

## Case Report

# Pulmonary Lymphangitis Carcinomatosis of Clear Cell Renal Cell Carcinoma After Angiogenesis Inhibition

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## Abstract

**Background:** Over eighty percent of renal cell carcinomas of the clear cell type (ccRCC) constitutively secrete Vascular Endothelial Growth Factor-A (VEGF-A), due to a defect in the von Hippel Lindau (VHL) gene. These tumors are therefore highly angiogenic, making metastasized ccRCC (m-ccRCC) patients the prime candidates for anti-angiogenic therapy. Angiogenesis inhibition nowadays forms the backbone of first-line treatment of m-ccRCC patients. Despite prolongation of disease-free and overall survival, common experience is that resistance develops.

**Objective:** To get more insight in the pathophysiological mechanisms that underlie disease progression under anti-angiogenic therapy.

**Methods:** We extensively analyzed from a 68-year-old male m-ccRCC patient the primary tumor and the corresponding pulmonary metastasis that initially responded well to anti-angiogenic treatment, but ultimately progressed.

**Results:** We show that anti-angiogenic treatment induced a phenotypic adaptation in the lung lesion, characterized by infiltration of tumor cells along and even in the pulmonary vasculature, resembling pulmonary lymphangitis carcinomatosis. This phenotype was radiologically reflected by a cloudy pattern on CT.

**Conclusions:** These observations suggest that pulmonary metastases of renal cell carcinoma can respond to anti-angiogenic therapies by adopting a diffuse phenotype that allows progression in an angiogenesis-independent fashion through co-option.

**Keywords:** Angiogenesis; Cediranib; Pulmonary metastases; Renal cell cancer

## Introduction

Starting with the study of Hurwitz that demonstrated benefit of treatment with bevacizumab (the humanized anti-VEGF-A antibody) combined with chemotherapy on progression-free and overall survival of patients with disseminated colorectal carcinoma [1], angiogenesis inhibitors have made a firm entry into oncology practice. The effects of bevacizumab in combination with chemotherapy have now been investigated in a wide range of tumor types. Although initially beneficial, almost without exception resistance develops. In tumor types such as adenocarcinomas of the breast and glioblastomas, bevacizumab failed to prolong overall survival [2-4].

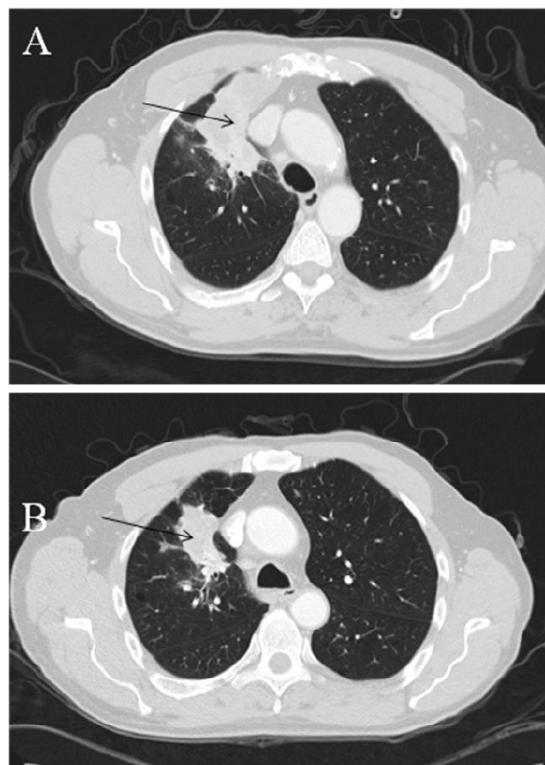
One of the few tumor types that is responsive to monotherapy with angiogenesis inhibitors is clear cell renal cell cancer (ccRCC) [5]. The large majority of ccRCCs have a defective Von Hippel Lindau gene (VHL) [6,7], resulting in increased half-life and accumulation of the transcription factors hypoxia inducible factors HIF1 $\alpha$  and/or HIF2 $\alpha$  [8]. Because VEGF-A is one of the prototype target genes of HIF1 $\alpha$ , ccRCC is highly vascularized and, indeed, responds relatively well to angiogenesis inhibitors. Currently, multispecific inhibitors of angiogenic receptors VEGFR2 and PDGFR $\beta$  are used for first-line treatment of patients with m-ccRCC [9]. Despite the impact on progression free survival also these patients develop resistance, reflected by a limited effect on overall survival. Different mechanisms of resistance to angiogenesis inhibitors have been proposed [10]. One of these is a phenotypic shift to non-angiogenic vessel co-option, a phenomenon that can be especially expected in tissues with a high vessel density. Recently, vessel co-option has been demonstrated as a resistance mechanism in hepatic metastases of colorectal carcinoma [11] and increased vessel co-option has also been observed in glioblastoma [12].

