Case Reports in Ophthalmology

Case Rep Ophthalmol 2012;3:443–451
DOI: 10.1159/000346045

Published online:
December 22, 2012

ISSN 1663–2699
www.karger.com/cop

A Clear Cell Renal Cell Carcinoma Inhibiting the Response to Intravitreal Antivascular Endothelial Growth Factor Therapy in Wet Age-Related Macular Disease

Manuel S. Falcão[a,d] João Vinagre[a–c] Paula Soares[a,b]
José Manuel Lopes[a,b,e] Elisete Brandão[d]
Ângela M. Carneiro[a,d]

[a]Faculty of Medicine and [b]Institute of Molecular Pathology and Immunology (IPATIMUP), University of Porto, [c]Institute of Biomedical Sciences Abel Salazar (ICBAS), and Departments of [d]Ophthalmology and [e]Pathology, Hospital de São João, Porto, Portugal

Key Words
Wet age-related macular degeneration · Vascular endothelial growth factor · Von Hippel-Lindau · Renal cell carcinoma · Pseudohypoxia · Bevacizumab · Ranibizumab

Abstract
Purpose: Wet age-related macular degeneration (AMD) is an ocular disorder that can be successfully treated with intravitreal antivascular endothelial growth factor (VEGF) therapy. We report a case of incomplete response to intravitreal therapy associated with a clear cell renal cell carcinoma (ccRCC).
Methods: A 72-year-old male with wet AMD responded poorly to intravitreal bevacizumab and ranibizumab injections. The removal of a ccRCC led to the spontaneous stabilization of the choroidal neovascular lesion. The renal carcinoma was examined for Von Hippel-Lindau *(VHL)* gene alterations. Immunohistochemical profiling of the hypoxia-inducible factor (HIF) pathway addressing the marker HIF-1α and its downstream targets VEGF, glucose transporter 1 and carbonic anhydrase IX was performed.
Results: Genotyping of the ccRCC revealed the presence of a truncating *VHL* mutation (p.E134fs*25). Immunohistochemistry displayed HIF pathway target activation and VEGF expression in the ccRCC tumour cells. Following tumour removal, the neovascular lesion remained stable for 6 months without any further anti-VEGF therapy.

José Manuel Lopes
Institute of Molecular Pathology and Immunology (IPATIMUP)
Rua Dr. Roberto Frias, s/n
PT–4200-465 Porto (Portugal)
E-Mail jmlopes@ipatimup.pt

Case Rep Ophthalmol 2012;3:443–451
DOI: 10.1159/000346045
Published online: December 22, 2012

ISSN 1663–2699
www.karger.com/cop

Conclusion: The somatic *VHL* mutation correlates with persistent high levels of HIF-1α pathway targets and VEGF expression in the ccRCC. We postulate that this increased VEGF in the tumour and subsequently in the plasma levels could have caused the incomplete response to intravitreal anti-VEGF therapy. Stabilization of the wet AMD following tumour removal indicates that the angiogenic secreting tumour (ccRCC) abrogates the response to VEGF inhibitor therapy. Thus, in cases of poor response to intravitreal anti-VEGF therapy, systemic evaluation including plasma levels of VEGF and/or systemic screening for VEGF-producing tumours should be considered.

Introduction

Age-related macular degeneration (AMD) is a leading cause of blindness in industrialized nations [1]. In wet AMD, subfoveal choroidal neovascularization (CNV) severely alters macular morphology and physiology, causing serious central vision loss. CNV is an angiogenic process in which vascular endothelial growth factor (VEGF) plays an important role. Therapy with intravitreal anti-VEGF agents has significantly improved the management of AMD. Both ranibizumab and bevacizumab have been shown to recover visual acuity and retinal morphology either with monthly treatments or with optical coherence tomography (OCT)-guided treatments [2, 3]. In OCT-guided treatments, patients are reinjected whenever OCT B-scans show CNV activity, which is usually defined by the presence of intraretinal or subretinal fluid.

Increased levels of VEGF have been demonstrated both in the aqueous and vitreous humour of patients with active CNV. Intraocular anti-VEGF therapy may decrease systemic VEGF levels [4]. However, the influence of persistent high levels of systemic VEGF on the activity of AMD has not been reported so far.

We report a case of CNV secondary to wet AMD who partially responded to monthly intravitreal anti-VEGF therapy. Following the removal of a VEGF-producing clear cell renal cell carcinoma (ccRCC), the ocular disease was controlled spontaneously.

