Published in final edited form as:

Cancer. 2009 May 15; 115(10 0): 2346–2354. doi:10.1002/cncr.24228.

Circulating Biomarkers for Vascular Endothelial Growth Factor Inhibitors in Renal Cell Carcinoma*

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Abstract

In recent years, there has been significant progress in the clinical development and application of antiangiogenic therapies in renal cell carcinomas, particularly inhibitors of the vascular endothelial growth factor (VEGF) pathway. Despite this progress, no validated methods are currently available for identifying which patients are most likely to respond to treatment or experience toxic effects, selecting the optimal dose, or determining whether the intended molecular target has been effectively inhibited. However, recent studies have suggested that some of the biomarkers currently under investigation in clear cell renal cell carcinoma for VEGF pathway inhibitors are promising. These biomarkers include circulating proangiogenic factors and receptors; markers of hypoxia and endothelial damage; and cellular populations in peripheral blood, such as circulating endothelial cells. Further preclinical and translational validation studies are still needed to determine their practical utility in the clinical setting.

Keywords

blood-based biomarkers; vascular endothelial growth factor; angiogenesis inhibitors; renal cell carcinoma; circulating endothelial cells

During the past decade, antiangiogenic therapy has moved from theory to clinical practice. Bevacizumab, amonoclonal antibody directed against vascular endothelial growth factor (VEGF), has been demonstrated to provide clinical benefit when combined with chemotherapy for colorectal, lung, and breast cancer. Furthermore, bevacizumab and multitargeted inhibitors blocking the VEGF receptor (VEGFR) pathway, such as sunitinib

*Innovations and Challenges in Renal Cancer: Proceedings of the Third Cambridge Conference, Supplement to Cancer

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and sorafenib, have demonstrated significant clinical activity in chemotherapy-refractory tumors, such as renal cell carcinoma (RCC).[1–3] Despite these advances, the biologic activity of these and newer agents remains difficult to assess. Given the large number of targeted agents now entering clinical testing in RCC and the escalating costs of drug development, it is generally not feasible to perform large randomized trials for drugs without extra evidence of biologic activity early in their development. Consequently, a real risk remains that drugs that could ultimately benefit patients may not be developed and even that many patients might be treated with less effective drug doses or schedules in phase 2 or 3 trials because of a lack of correlates of activity in earlier clinical testing. Therefore, surrogate biomarkers are clearly needed to advance the clinical development of VEGF inhibitors. These markers may serve some, or all, of the following uses: 1) assessment of expected biologic activity; 2) optimization of dosing; 3) identification of patients most likely, or least likely, to benefit from a given treatment; and/or 4) monitoring response to treatment and investigating potential mechanisms of resistance.

SOLUBLE MARKERS IN SERUM AND PLASMA

Circulating Angiogenic Growth Factors, Inhibitors, and Related Vascular Molecules

Most patients with advanced RCC demonstrate clear cell histology, which is typically characterized by von Hippel-Lindau gene (*VHL*) inactivation. This process leads to an abnormal accumulation of hypoxia-inducible factor (HIF) and a deregulated HIF-1 activity that results in the transcription of >200 hypoxia-inducible genes, including mediators of angiogenesis such as VEGF, platelet- derived growth factor, transforming growth factor–α, erythropoietin, and carbonic anhydrase IX (CAIX).[4] Several molecules implicated in angiogenesis can be detected in meaningful amounts in circulation (serum or plasma) and other biologic fluids in patients with RCC and serve as biomarkers for monitoring anti-VEGF therapies.

The majority of antiangiogenic drugs used in clinical practice or currently under development in RCC target the VEGF signaling pathway directly or indirectly. Patients with RCC demonstrate increased VEGF levels compared with healthy controls.[5] High serum VEGF levels have been associated with tumor stage, tumor grade, disease progression, and poor prognosis.[5–7] The Groupe Français d'Immunotherapie recently presented data indicating an independent correlation between VEGF and event-free and overall survival in metastatic RCC patients with good and intermediate prognostic characteristics.[8] Similar results were found in patients treated with placebo or bevacizumab in combination with interferon-α in the phase 3 TARGET (sorafenib) and AVOREN clinical trials, respectively. Above–median VEGF concentrations were found to be correlated with significantly shorter progression-free survival (PFS),[7,9] supporting the notion of VEGF being a prognostic biomarker in clear cell RCC. Interestingly, patients with high and low pretreatment VEGF levels benefited equally from sorafenib (5.5 months in terms of PFS) in the TARGET trial.[7]