One of the problems in studying the morphological response of metastatic lesions to anti-angiogenic treatment, is the scarcity of post-treatment tumor specimen. Here we report on a patient with metastasized renal cell carcinoma who became amenable for metastasectomy after treatment with the VEGFR2/PDGFR $\beta$  inhibitor cediranib.

## Patient History

A 68-year-old male underwent radical tumor nephrectomy in April 1999. The histopathological workup of the specimen showed a pT3bN0M0 renal cell carcinoma of the clear cell type (revised according to WHO 2010 classification). In May 2002 metastatic disease in lung and mediastinal lymph nodes was diagnosed on a routine follow-up chest CT scan. IFN- $\alpha$  treatment was initiated in October 2002 and discontinued nine months later because the patient had stable disease. In December 2004, there was evident

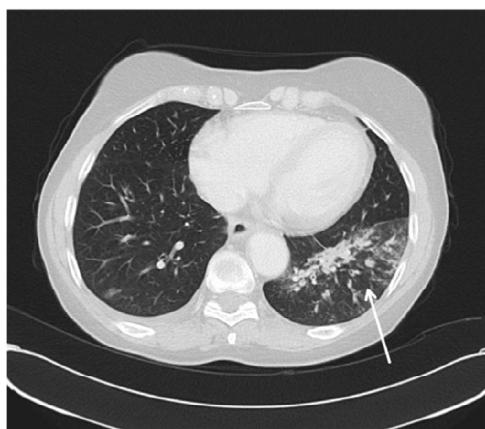
disease progression and the patient was enrolled in a phase I study (NCT00502060) in which he was treated with the combination of the angiogenesis inhibitor cediranib (20 mg p.o. daily), a VEGF receptor 2 (VEGFR2)- and Platelet Derived Growth Factor Receptor (PDGFR)-inhibitor, and gefitinib (500 mg p.o. daily), an Epidermal Growth Factor Receptor (EGFR) inhibitor. After 4 weeks of therapy the lung metastases showed a partial response with tumor downsizing from 7.5 cm to 4.5 cm (Figures 1A and 1B, respectively). Metastasectomy followed in October 2005 after a two week drug holiday to prevent wound healing complications. Subsequent histopathological analysis revealed extensive infiltration of tumor in the lung tissue, reaching in the resection plane.



**Figures 1(A,B):** CT scans of a 68-year-old male patient at baseline (A, December 2004) and after 4 weeks of therapy with cediranib/gefitinib (B, January 2005). Note the regression of the tumour at the upper left part of the lung. This partial response enabled metastasectomy.

In July 2006, tumor progression was observed in the conventional thorax X-ray. Malignancy was confirmed in August 2006 in bronchus brush cytology material from the lower left lung. As third line therapy, patient was treated with the multi-target angiogenesis inhibitor sorafenib (400 mg orally daily). During this treatment, the pulmonary lesions showed progression to a cloudy pulmonary lymphangitis carcinomatosis-like (PLC-like) pattern in the CT scan. In February 2007 a high-resolution CT scan (HRCT) of the chest showed a diffuse infiltrative process suggestive of

PLC (Figure 2). The patient died in January 2008. An autopsy was not performed. This study was performed in accordance with Radboudumc guidelines, and included informed consent from the patient.



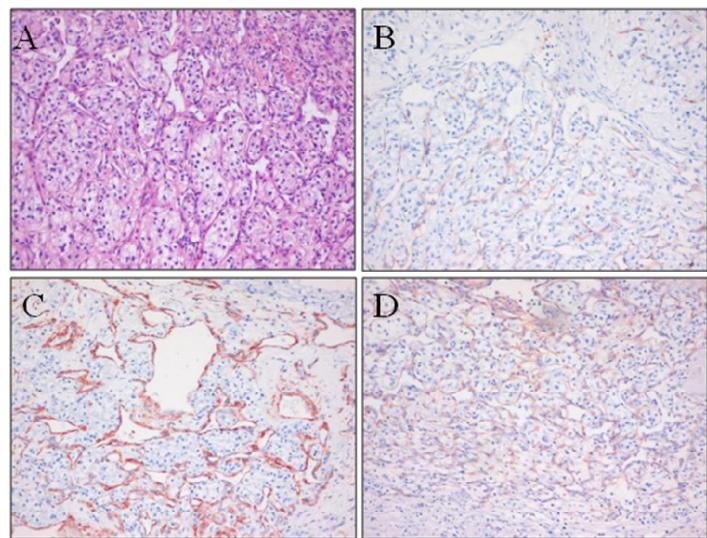
**Figure 2:** Chest high resolution CT (HRCT) scan of patient after sorafenib treatment. Arrow points at a region suspect for infiltrative growth.