Case Presentation

A 72-year-old male was referred to the retinal department after sudden visual loss in his left eye. At presentation, best-corrected visual acuity of his left eye was 20/200. This eye was aphakic due to cataract surgery performed several years before. He used a contact lens and spectacles to correct his refractive error. Funduscopy showed a subretinal macular haemorrhage and drusen (**fig. 1**a). Fluorescein angiography and OCT confirmed the diagnosis of an occult CNV lesion (fig. 1b, c) secondary to AMD.

The patient started treatment with 1.25 mg intravitreal bevacizumab. The aim was to employ an 'as-needed' treatment strategy, using any intraretinal or subretinal fluid on OCT or presence of retinal haemorrhage as retreatment criteria. There was an immediate favourable response to treatment with an improved visual acuity of 20/40 and a significant reduction of the macular haemorrhage. However, subretinal fluid persisted despite monthly treatments. After 17 injections, bevacizumab was replaced by ranibizumab when the latter became available in our hospital. Despite the change in medication, there was no improvement in the amount of subretinal fluid or visual acuity. The patient received further 18 monthly ranibizumab injections. During this time, combination therapy with photodynamic therapy and verteporfin was attempted. Nevertheless, visual acuity remained stable at 20/40 and subretinal fluid persisted on OCT.

Case Rep Ophthalmol 2012;3:443–451
DOI: 10.1159/000346045
Published online: December 22, 2012

ISSN 1663–2699
www.karger.com/cop

At a routine systemic medical visit unrelated to the ophthalmologic treatments, a renal tumour of the left kidney was diagnosed. The tumour was removed by partial nephrectomy. The histological exam disclosed a ccRCC featuring an alveolar pattern with a network of thin-walled blood vessels, Fuhrman grade 2 and focally grade 3, cystic areas and necrosis. No signs of capsular invasion were detected. The tumour was classified as pT1a.

In the perioperative period, the patient neglected the ophthalmologic evaluations. When he returned to the ophthalmology department 8 weeks after his last intravitreal injection of ranibizumab, his vision remained stable but OCT did not show any subretinal fluid (**fig. 2**). The patient's retina and visual acuity remained stable for 6 months without further anti-VEGF treatment.

Molecular analysis of the ccRCC disclosed the presence of a frameshift mutation in the Von Hippel-Lindau *(VHL)* gene (p.E134fs*25) that leads to a predicted premature stop codon (**fig. 3**a). This mutation was not detected in the normal adjacent tissue of the tumour.

VHL mutations are known to induce a pseudohypoxic state [5] with activation of the hypoxia-inducible factor (HIF) pathway. Thus, we decided to perform an immunophenotypical analysis of the HIF-1α protein and its pathway downstream targets VEGF, glucose transporter 1 (GLUT-1) and carbonic anhydrase IX (CA-IX). Evaluation of the immunophenotype of the tumour cells revealed nuclear staining of HIF-1α and increased expression of GLUT-1, CA-IX and VEGF when compared to normal adjacent tissue (fig. 3c–e).

One year after surgery, the patient remains without evidence of any tumour (local/distant) recurrence. Reactivation of CNV occurred 6 months later but it was controlled with 2 injections of bevacizumab. His vision remains stable at 20/40.

Discussion

Wet AMD is characterized by the development of pathologic CNV that is highly dependent on VEGF. Increased ocular levels of VEGF have been described in the aqueous and vitreous humour of affected patients. Intravitreal anti-VEGF therapy is recognized as the gold standard for treatment. Intravitreal VEGF inhibition with either bevacizumab or ranibizumab has shown significant visual and anatomical results [2]. Even systemic VEGF inhibition with intravenous bevacizumab leads to improvements in wet AMD [6].

We report a case of a wet AMD patient showing partial response to intravitreal anti-VEGF therapy. There was a significant improvement in visual acuity at the beginning of the treatment. However, despite successive monthly injections, subretinal fluid persisted, indicating persistent exudation from the neovascular complex. Even though the patient was proposed for an 'as-needed' treatment strategy, constant neovascular activity led to 35 consecutive monthly treatments. However, following the detection and removal of the ccRCC, CNV regressed and stabilized without further treatment. This favourable evolution was remarkable and intriguing. Thus, we hypothesized that the tumour was producing growth factors that interfered with anti-VEGF therapy. The *VHL* mutation detected led to increased VEGF expression in the tumour cells, which may have led to an increase in circulating VEGF levels.

