A Continuing Medical Education Activity sponsored by InforMEDical Communications, Inc.
InforMEDical Communications, Inc. • tel: 978-318-9582 • fax: 978-246-8044 • email: info@informedicalcme.com

TRANSLATIONAL RESEARCH STUDIES IN RENAL CANCER:
Mechanisms and Management of Resistance to Anti-VEGF Therapy in Renal Cell Carcinoma

Rupal S. Bhatt MD, PhD and Michael B. Atkins MD, Beth Israel Deaconess Medical Center, Boston, MA

Supported by: DF/HCC Kidney Cancer SPORE NIH P50 CA101942

Introduction

The greatest advance in the treatment of patients with renal cell carcinoma (RCC) over the last few years has been the introduction into clinical practice of antitumor agents that function primarily as inhibitors of vascular endothelial growth factor (VEGF)-driven angiogenesis. The prospect that VEGF receptor (VEGFR) antagonists might be particularly useful in the treatment of patients with clear cell RCC was predicted from the genetic alterations peculiar to the disease.[1] Approximately 60% of clear cell RCC lacks a functional von Hippel-Lindau (VHL) gene as a result of biallelic loss from mutation and/or hypermethylation. The VHL gene encodes an E3 ligase involved in the oxygen-dependent ubiquitination and proteasomal degradation of HIF-1α and HIF-2α, subunits of transcriptional factors involved in the expression of VEGF and other hypoxia-driven genes. The loss of VHL results in the accumulation of HIF (even in normoxic conditions) leading to increased expression of HIF regulated genes such as VEGF and platelet derived growth factor (PDGF).[1] This feature of clear cell RCC is thought to account for the unique initial sensitivity of these tumors to VEGF pathway antagonists.

Several VEGFR antagonists, (sunitinib, sorarenib, and pazopaninb) have proven effective in randomized clinical trials at producing tumor shrinkage and prolonging median progression free survival resulting in their FDA approval.[2-4] Other agents (e.g. axitinib and tivozanib) are currently under late stage investigation and may shortly be added to the therapeutic armentarium.[5,6] While these results are exciting and have revolutionized the treatment of patients with advanced RCC, they still leave considerable opportunity for improvement. The various VEGF pathway inhibitors produce few if any complete or durable responses; tumors typically acquire resistance to VEGFR inhibition at a median of 5-12 months at which point tumor growth resumes, sometimes at an accelerated pace, even with continued VEGF pathway blockade.

Mechansims of resistance

In some malignancies, such as lung cancer or CML, the development of resistance to a targeted therapy (e.g. erlotinib, imatinib) is often due to a mutation in a gene encoding a key receptor tyrosine kinase targeted by the drug.[7,8] VEGFR antagonism, however, likely capitalizes on the unique vulnerability of tumor endothelial cells, leaving damage to the tumor as a secondary effect. Thus, the mechanisms underlying the acquired resistance to VEGFR targeted therapy likely involve an adaptive response to increasing tumor hypoxia resulting from treatment-induced pruning of the tumor microcirculation rather than a stable genetic mutation in a tumor cell. In support of this possibility, we have shown that acquired resistance to sorafenib or sunitinb therapy is accompanied by a restoration of tumor perfusion as assessed by Arterial Spin Labeled perfusion MRI (ASL MRI).[9] Moreover we have found that tumors maintain their ability to respond to sorafenib upon tumor excision and reimplantation into a naive host and that these perfusion changes also reverse in the setting of re-exposure to treatment.[10] Thus, resistance to VEGFR inhibition is likely due in part to up-regulation of angiogenic factors, the loss of angiostatic pathways or the adaptation of a tumor to survive hypoxic conditions.

Biologic pathways contributing to acquired resistance

A number of adaptive responses to VEGFR have been proposed and investigated as mechanisms of resistance. One mechanism proposed is the up-regulation of HIF due to VEGFR inhibitor induced hypoxia. This theoretically could lead to the increases in circulating VEGF that is seen in the setting of VEGFR blockade.[11] It remains uncertain to what extent this increase in these HIF driven factors is sufficient to overcome or circumvent the receptor blockade mediated by the various VEGFR blockers. To the extent that increased HIF is relevant to the resistance mechanism, mTOR inhibitors that can block HIF 1 alpha production might have potential utility.