## Methods

Routine thorax CT-scans were used for the evaluation of metastases. To examine the effect of anti-angiogenic treatment, tissue blocks from the primary ccRCC specimen and metastasectomy specimen were used. Immunohistochemical stainings were performed with antibodies against cytokeratin 7 (CK7; to detect pulmonary epithelial cells), carbonic anhydrase-IX (CA-IX; antibody G250, to detect tumour cells) [13], CD34 (NeoMarkers, USA, to detect endothelial cells), laminin (DakoCytomation, Denmark, to detect vessel basement membrane), D2-40 (to stain for lymph vessels),  $\alpha$ -SMA ( $\alpha$ -smooth muscle actin, to detect pericytes, Sigma, Zwijndrecht, The Netherlands), Ki67 (DakoCytomation, Denmark, to detect tumour cell proliferation) and cleaved caspase 3 (BD Pharmingen, to detect apoptosis) essentially as described [14].

## Results

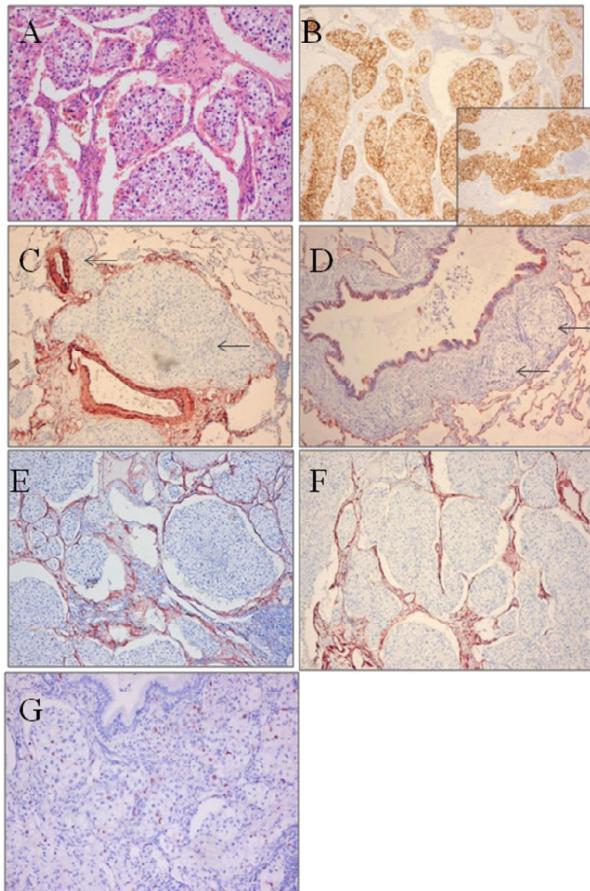
Histological analysis of the tumour nephrectomy specimen showed a typical ccRCC morphology (H&E staining in Figure 3A) with central areas of necrosis and at the tumour rim a micronodular phenotype with clusters of cancer cells, surrounded by endothelial cells (CD34 staining in Figure 3B), pericytes ( $\alpha$ -SMA staining, Figure 3C) and basement membrane (laminin staining in Figure 3D) [15]. The tumor was homogeneously positive for carbonic anhydrase IX (CA-IX), a known marker of ccRCC [16] (not shown).



**Figures 3 (A-D):** Morphology of the primary tumour. A characteristic clear cell phenotype is observed (H&E in A) with high vessel density as seen with CD34 (B),  $\alpha$ -smooth muscle actin (C) and laminin (D) staining. Magnification x50.

Immunohistochemical analysis of the treated metastasis is depicted in figure 4. Overall, the centre of the tumour was necrotic and viable tumour was present at the rim (Figure 4A). Distant from the main tumour mass, and throughout the entire surgically removed tissue, numerous small tumour deposits were observed that infiltrated the normal tissue (G250 staining for CA-IX in Figure 4B). Central hypoxia was observed only occasionally (determined by glut-1 expression [14], not shown). The appearance of tumor deposits was often branched (inset in Figure 4B) suggestive of an association with vasculature. Further analysis revealed that tumour deposits were almost always localized directly adjacent to larger pulmonary arteries ( $\alpha$ -SMA staining in Figure 4C, arrows point at peri-arterial tumour deposits) but association around bronchi was also frequently observed (CK7 staining in Figure 4D). Importantly, large parts of the viable tumour mass were characterized by the presence of a fibrovascular network, positive for CD34 (Figure 4E),  $\alpha$ -SMA (Figure 3F), laminin and collagen IV (not shown), similar to the network that is characteristically observed in primary ccRCC. In the treated metastasis, however, this vascular network formed a relatively open structure with large spaces between tumour cell clusters and vessel wall elements. In these spaces erythrocytes were present (Figure 4A), suggesting an intravascular localization of tumour aggregates and at least some degree of circulation. Tumour cells in these aggregates appeared viable. Intriguingly, a locally high proliferation index was observed (approximately 50%, Ki67 staining in Figure 4G), although this was only apparent

in regions with large spaces between tumour cells and vessel wall elements. Fibrovascular structures in the metastasis were positive for the lymphatic endothelial marker D2-40 (not shown) suggestive of the presence of tumour in the lymphatic vasculature; a large proportion of D2-40 positive vessel structures were also CD31- and CD34-positive and associated with pericytes.