The choroid is a highly vascularized tissue. When high levels of circulating VEGF are present, the continuous supply of this growth factor may abrogate the effectiveness of anti-VEGF therapy in controlling CNV activity. Removal of the ccRCC, and a putative consequent decrease in plasma VEGF levels allowed better control of the disease. After tumour removal, there was no evidence of CNV activity for 6 months. Reactivation was controlled with 2 consecutive monthly intravitreal bevacizumab injections. The patient

Case Rep Ophthalmol 2012;3:443–451
DOI: 10.1159/000346045
Published online: December 22, 2012

ISSN 1663–2699
www.karger.com/cop

remained injection-free for another 4-month spell. In the natural history of treated CNV lesions, disease reactivation may occur many months after initial stabilization. The reactivation can be attributed to the ocular pathology and is not related to recurrence of the ccRCC or increased systemic VEGF levels. In fact, the systemic evaluation performed was negative for neoplastic recurrence or metastasis. After CNV reactivation, the response to treatment was distinct from that before removal of the ccRCC. This supports our claim that the tumour was producing systemic factors that abrogated response to treatment, and that tumour removal allowed a more predictable response to CNV.

ccRCCs frequently harbour allelic inactivation of the *VHL* gene [7]. *VHL* loss can be due to gene mutations, promoter hypermethylation or chromosome 3 losses, and is reported to occur in up to 91% of the sporadic ccRCCs [8, 9]. The case herein reported was consistent with the *VHL*-mutated ccRCC spectrum: we detected a frameshift mutation that leads to a predicted truncated protein. We excluded the possibility of a germline mutation by genotyping the normal adjacent tissue that did not disclose *VHL* mutations. It is worth mentioning that *VHL* loss affected the tumour but was not a systemic alteration, which is consistent with the observed improvement in the subsequent evolution of the wet AMD condition after removal of the ccRCC.

We then sought to determine how *VHL* loss could be involved in the objective clinical benefit detected in our wet AMD patient. The protein product of *VHL*, pVHL, holds multiple functions; a pivotal one relates to its ability to target HIF-1α for polyubiquitination and its proteasomal degradation. Such imbalance in this mechanism, as observed in our pVHL-defective ccRCC case, fits with the expression of proteins under the transcriptional control of HIF-1α [10]. We observed that HIF-1α displayed transcriptionally active features (nuclear staining; fig. 3b) that may sustain a pseudohypoxic state featuring higher GLUT-1 and CA-IX expression than in normal tissue (fig. 3c and d). In accordance with our hypothesis that a tumour factor could restrain anti-VEGF therapy response, increased VEGF expression in the ccRCC cells was detected (fig. 3e). We claim that VEGF secreted from ccRCC tumour cells played a role in abrogating the response to VEGF inhibitor therapy for the wet AMD of this patient.

So far, this is the first report describing a wet AMD patient with an incomplete response to anti-VEGF therapy probably due to a ccRCC secreting proangiogenic factors. Indeed, a study [11] addressing circulating VEGF detection in cancer patients found that VEGF could only be substantially increased in RCC cancer patients, and this was due to a genetic defect (*VHL* alterations) that leads to increased VEGF production. In the same report, VEGF plasma levels were four and five times higher than in non-RCC cancer patients and normal controls, respectively, which is in accordance with our observations. Unfortunately, we could not retrieve blood from the patient before removal of the ccRCC to evaluate the plasma levels of VEGF.

In conclusion, our report shows that increased circulatory levels of VEGF, due to an angiogenic-factor secreting tumour, may inhibit the therapeutic effect of intravitreal anti-VEGF in wet AMD patients. Removal of the VEGF-secreting ccRCC led to a fading of angiogenic activity at the choroidal level and withheld the need for re-treatment. As far as we are aware, this might correspond to an incidental finding. Importantly, it represents a novel mechanism in a patient with wet AMD never addressed before. In cases of wet AMD that maintain neovascular activity despite aggressive intravitreal

Case Rep Ophthalmol 2012;3:443–451
DOI: 10.1159/000346045
Published online: December 22, 2012

ISSN 1663–2699
www.karger.com/cop

anti-VEGF therapy, poor response to treatment may be due to increased levels of circulating VEGF caused by proangiogenic neoplasias elsewhere. Therefore, we postulate that a systemic evaluation, including plasma levels of VEGF and/or systemic screening for tumours, should be considered in wet AMD whenever resistance to anti-VEGF therapy remains to be clarified.