Preclinical investigations have begun to identify other factors potentially contributing to the acquired resistance to VEGF pathway blockade. In a study of immunosuppressed mice bearing pancreatic islet tumors undergoing treatment with a neutralizing monoclonal rat anti-VEGFR2 antibody,[12] an initial reduction in tumor size and microvessel density was followed by tumor regrowth. Tumor regrowth on treatment was associated with extensive capsular invasion and other stigmata of increased aggressiveness. Analysis of resistant tumor tissue demonstrated an increase in transcripts corresponding to several members of the fibroblast growth factor (FGF) family. The administration of an adenovirus encoding a soluble form of FGF receptor-2 (which bound several members of the FGF family) reduced tumor regrowth and revascularization. This finding implicated members of the FGF family as critical factors responsible for VEGF-independent tumor growth in this model. In support of this, FGFR1 expression has recently been described to be present in the vast majority of both primary and metastatic RCC specimens.[13] Although it is unclear whether this FGFR expression is found on tumor or endothelial cells (ECs), its presence on ECs might allow FGF secreting renal carcinoma cells to stimulate sufficient angiogenesis, even in the presence of VEGF pathway blockade, to restore tumor growth.

Several stromal elements, especially in the setting of hypoxia, are also thought to produce factors that contribute to tumor invasiveness and angiogenesis. Tumor-infiltrating fibroblasts, for example, secrete abundant SDF-1 and drive angiogenesis in invasive human breast carcinomas through a CXCR4-dependent mechanism.[14] Recent studies have suggested that tumor-infiltrating $CD11b^{+}Gr1^{+}$ myeloid cells not only tend to accumulate in tumors inherently resistant to VEGF antagonists, but actually produce factors that mediate the resistance.[15] Placental growth factor (PlGF) is a particularly interesting angiogenic factor that has been found to increase in during treatment with sunitinib.[16] PlGF is a HIF dependent ligand for VEGFR1. In a study by Fischer et al., the authors reported that an antibody against PlGF inhibited growth and metastasis of various nonRCC tumors including those resistant to VEGFR inhibition.[17] However, given that sunitinib blocks signaling through multiple VEGF receptors including VEGFR1, the potential contribution of PlGF to the acquired resistance to sunitinib in patients with RCC remains conjectural.

There is also considerable evidence suggesting that the angiopoietin 2 (Ang2)/Tie2 axis has angiogenic potential that could parallel the VEGF axis and potentially overcome VEGFR blockade. In preclinical

studies, inhibition of Ang2 led to suppression of tumor growth.[18] Additionally we have shown that Ang2 rises in the plasma of the majority of patients with RCC at the time of resistance to sunitinib.[19] Efforts to prevent this potential mechanism of resistance clinically are currently underway (see below).

Interleukin-8 (IL-8) has also been implicated as a mediator of angiogenic escape. In a study involving colon carcinoma cells rendered deficient in HIF transcription factors, IL-8 was shown to play a dominant role in the generation and maintenance of the tumor microcirculation. Tumor angiogenesis could be blocked in this model with a neutralizing anti-IL-8 antibody.[20] This finding demonstrates that IL-8 is able to promote tumor angiogenesis in a setting in which VEGF production is impaired and suggests that it might play a similar role in circumstances in which VEGF is rendered irrelevant due to drug-mediated receptor blockade. This conjecture is further supported by a recent study in which administration of a neutralizing IL-8 antibody to mice harboring sunitinib-resistant RCC xenografts resensitized the tumor to sunitinib treatment.[21] We have also found that interferon gamma (IFNα) regulated pathways are down-modulated at the time of resistance and that similar to IL-8 blockade, restoration of such angiostatic pathways can also delay resistance to therapy in RCC xenograft models.[10] Taken together these findings suggest that acquired resistance to VEGFR blockade represents a combination of enhanced proangiogenic and diminished angiostatic forces that conspire to overcome the lack of VEGF and support sufficient endothelial cell proliferation necessary to restore tumor growth.