Plasma and serum VEGF levels are also currently being actively investigated as biomarkers of activity of VEGF inhibitors. In preclinical models, blood plasma levels of VEGF are rapidly and significantly increased by VEGFR-2 blockade in a dose-dependent manner,[10] with maximum values peaking when doses previously determined to be optimal for therapy

were used. The mechanisms responsible for these fluctuations in serum or plasma VEGF are not known. Likewise, VEGF levels increase during VEGF-targeted therapy in RCC patients,[7,11,12] but the extent of VEGF modulation varies widely in different individuals and depends on the specific drug, its potency, and its mechanism of action.[13]

VEGF-A binds to 2 receptor tyrosine kinases: VEGFR-1 (Flt-1) and VEGFR-2 (KDR, flk-1). Naturally occurring, soluble forms of VEGFR-1 (sVEGFR-1), VEGFR-2, and VEGFR-3 (which is involved in lymphangiogenesis) have been described previously.[13–15] Soluble VEGFR-1 has been studied as a surrogate marker for inhibition of angiogenesis in RCC.[16] The functional significance of sVEGFR-2 and sVEGFR-3 remains unclear, but decreases in plasma sVEGFR-2 appear to be related to VEGF-induced down-regulation from the cell surface.[17] VEGF and sVEGFR-2 change in a reciprocal manner during the treatment of patients with RCC and other cancers with tyrosine kinase inhibitors.[7,13,18–20] Initial experiences in assessing changes in serum or plasma levels of these factors as a surrogate for exposure to anti-VEGF agents and even patient benefit suggest a clear potential value. Most of the available results in RCC come from sunitinib-treated patients, but similar data have been shown for sorafenib and pazopanib (Table 1). Overall, larger changes in VEGF, sVEGFR-2, and sVEGFR-3 levels have been observed in patients achieving treatment response, suggesting their potential value as biomarkers of pharmacologic and clinical activity.[13,21]

Other Molecules Regulatory of Angiogenesis

Circulating serum and/or plasma levels of fibroblast growth factor–2, placental growth factor, hepatocyte growth factor, thrombospondin-1, soluble Tie-2, and interleukin–8 have been explored for their potential as surrogates of angiogenesis and to monitor response to VEGF-targeted therapies. In RCC, elevations in plasma levels of placental growth factor have been reported after therapy with sunitinib.[12,13,21]

Markers of Endothelial Cell Damage and Hemostasis

VEGF inhibition markedly increases the thromboembolic risk in cancer patients.[22–25] Markers of endothelial cell function or damage include von Willebrand factor, soluble thrombomodulin, soluble tissue factor, and soluble E-selectin. To our knowledge, few data are available regarding modulation of these factors by antiangiogenic therapy in RCC.

Markers of Hypoxia

Hypoxia promotes the production of proangiogenic factors, many of which are regulated by the HIF-1α pathway.[4] High levels of HIF-1α have been shown in RCC.[26] Because VEGF inhibitors may induce tumor hypoxia,[27] the role of the HIF-1α pathway in the response to antiangiogenics is an area of active investigation. Data preliminarily presented at the 2008 annual meeting of the American Society of Clinical Oncology suggest that high tumor levels of HIF-2a as assessed by Western blot analysis are predictive of sunitinib response in patients with metastatic RCC.[28] Levels of the circulating hypoxia-regulated proteins osteopontin and soluble CAIX have been shown to have prognostic value in RCC[29] and hold promise for monitoring the effects of VEGF-targeted therapies. High levels of CAIX

expression in tissue (along with GLUT-1, another HIF-regulated marker) have been preliminarily linked to response to sorafenib and improved PFS in RCC patients.[30]

In summary, several circulating angiogenic growth factors and related vascular molecules appear to have a role as pharmacodynamic biomarkers of exposure to and effect of anti-VEGF therapies. More studies, however, are needed to evaluate their role in response prediction and to validate these initial findings.