Acknowledgment

This study was supported by Fundação para a Ciência e Tecnologia through the grant SFRH/BD/81940/2011. IPATIMUP is an Associate Laboratory of the Portuguese Ministry of Science, Technology and Higher Education that is partially supported by the Fundação para a Ciência e Tecnologia.

Case Rep Ophthalmol 2012;3:443–451
DOI: 10.1159/000346045
Published online: December 22, 2012

ISSN 1663–2699
www.karger.com/cop

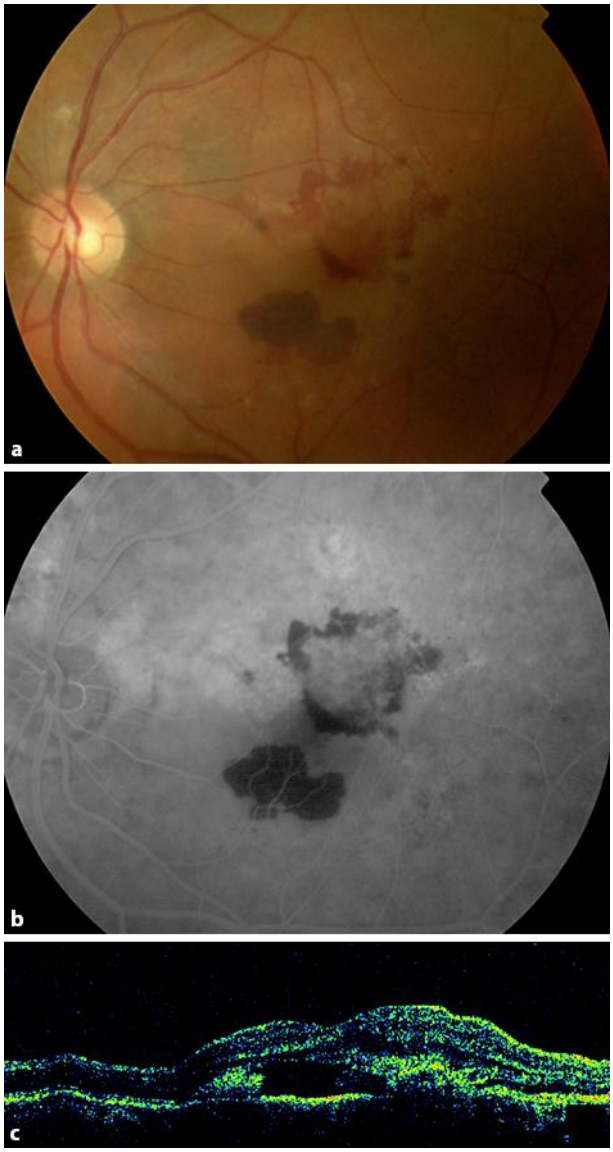

Fig. 1. Occult choroidal neovascular lesion secondary to AMD. Funduscopically (**a**), a large subretinal haemorrhage associated with drusen is observed. Fluorescein angiography shows a subfoveal occult neovascular lesion with late leakage (**b**). Time domain OCT demonstrates intraretinal and subretinal fluid associated with the subretinal choroidal neovascular membrane (**c**).

Case Rep Ophthalmol 2012;3:443–451
DOI: 10.1159/000346045
Published online: December 22, 2012

ISSN 1663–2699
www.karger.com/cop

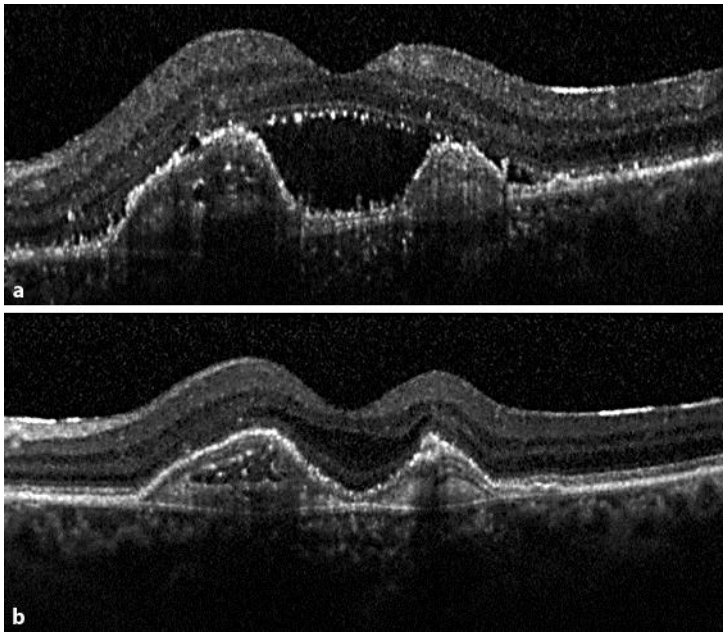

Fig. 2. Spectral domain OCT of the retina before and after removal of the ccRCC. Before surgery (**a**), subretinal fluid persisted despite 35 intravitreal injections. After surgery (**b**), subretinal fluid disappeared. Note the existence of subretinal fibrosis secondary to the choroidal neovascular complex both before and after surgery.