Clinical investigation aimed at overcoming VEGFR inhibitor resistance

Clinical trials in the setting of VEGF pathway resistance have focused on either the sequential administration of distinct VEGF pathway blockers or inhibitors of non-VEGF related pro-angiogenic factors. Several studies involving sequential administration of VEGF pathway blocking agents have shown retained anti-tumor activity. For instance, sunitinib produced tumor responses in 23% and some tumor shrinkage in 85% of patients with metastatic RCC with RECIST-defined disease progression following bevacizumab-based therapy.[23] Similarly axitinib produced tumor responses in 23% and tumor shrinkage in 80% of patients with metastatic RCC who had previously shown resistance to sorafenib and a subset of whom who were also refractory to sunitinib.[24] Anti-tumor activity was particularly prominent in patients who had not received prior suninitib, suggesting that the level of tumor susceptibility to sequential VEGF inhibitors may depend on features of prior VEGF-targeting, drug exposure including duration of prior therapy, and the relative potency of each agent against VEGFR. Finally, recent anecdotal reports have suggested restored antitumor activity with re-administration of the same VEGF pathway inhibitor following a drug holiday[25] clearly supporting the, at least partial, reversibility of resistance mechanisms.

Table 1: Selected ongoing or proposed clinical trials aimed a preventing or overcoming VEGFR TKI resistance in patients with RCC

Setting	Trial Design	Phase	Strategy/Question
Sunitinib-refractory	temsirolimus vs. sorafenib	III	Role of mTor inhibition vs sequential VEGFR inhibition
TKI-refractory	everolimus +/- bevacizumab	III	Role of maintenance of VEGF pathway blockade in the setting of mTOR inhibition
Front-line refractory	axitinib vs. sorafenib	III	Role of potency of VEGFR blockade in overcoming VEGFR resistance
Sunitinib and everolimus refractory	sorafenib vs. dovitinib (TKI 258)	III	Role of FGFR blockade in overcoming resistance to VEGFR and mTor inhibition
Front-line	sorafenib +/- AMG386	II	Role of blocking angiopoeitins in delaying resistance to VEGFR TKI therapy
Front-line	sunitinib + AMG386	II	Role of blocking angiopoeitins in delaying resistance to VEGFR TKI therapy

A potential role for HIF1α up-regulation in resistance is supported by studies showing a significant benefit for the administration of everolimus relative to placebo (PFS of 4.0 months vs. 1.9 months HR 0.3, 95% CI 0.22 – 0.40 p<0.001) in patients with RCC that was resistant to sunitinib, sorafenib or both agents.[27] It should be noted, however, that the overall effect of mTOR inhibition in this setting was modest and the comparator arm was inactive, likely exaggerating the relative benefit. The value of mTOR inhibition relative to VEGF pathway blockade and the extent to which maintenance of VEGF pathway blockade in setting of mTOR inhibition is important are being in evaluated in several ongoing or proposed clinical trials (Table 1).

Approaches that aim to inhibit other non-VEGF dependent pathways of resistance are less well advanced clinically. As with mTOR inhibition, such approaches could be instituted either concurrent with VEGF pathway blockade in an effort to delay or prevent the onset of resistance or in sequence with therapy, a strategy that may reduce toxicity associated with combination therapy. Clinical trials assessing the utility of Ang2 inhibition with AMG386 administered in combination with either sorafenib or sunitinib are currently underway, while studies of the dual VEGFR and FGFR inhibitor, dovitinib (TKI258), in patients exhibiting disease progression on both VEGFR and mTOR inhibitors are in the planning stages (Table 1). Efforts to target IL-8 or enhance IFNα pathway mediated angiostasis await the development of agents suitable for clinical administration.