CIRCULATING ENDOTHELIAL CELLS AND OTHER CELLULAR MARKERS IN PERIPHERAL BLOOD

Rationale

It has been recognized for more than 30 years that cells bearing characteristics of endothelial cells can be detected in peripheral blood. The majority of these cells, termed circulating endothelial cells (CECs), appear to arise from blood vessel walls and are increased after vascular damage.[31–33] However, in recent years it has been demonstrated that a subset of CECs derived from bone marrow, the so-called circulating endothelial precursors (CEPs), can differentiate into mature endothelial cells and contribute to neovascularization in both murine models and humans.[34] The contribution of CEPs to the tumor vasculature appears to be highly variable and depends on both host and tumor contexts.[34–37] In humans, mature CECs and CEPs are distinguished from other peripheral blood mononuclear cells based on surface markers such as VEGFR-2 (KDR) and CD133.[38] The rationale for investigating these cells as a potential biomarker is as follows. First, CECs are known to be increased in cancer patients and are known to be associated with disease progression.[39,40] Second, these cells are known to be mobilized in response to VEGF in both murine models[35,41] and humans[42,43] and to express molecular targets for many of the angiogenesis inhibitors currently in development, including VEGFR-1 and VEGFR-2. Finally, CECs may serve as a marker of damage to tumor vasculature and, therefore, reflect activity and potential benefit. Therefore, distinguishing between CEPs and mature CECs is important because one would expect VEGFR inhibitors to decrease the number of CEPs by inhibiting their mobilization but increase the number of sloughed vessel wall derived CECs (Fig. 1).

A variety of different methods have been used to detect CECs. Most recent studies applied within the clinical setting have used multicolor flow cytometry using a panel of antibodies to endothelial, hematopoietic, and progenitor cells.[44,45] Flow cytometry-based methods have significant advantages in that they permit multiparametric analyses and high-speed measurement. There are, however, significant methodologic issues related to differences in the antibodies and markers used (cell preparation; use of nucleated, activated, replicating, or viable cell staining; absolute or relative counts; and gating strategies) that make it difficult to compare results among different investigators and studies. Furthermore, to the best of our knowledge, no single antigen identified to date is entirely specific to endothelium. A variety of definitions of these different populations have been proposed based on different markers. Most commonly, CD133 has been used as a marker of CEPs; with CD31, CD146, VEGFR-2, and VE-cadherin being common endothelial markers and CD45 being used to

identify hematopoietic cells. Clearly, ongoing efforts standardizing these different methods will help compare results from different investigators and from different studies.[47]

Clinical Results: Changes in CECs in Response to Treatment

CECs (including both mature CECs and CEPs) have been shown to be elevated in RCC patients compared with healthy controls. Ebbinghaus et al investigated blood samples from 77 RCC patients and 19 healthy subjects and found significantly higher CEC and CEP counts in cancer patients.[48] More recently, Bhatt et al[49] analyzed blood samples from patients with von Hippel-Lindau (VHL) syndrome and RCC and found an increased ratio of CEPs and CECs only in RCC patients with and without VHL syndrome compared with those with VHL syndrome with no RCC and healthy controls.

CECs have been evaluated after treatment with angiogenesis inhibitors in several clinical studies in RCC. It is not yet possible to draw definitive conclusions from those studies to date because they have been of modest size and used different detection methods and phenotypic markers. Nevertheless, they suggest that CECs may be a useful marker that merits further investigation in larger trials (Table 2). In 1 case, baseline levels of CECs were shown to stratify patients with advanced RCC who were receiving treatment with the thrombospondin-1 mimetic peptide ABT-510 with regard to time to disease progression.[48] Patients with higher baseline CEC levels had a significantly shorter time to disease progression compared with those with low and stable CECs counts. However, in a smaller study of 21 RCC patients receiving treatment with sunitinib or sorafenib, elevated baseline levels of CECs and low CEPs were found to be correlated with improved treatment response, whereas high CEC counts by Day 14 of treatment predicted poor response.[50] A sunitinib- induced increase in CEPs has also been shown to be predictive of treatment benefit.[51]

These and additional studies in other cancers illustrate that CECs change in a dynamic manner after treatment with an angiogenesis inhibitor and that the kinetics of change, as well as the specific population of cells that are changing, are critical parameters to assess. The development and standardization of robust, reproducible, and multiparametric analytical methods are clearly an important unmet need in the field.

