L, Ali MA, Esencay M, Mendez O, Yee H, Voura EB, Newcomb EW (2006) Hypoxia-inducible factor 1 and VEGF upregulate CXCR4 in glioblastoma: implications for angiogenesis and glioma cell invasion. *Lab Invest* 86(12):1221–1232. doi:10.1038/labinvest.3700482
3. Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS, Ferrara N (1993) Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. *Nature* 362(6423):841–844. doi:10.1038/362841a0
4. Kerbel RS (2008) Tumor angiogenesis. *N Engl J Med* 358(19):2039–2049. doi:10.1056/NEJMr0706596
5. Wilson WR, Hay MP (2011) Targeting hypoxia in cancer therapy. *Nat Rev Cancer* 11(6):393–410. doi:10.1038/nrc3064
6. Gaur P, Bose D, Samuel S, Ellis LM (2009) Targeting tumor angiogenesis. *Semin Oncol* 36(2 suppl 1):S12–S19. doi:10.1053/j.seminoncol.2009.02.002
7. Martin DF, Maguire MG, Ying GS, Grunwald JE, Fine SL, Jaffe GJ (2011) Ranibizumab and bevacizumab for neovascular age-related macular degeneration. *N Engl J Med* 364(20):1897–1908. doi:10.1056/NEJMoA1102673
8. Folkman J (1971) Tumor angiogenesis: therapeutic implications. *N Engl J Med* 285(21):1182–1186. doi:10.1056/NEJM197111182852108
9. Moshkin O, Syro LV, Scheithauer BW, Ortiz LD, Fadul CE, Uribe H, Gonzalez R, Cusimano M, Horvath E, Rotondo F, Kovacs K (2011) Aggressive silent corticotroph adenoma progressing to pituitary carcinoma. The role of temozolomide therapy. *Hormones (Athens)* 10(2):162–167
10. Krause DS, Van Etten RA (2005) Tyrosine kinases as targets for cancer therapy. *N Engl J Med* 353(2):172–187. doi:10.1056/NEJMr044389
11. Ferrara N, Hillan KJ, Novotny W (2005) Bevacizumab (Avastin), a humanized anti-VEGF monoclonal antibody for cancer therapy. *Biochem Biophys Res Commun* 333(2):328–335. doi:10.1016/j.bbrc.2005.05.132
12. Presta LG, Chen H, O'Connor SJ, Chisholm V, Meng YG, Krummen L, Winkler M, Ferrara N (1997) Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. *Cancer Res* 57(20):4593–4599
13. Chamberlain MC, Johnston S (2009) Salvage chemotherapy with bevacizumab for recurrent alkylator-refractory anaplastic astrocytoma. *J Neurooncol* 91(3):359–367. doi:10.1007/s11060-008-9722-2
14. Jouanneau E (2008) Angiogenesis and gliomas: current issues and development of surrogate markers. *Neurosurgery* 62(1):31–50. doi:10.1227/01.NEU.0000311060.65002.4E00006123-200801000-00003 discussion 50–32
15. Norden AD, Drappatz J, Wen PY (2008) Antiangiogenic therapy in malignant gliomas. *Curr Opin Oncol* 20(6):652–661. doi:10.1097/CCO.0b013e32831186ba00001622-200811000-00009
16. Norden AD, Drappatz J, Muzikansky A, David K, Gerard M, McNamara MB, Phan P, Ross A, Kesari S, Wen PY (2009) An exploratory survival analysis of anti-angiogenic therapy for recurrent malignant glioma. *J Neurooncol* 92(2):149–155. doi:10.1007/s11060-008-9745-8
17. Kioi M, Vogel H, Schultz G, Hoffman RM, Harsh GR, Brown JM (2010) Inhibition of vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice. *J Clin Invest* 120(3):694–705. doi:10.1172/JCI4028340283
18. Torcuator R, Zuniga R, Mohan YS, Rock J, Doyle T, Anderson J, Gutierrez J, Ryu S, Jain R, Rosenblum M, Mikkelsen T (2009) Initial experience with bevacizumab treatment for biopsy confirmed cerebral radiation necrosis. *J Neurooncol* 94(1):63–68. doi:10.1007/s11060-009-9801-z
19. Syro LV, Ortiz LD, Scheithauer BW, Lloyd R, Lau Q, Gonzalez R, Uribe H, Cusimano M, Kovacs K, Horvath E (2011) Treatment of pituitary neoplasms with temozolomide: a review. *Cancer* 117(3):454–462. doi:10.1002/ncr.25413
20. McCormack AI, Wass JA, Grossman AB (2011) Aggressive pituitary tumours: the role of temozolomide and the assessment of MGMT status. *Eur J Clin Invest*. doi:10.1111/j.1365-2362.2011.02520.x
21. Tebbutt NC, Murphy F, Zannino D, Wilson K, Cummins MM, Abdi E, Strickland AH, Lowenthal RM, Marx G, Karapetis C, Shannon J, Goldstein D, Nayagam SS, Blum R, Chantrill L, Simes RJ, Price TJ (2011) Risk of arterial thromboembolic events

- in patients with advanced colorectal cancer receiving bevacizumab. *Ann Oncol* 22(8):1834–1838. doi:[10.1093/annonc/mdq702](https://doi.org/10.1093/annonc/mdq702)
22. Norden AD, Drappatz J, Ciampa AS, Doherty L, LaFrankie DC, Kesari S, Wen PY (2009) Colon perforation during antiangiogenic therapy for malignant glioma. *Neuro Oncol* 11(1):92–95. doi:[10.1215/15228517-2008-071](https://doi.org/10.1215/15228517-2008-071)
23. Helfrich I, Scheffrahn I, Bartling S, Weis J, von Felbert V, Middleton M, Kato M, Ergun S, Schadendorf D (2010) Resistance to antiangiogenic therapy is directed by vascular phenotype, vessel stabilization, and maturation in malignant melanoma. *J Exp Med* 207(3):491–503. doi:[10.1084/jem.20091846](https://doi.org/10.1084/jem.20091846)
24. Azam F, Mehta S, Harris AL (2010) Mechanisms of resistance to antiangiogenesis therapy. *Eur J Cancer* 46(8):1323–1332. doi:[10.1016/j.ejca.2010.02.020](https://doi.org/10.1016/j.ejca.2010.02.020)
25. Paez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, Vinals F, Inoue M, Bergers G, Hanahan D, Casanovas O (2009) Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. *Cancer Cell* 15(3):220–231. doi:[10.1016/j.ccr.2009.01.027](https://doi.org/10.1016/j.ccr.2009.01.027)
26. Cheshier SH, Kalani MY, Lim M, Ailles L, Huhn SL, Weissman IL (2009) A neurosurgeon's guide to stem cells, cancer stem cells, and brain tumor stem cells. *Neurosurgery* 65(2):237–249. doi:[10.1227/01.NEU.0000349921.14519.2A00006123-200908000-00003](https://doi.org/10.1227/01.NEU.0000349921.14519.2A00006123-200908000-00003); discussion 249–250; quiz N236
27. Friedman HS, Prados MD, Wen PY, Mikkelsen T, Schiff D, Abrey LE, Yung WK, Paleologos N, Nicholas MK, Jensen R, Vredenburgh J, Huang J, Zheng M, Cloughesy T (2009) Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. *J Clin Oncol* 27(28):4733–4740. doi:[10.1200/JCO.2008.19.8721](https://doi.org/10.1200/JCO.2008.19.8721)