Conclusion

Acquired resistance to VEGF pathway blockade represents a critical

obstacle to improved therapy in patients with advanced RCC. Preclinical studies are increasing our understanding of this process and clinical investigations are actively testing a variety of strategies to ameliorate this condition. The current state of the field involves the testing of sequential VEGFR or mTOR inhibition, alone or in combination, in the clinical setting, and the elucidation of novel resistance pathways in murine models. These novel pathways will likely be targeted in future clinical trials and hopefully produce additional opportunities for therapeutic benefit in patients with RCC.

Discussion

Dr. George: Does the fact that the addition of IL-8 only produces benefit at time of resistance in mouse models mean that IL-8 is not an important driver of angiogenesis at baseline?

Dr. Atkins: That is a good question. The murine xenograft data suggests this is the case, although there are some inconsistencies. For example, IL-8 levels upregulated in the plasma of control treated mice bearing A498 tumors at day 124 suggesting that it may be a measure of tumor burden. Furthermore, the Huang et al data suggest that only a subset of the sunitinib treated mice actually retain or up-regulate IL-8 expression at resistance. This suggests that even in the identical tumor in the identical mouse, some variability exists in the upregulation of IL-8 under hypoxic stress. Finally, in looking at the clinical specimens although the amount of data is small, it appears that some tumors express IL-8 at baseline and others do not and that this expression is associated with resistance to sunitinib. This suggests that in the more heterogenous situation of human RCC, that there may be a role for administering IL-8 and sunitinib concurrently. Clearly, a lot needs to be sorted out regarding this question not only for IL-8, but for some of the other factors that might drive angiogenic escape as well.

Dr. Choueiri: Animal models suggest that FGF plays are role in angiogenic escape. Do your group's experiments suggest that FGF plays a significant role in mediating the resistance to VEGF targeted therapy in patients with RCC?

Dr. Atkins: In our murine RCC xenograft models we do not see upregulation of FGF at the time of resistance. This is at a time when we see upregulation of IL-8 and of course VEGF and down modulation of various angiostatic factors such as IP-10. In patients the results are more variable. We see some patients who appear to have upregulation of bFGF in their plasma at the time of disease progression while others do not. Interestingly, it appears that upregulation of FGF at the time of resistance is associated with a long time to progression. The significance/validity of this observation is unclear, but could conceivably mean that FGF is lower priority and less powerful means of angiogenic escape that come into play only if some of the more potent approaches are insufficient to overcome the VEGFR blockade induced hypoxic drive. Once again this is something that will require additional investigation. Given that some agents that inhibit FGF will soon be tested in the setting of VEGFR TKI refractory RCC, hopefully we will be able to get some meaningful correlative data from clinical trials that will address this question.

References

Mechanisms and Management of Resistance to Anti-VEGF Therapy in Renal Cell Carcinoma