Other Cellular Populations in Peripheral Blood as Potential Biomarkers

Although it was initially assumed that the contribution of bone marrow-derived cells to angiogenesis was through CEPs, it recently has been appreciated that circulating cell populations with a clear hematopoietic origin may contribute as well. Such cells have in common their commitment to the myeloid lineage in a more or less advanced stage of differentiation.

The majority of available data are related to myelomonocytic cells that home to areas of vascular injury and contribute to angiogenesis and metastasis by secreting angiogenic growth factors, facilitating extracellular matrix remodeling, promoting tumor cell motility, and providing structural support to the adjacent endothelial cells by establishing themselves as mural cells or pericyte precursors.[52–54] These results suggest that distinct populations of hematopoietic cells may functionally contribute to angiogenesis and metastasis. Also

relevant are recent data implicating $CD11b^+$ $Gr\text{-}1^+$ cells, a phenotype that corresponds to murine immunosuppressive myeloid cell populations,[55] in therapeutic resistance to VEGF pathway inhibition in animal models of cancer.[56]

However, it is unknown in humans whether, after treatment with antiangiogenic agents, these cell populations may serve as a target or as a biomarker or both. Circulating myeloid-derived cells with immunosuppressive activity, defined as arginase-producing or displaying a $CD15^+$ $CD14^-$ $CD33^+$ $HLA\text{-}DR^-$ phenotype, have been found to be significantly increased in the peripheral blood of patients with metastatic RCC compared with healthy controls.[57–59] As a step toward identifying new potential cellular biomarkers for the VEGF pathway, we screened peripheral blood for cellular populations binding VEGF and the expression of VEGFRs. We found that most VEGF-binding cells in peripheral blood were $VEGFR\text{-}1^+$ cells coexpressing the monocyte marker CD14 (CECs also expressed VEGFR-1 and VEGFR-2, but were present at lower levels).[60] Next, we monitored changes in CEC and monocyte numbers during treatment with sunitinib in patients with metastatic gastrointestinal stromal tumors and correlated these findings with clinical benefit. Mean monocyte numbers decreased by 54% from baseline to Day 14 of treatment, and there was a greater decrease noted in the progressive disease group compared with the clinical benefit group (58% vs 48%). Monocyte levels rebounded toward baseline during a rest period and then repeated the pattern when sunitinib therapy was restarted. In patients with metastatic RCC, initial experience suggests that treatment with sunitinib reverses the accumulation of myeloid-derived suppressor cells.[58,59] Therefore, accumulating data indicate that myelomonocytic cells in peripheral blood hold potential as a pharmacodynamic marker for sunitinib activity and clinical benefit in cancer patients, including those with RCC. Because such cells express VEGFR-1 and other potential targets for angiogenesis inhibitors such as platelet-derived growth factor receptor-β, Kit, and Tie-2,[60–65] we are currently assessing distinct subpopulations to determine whether that may serve as a more specific biomarker of activity.

FUTURE DIRECTIONS

In part due to recent randomized phase 3 trials in RCC demonstrating that angiogenesis inhibitors can benefit cancer patients, there has been an explosion in the number and variety of these agents currently under development and clinical testing. These advances, although welcomed, present a new set of challenges and obstacles for the field. First, there is a major yet unmet need for standardized, validated, noninvasive markers for evaluating the activity of these agents and for assessing the degree of target inhibition caused by treatment. This is needed for both selecting drugs to advance in clinical development and choosing the optimal dose for a given patient. Second, it will be necessary to identify the mechanisms by which tumors become resistant to these agents and to develop markers to monitor for this resistance. Biomarkers will play a critical role in the rational design of regimens, including combinations of different angiogenesis inhibitors. Third, it will be critical to develop robust predictive markers to guide the selection of the most appropriate agent or combination for the individual patient before initiating therapy. For all these reasons, biomarkers are likely to play an increasingly important role in the clinical development of VEGF inhibitors and other antiangiogenic agents in RCC and the cancer field in general.