**Figures 4(A-G):** Morphology of pulmonary post-treatment metastases. Multiple cuffs of tumour cells are detected in a vascular cast with H&E staining (A) and CA-IX staining (B). Inset in B shows branched tumour ‘rods’, suggestive of vessel association. Peri-arterial tumour localization is illustrated in  $\alpha$ -SMA staining (C, arrows point at tumour cell cuffs) but frequently the tumour was also present in the wall of bronchi (CK7 staining in D). E-G represent immunostained sections of tumour regions, adjacent to the necrotic centre of the tumour. Note the similarity of the vascular architecture with that in the primary tumour (Figure 1B) whereas numerous small, apparently shrunk, tumour aggregates are located within a vascular cast that is CD34 (E) and  $\alpha$ -SMA (F) positive. Note also the presence of erythrocytes in the spaces between tumour aggregates and vascular elements, suggestive of intravascular tumour localization (A). Panel G indicates relatively high proliferation via Ki67 staining in some areas. Magnification x100.

## Discussion

The highly angiogenic character of ccRCC, a result of constitutive VEGF-A expression, has resulted in therapeutic approaches that make use of VEGF pathway inhibition. The clinical implementation of tyrosine kinase inhibitors (TKIs) directed against VEGFR and PDGFR has considerably improved the prognosis of ccRCC patients [17,18] yet does not prevent cancer progression in the end. In preclinical models of brain metastases of melanoma and orthotopic models of glioma, inhibitors of angiogenesis induce a shift from an expansive to an invasive growth mode in which tumor cells use pre-existent blood vessels (vessel co-option) [19-21] and recently the clinical relevance of these findings has been demonstrated in liver metastases of colorectal carcinoma and breast adenocarcinoma under bevacizumab treatment [11], as well as in glioblastoma under bevacizumab/temozolomide treatment [12]. The data presented here suggest that also in other vessel-dense tissues such as lung, vessel co-option can be an effective way for a tumor to escape the effects of anti-angiogenesis. Furthermore, it appears that tumor deposits not only coopt blood vessels but also bronchi, which may be an alternative method for cancer cells to ensure an effective supply of oxygen.

This observation has important consequences. First, whereas chest CT scans suggest response to therapy based on the shrinkage of the bulk of tumor, small cancer cell deposits that invade the lung lymphatics or lung vasculature are less easily recognized on conventional CT scans. These deposits prohibit curative surgery. Our observation may also explain massive but reversible flare up of pulmonary metastases of renal cell cancers that can be observed once treatment with anti-angiogenic drugs is terminated [22]. Indeed, progression of cancer in the lung via diffuse growth while suppressing VEGF-effects may result in a significant increase of tumor burden and high levels of local VEGF. Upon withdrawal of VEGF suppression, the high levels of VEGF will rapidly induce pleural effusion and dyspnoea and pain, which reduces upon readministration of drugs.

The patient in this study was treated in third line with sorafenib which, again, ultimately could not prevent tumor progression. In high resolution CT scans lymphangitis carcinomatosis was observed. Although no post-mortem examination was performed, and no histopathological confirmation is available, the CT scans in Figure 2 also strongly suggest extensive tumor infiltration in the lung, although with a different phenotype than the “cannon ball metastases”; large, round and sharply demarcated lesions, that are normally observed for renal cell cancer metastases in lung [23]. Pulmonary lymphangitis carcinomatosis is rarely seen in m-ccRCC, but has been described more often in the context of angiogenesis inhibition [22]. Because we did not have pre-treatment

HRCT scans available, it is difficult to attribute the development of this phenotype to the cediranib or sorafenib treatment.

In this report we describe for the first time using extensive (immuno)histopathology and radiology that pulmonary metastases of renal cell cancer can adapt to anti-angiogenic treatment by vessel and bronchus co-option. As this report is based on one patient, expanding this work to other similar cases to find validation of our findings is important, but also difficult because post-treatment metastatic cancer samples are scarce. Still, the biological concept of vessel co-option as a mechanism of resistance to angiogenesis inhibitors has already been demonstrated in preclinical models and in patients, giving credibility to our findings.

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## Conflicts of Interest

None of the authors have competing interests to declare.

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