1. Kaelin, WG, Jr. The von Hippel-Lindau protein, HIF hydroxylation, and oxygen sensing. Biochem Biophys Res Commun. 2005; 338: 627.
2. Motzer RJ; Hutson TE; Tomczak P; Michaelson MD; Bukowski RM; Rixe O; Oudard S; Negrier S; Szczylik C; Kim ST; Chen I; Bycott PW; Baum CM; Figlin RA. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med. 2007; 356(2): 115-24.
3. Escudier, B, Eisen, T, Stadler, WM, et al: Sorafenib for treatment of renal cell carcinoma: Final efficacy and safety results of the phase III Treatment Approaches in Renal Cancer Global Evaluation Trial. J Clin Oncol. 2009; 27: 3312.
4. Sternberg CN, Davis ID, Mardiak J, et al: Pazopanib in locally advanced or metastatic renal cell carcinoma: results of a randomized phase III trial. J Clin Oncol. 2010; 28: 1061-8.
5. Rini BI, Wilding G, Hudes G, et al: Phase II study of axitinib in sorafenib-refractory metastatic renal cell carcinoma. J Clin Oncol. 2009; 27: 4462-8.
6. Bhargava P, Esteves B, Al-Adhami M, et al: Activity of tivozanib (AV-951) in patients with renal cell carcinoma (RCC): Subgroup analysis from a phase II randomized discontinuation trial (RDT) Tivozanib, J Clin Oncol. 2010; 28:15s, Abstract 4599.
7. Jänne PA, Engelman JA, Johnson BE., Epidermal growth factor receptor mutations in non-small-cell lung cancer: implications for treatment and tumor biology. J Clin Oncol. 2005; 23: 3227-34.
8. Deininger M, Buchdunger E, Druker BJ., The development of imatinib as a therapeutic agent for chronic myeloid leukemia. Blood. 2005; 105: 2640-53.
9. Schor-Bardach R, Alsop DC, Pedrosa I, et al: Response of renal cancer mouse model to antiangiogenic therapy correlates with tumor perfusion as measured with arterial spin labeling MRI. Radiology. 2007; 245(s): 343.
10. Bhatt RS, Want X, Zhang L, et al. Renal Cancer Resistance to Antiangiogenic Therapy Is Delayed by Restoration of Angiostatic Signaling. Mol Cancer Ther. 2010 Sep 21. [Epub ahead of print]
11. Deprimo SE, Bello CL, Smeraglia J, Baum, et al: Circulating protein biomarkers of pharmacodynamic activity of sunitinib in patients with metastatic renal cell carcinoma: modulation of VEGF and VEGF-related proteins. J Transl Med. 2007; 5: 32.
12. Casanovas O, Hicklin D, Bergers G, Hanahan D. Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. Cancer Cell. 2005; 8: 299-309.
13. Bhatt R, Atkins MB. FGFR: Another potential target in RCC? British Journal of Cancer, 2010 (in press)
14. Orimo A, Gupta PB, Sgroi DC, et al: Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell. 2005; 121: 335-48.
15. Shojaei F, Wu X, Malik AK, et al: Tumor refractoriness to anti-VEGF treatment is mediated by CD11b(+)Gr1(+) myeloid cells. Nat Biotechnol. 2007; 8: 911-20.
16. Deprimo SE, Bello CL, Smeraglia J, et al: Circulating protein biomarkers of pharmacodynamic activity of sunitinib in patients with metastatic renal cell carcinoma: modulation of VEGF and VEGF-related proteins. J Transl Med. 2007; 5: 32.
17. Fischer C, Jonckx B, Mazzone M, et al: Anti-PlGF inhibits growth of VEGF(R)-inhibitor-resistant tumors without affecting healthy vessels. Cell. 2007; 131: 463-75.
18. Oliner J, Min H, Leal J, et al: Suppression of angiogenesis and tumor growth by selective inhibition of angiopoietin-2. Cancer Cell. 2004; 6: 507-16.
19. Bullock A, Zhang L, O'Neill A, et al: Plasma Angiopoietin-2 (ANG2) as an angiogenic biomarker in renal carcinoma. J Clin Oncol. 2010; 28:15s, Abstract 4630.
20. Mizukami Y, Jo WS, Duerr EM, et al: Induction of interleukin-8 preserves the angiogenic response in HIF-1alpha-deficient colon cancer cells. Nat Med. 2005; 11: 992-7.
21. Huang D, Ding Y, Zhou M, et al: Interleukin-8 mediates resistance to antiangiogenic agent sunitinib in renal cell carcinoma. Cancer Res. 2010; 70(3): 1063-71.
22. Rini BI, Michaelson MD, Rosenberg JE, et al: Antitumor activity and biomarker analysis of sunitinib in patients with bevacizumab-refractory metastatic renal cell carcinoma. J Clin Oncol. 2008; 26: 3743-8.
23. Rini BI, Wilding G, Hudes G, et al: Phase II study of axitinib in sorafenib-refractory metastatic renal cell carcinoma. J Clin Oncol. 2009; 27: 4462-8.
24. Elfiky AA, Cho DC, McDermott DF, et al: Predictors of response to sequential sunitinib and the impact of prior VEGF-targeted drug washout in patients with metastatic clear-cell renal cell carcinoma. Urol Oncol. 2010 May 5.
25. Rini BI, et al Cancer (in press)
26. Motzer RJ, Escudier B, Oudard S, et al; RECORD-1 Study Group. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. Lancet. 2008; 372(9637): 449-56.