Acknowledgments

Conflict of Interest Disclosures

The program was made possible by educational grants provided by Genentech; Novartis Pharmaceuticals, Pfizer, Inc., and Wyeth Pharmaceuticals. Program management and CME sponsorship were provided by InforMEDical Communications, Inc., Carlisle, Mass.

OPEN DISCUSSION

The questions and discussion below follow from the oral presentation given at the Third Cambridge Conference on Innovations and Challenges in Renal Cancer and do not correspond directly to the written article, which is a more general review.

Dr. Bernard Escudier: How can we standardize biomarker thresholds?

Dr. Amado J. Zurita: Only those markers that showed statistically significant interaction with the treatment arm were chosen.

Dr. Gary Hudes: Don't you need to look at each biomarker separately and determine whether it is a mean value or whether there is a relationship between a certain cutoff value and whatever your endpoint is ?

Dr. Zurita: We did that for the univariate analysis.

Dr. Jeffrey Sosman: Have you looked further at what changes occur with therapy ?

Dr. Zurita: Yes. We accessed changes in biomarker concentrations from baseline to 4 weeks and 8 weeks on treatment.

Dr. Michael Atkins: This multiplex bead profiling technology can be applied to a lot of different diseases. The ideal place to look at this is with antiangiogenic and targeted agents in kidney cancer, where these treatments work. You can gain a sense of what the treatments are doing, you can look at the various pathways and how they change, and you can determine whether they might predict for a particular therapy. We have an advantage in kidney cancer relative to other diseases and an opportunity to test this technology.

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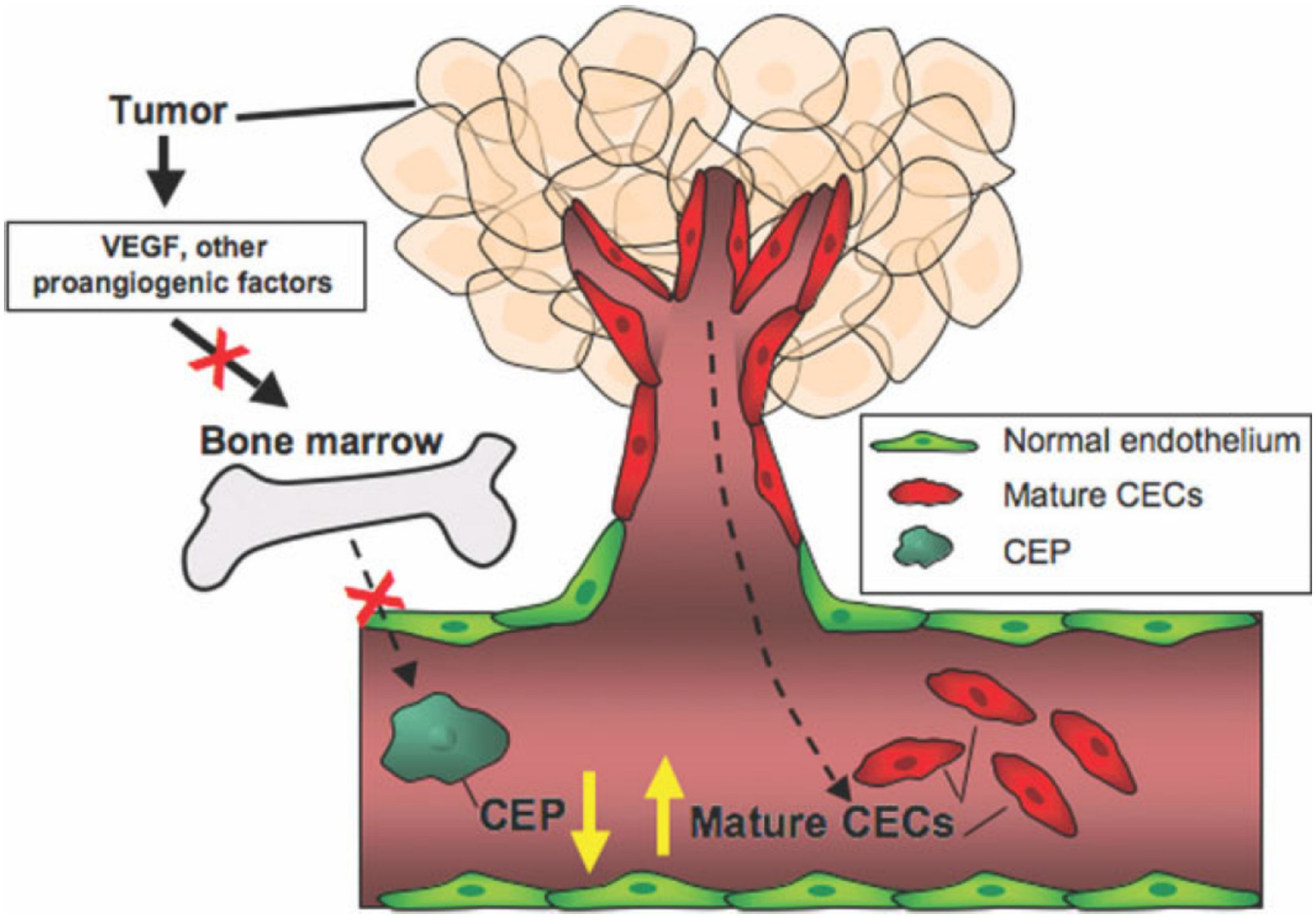