Case Rep Ophthalmol 2012;3:443–451
DOI: 10.1159/000346045
Published online: December 22, 2012

ISSN 1663–2699
www.karger.com/cop

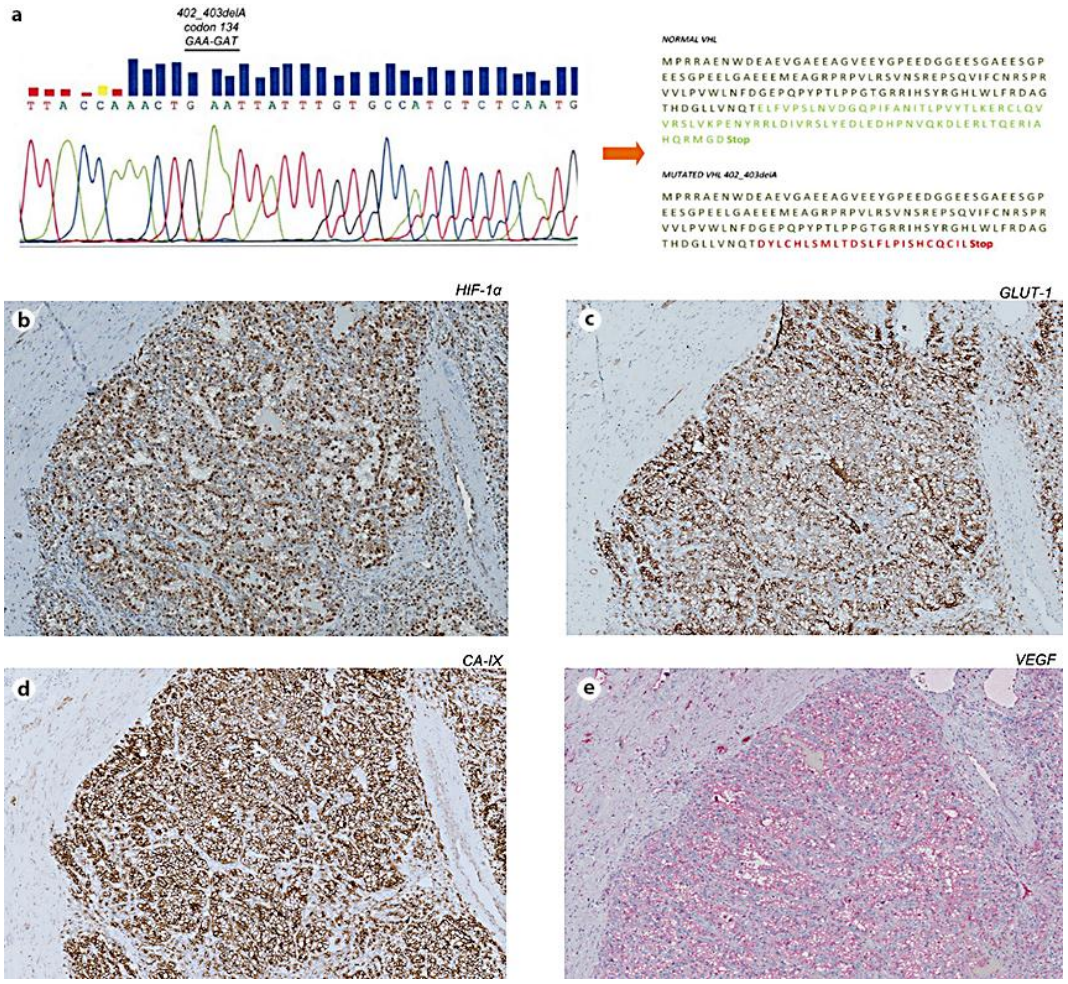

Fig. 3. Chromatogram of the *VHL* mutation (p.E134fs*25) with a deletion in nucleotide 402 and prediction of the deletion effect, resulting in a premature stop codon (**a**). Immunostaining of the ccRCC for proteins: HIF-1α (**b**); GLUT-1 (**c**); CA-IX (**d**), and VEGF (**e**). The presence of nuclear staining for HIF-1α indicates that it is transported to the nucleus where it acts as a transcription factor, leading to the expression of downstream targets such as GLUT-1, CA-IX and VEGF.