Figure 1.

Potential changes in circulating endothelial progenitors (CEPs) and mature circulating endothelial cells (CECs) in response to treatment with antiangiogenic agents. Angiogenesis inhibitors may decrease the mobilization of bone marrow-derived CEPs induced by vascular endothelial growth factor (VEGF) and other proangiogenic cytokines but at least transiently increase the shedding of mature endothelial cells by preferentially targeting fragile tumor endothelium (2-compartment model).[66]

Table 1

Reported Effects of Tyrosine Kinase Inhibitors on VEGF and sVEGFR-2 in Renal Cell Carcinoma Patients

Drug	Target	VEGF	sVEGFR-2	Study
Sorafenib	VEGF-1, VEGF-2, VEGF-3, PDGFR, RET, Raf	↑	↓	Bukowski 2007[7]
Sunitinib	VEGF-1, VEGF-2, PDGFR, KIT, RET	↑	↓	Motzer 2006[12] Deprimo 2007[13] George 2007[21]
Axitinib	VEGF-1, VEGF-2, VEGF-3, PDGFR, KIT	↑	↓	Rixe 2005[19]
Pazopanib	VEGF-1, VEGF-2, VEGF-3, PDGFR	↑	↓	Hutson 2008[18]
Cediranib	VEGF-1, VEGF-2, VEGF-3, PDGFR, KIT	NA	↓	van Herpen 2007[20]

VEGF indicates vascular endothelial growth factor; sVEGFR, soluble vascular endothelial growth factor receptor; PDGFR, platelet-derived growth factor receptor; NA, not applicable.

Table 2

Studies Describing Detection of CECs and CEPs by Flow Cytometry in Metastatic Renal Cell Carcinoma Patients

Study	Cell Phenotype	Drug	No.	Association
Ebbinghaus 2005[48]	$CD45^{-}CD31^{+}CD146^{+}LDS751^{+}$	ABT-510	77	CEC <15/μL–longer TTP
Escudier 2008[50]	CECs:	Sunitinib	21	High BL CEC–response
	$CD45^{-}CD31^{+}CD146^{+}7AAD^{-}$	Sorafenib		High BL CEP–poor prognosis
	CEPs:			High C1 on D 14 CEC–poor response
	$CD45^{dim}CD34^{+}VEGFR2^{+}7AAD^{-}$			
Vroling 2008[51]	CEPs:	Sunitinib	23	Increase in CEP C1–response
	$CD45^{-}CD133^{-}CD34^{bright}$			

CECs indicates circulating endothelial cells; CEPs, circulating endothelial progenitors; −, negative; +, positive; TTP, time to disease progression; BL, baseline; C1, cycle 1; D 14, Day 14.