References

1 Klein R, Peto T, Bird A, Vannewkirk MR: The epidemiology of age-related macular degeneration. Am J Ophthalmol 2004;137:486–495.

2 CATT Research Group, Martin DF, Maguire MG, Ying GS, Grunwald JE, Fine SL, Jaffe GJ: Ranibizumab and bevacizumab for neovascular age-related macular degeneration. N Engl J Med 2011;364:1897–1908.

3 Carneiro AM, Mendonca LS, Falcao MS, Fonseca SL, Brandao EM, Falcao-Reis FM: Comparative study of 1+PRN ranibizumab versus bevacizumab in the clinical setting. Clin Ophthalmol 2012;6:1149–1157.

4 Carneiro AM, Costa R, Falcao MS, Barthelmes D, Mendonca LS, Fonseca SL, Goncalves R, Goncalves C, Falcao-Reis FM, Soares R: Vascular endothelial growth factor plasma levels before and after treatment of neovascular age-related macular degeneration with bevacizumab or ranibizumab. Acta Ophthalmologica 2012;90:e25–e30.

5 Rohan SM, Xiao Y, Liang Y, Dudas ME, Al-Ahmadie HA, Fine SW, Gopalan A, Reuter VE, Rosenblum MK, Russo P, Tickoo SK: Clear-cell papillary renal cell carcinoma: molecular and immunohistochemical

Case Rep Ophthalmol 2012;3:443–451
DOI: 10.1159/000346045
Published online: December 22, 2012

ISSN 1663–2699
www.karger.com/cop

analysis with emphasis on the von Hippel-Lindau gene and hypoxia-inducible factor pathway-related proteins. Mod Pathol 2011;24:1207–1220.

6 Moshfeghi AA, Rosenfeld PJ, Puliafito CA, Michels S, Marcus EN, Lenchus JD, Venkatraman AS: Systemic bevacizumab (Avastin) therapy for neovascular age-related macular degeneration: twenty-four-week results of an uncontrolled open-label clinical study. Ophthalmology 2006;113:2002.e1–e12.

7 Sültmann H, von Heydebreck A, Huber W, Kuner R, Buness A, Vogt M, Gunawan B, Vingron M, Füzesi L, Poustka A: Gene expression in kidney cancer is associated with cytogenetic abnormalities, metastasis formation, and patient survival. Clin Cancer Res 2005;11:646–655.

8 Nickerson ML, Jaeger E, Shi Y, Durocher JA, Mahurkar S, Zaridze D, Matveev V, Janout V, Kollarova H, Bencko V, Navratilova M, Szeszenia-Dabrowska N, Mates D, Mukeria A, Holcatova I, Schmidt LS, Toro JR, Karami S, Hung R, Gerard GF, Linehan WM, Merino M, Zbar B, Boffetta P, Brennan P, Rothman N, Chow WH, Waldman FM, Moore LE: Improved identification of von Hippel-Lindau gene alterations in clear cell renal tumors. Clin Cancer Res 2008;14:4726–4734.

9 Moore LE, Nickerson ML, Brennan P, Toro JR, Jaeger E, Rinsky J, Han SS, Zaridze D, Matveev V, Janout V, Kollarova H, Bencko V, Navratilova M, Szeszenia-Dabrowska N, Mates D, Schmidt LS, Lenz P, Karami S, Linehan WM, Merino M, Chanock S, Boffetta P, Chow WH, Waldman FM, Rothman N: Von Hippel-Lindau (VHL) inactivation in sporadic clear cell renal cancer: associations with germline VHL polymorphisms and etiologic risk factors. PLoS Genet 2011;7:e1002312.

10 Gossage L, Eisen T: Alterations in VHL as potential biomarkers in renal-cell carcinoma. Nat Rev Clin Oncol 2010;7:277–288.

11 Niers TM, Richel DJ, Meijers JC, Schlingemann RO: Vascular endothelial growth factor in the circulation in cancer patients may not be a relevant biomarker. PLoS One 2011;6:e19873.

M.S.F. and J.V. contributed equally to this work.