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

ANTI-ANGIOGENIC AND TARGETED THERAPY IN RENAL CANCER:
Novel Targets in Metastatic Renal Cell Carcinoma
Toni K. Choueiri, MD, Dana-Farber Cancer Institute, Boston, MA

Introduction
In the past few years we have experienced a revolution in the treatment of patients with metastatic renal cell carcinoma (RCC) with the introduction of targeted therapies that affect pathways related to tumor angiogenesis and proliferation.. For years immunotherapy was the principal treatment option for patients with metastatic RCC with only a limited subset of patients experiencing a long-term clinical benefit. Deeper understanding of the molecular mechanisms underlying RCC, particularly the unique relationship between RCC and angiogenesis, enabled the development of effective targeted therapies. Currently, the vascular endothelial growth factor (VEGF) tyrosine kinase inhibitors (TKIs) sunitinib, pazopanib, and sorafenib, the anti-VEGF monoclonal antibody bevacizumab, as well as the rapamycin analogues temsirolimus and everolimus, have been approved for use in the United States in the treatment of patients with metastatic RCC.[1] Studies are starting to reveal mechanisms of resistance to current drugs and identify novel therapeutic targets, with many also inhibiting components of angiogenesis . This review will focus on therapeutic strategies that include blocking the non-vascular endothelial growth factor (VEGF) pro-angiogenic proteins, and targeting upstream signaling pathways along the VHL-HIF-VEGF and PI3k-AKT-mTOR.

Novel agents targeting VEGF
Based on the success of targeting VEGF directly, several novel targeted agents are currently being evaluated in clinical trials. Targeting the same protein is reasonable if a drug has a clear superior activity from pre-clinical or early clinical studies and/or better tolerability than available compounds. To that end, two novel VEGF TKI merit discussion at this stage: axitinib and tivozanib.

Axitinib.
Axitinib is a pan-VEGF receptor (VEGFR) TKI with lower nanomolecular concentrations than sunitinib and pazopanib on VEGFR-1, -2, and -3.[2] A phase II study involving 52 patients with cytokine refractory metastatic RCC demonstrated an overall response rate (ORR) of 44.2%, and a median time to progression of 15.7 months.[3] In a phase II study involving patients who were refractory to sorafenib (N=62), ORR to axitinib was 22.6% and progression-free survival (PFS) was 7.4 months, demonstrating activity of axitinib in VEGFR-TKI-refractory patients.[4] A phase III study (N=540), the AXIS trial, compares axitinib with sorafenib in the treatment of patients who have failed one prior systemic therapy. This trial finished enrollment and results are eagerly awaited (clinicaltrials.gov ID: NCT00678392).

Tivozanib (AV-951) is another highly potent and selective pan-VEGFR TKI that has being evaluated in a "randomized discontinuation" phase II study of patients with metastatic RCC who had not received prior VEGF-targeted therapy. Data from this trial were updated at the 2010 American Society of Clinical Oncology (ASCO) meeting and revealed that ORR was achieved in 27% of 245 evaluable patients and PFS was 11.8 months overall. Restricting the analysis to patients with clear cell histology who underwent prior nephrectomy (N=176, 72% of the population), median PFS was 14.8 months.[5] Hypertension and dysphonia were the most common all-grade, treatment-related side effects, affecting 50% and 22% of patients, respectively. Interestingly, minimal all-grade fatigue, diarrhea, and mucositis were observed (all <10%) suggesting a very favorable side effect profile for this drug. A randomized trial of sorafenib vs. tivozanib (N=500) is currently accruing VEGF-naive patients (clinicaltrials.gov ID: NCT01030783).

Targeting HIF-2: an upstream target. It is known that RCC produces very high levels of VEGF and a number of inhibitors of VEGF, or its receptor have demonstrated activity in this disease and led to the use of these drugs in common practice. These agents are, however, not curative, and patients invariably become refractory to these agents. In theory, it might be more effective to target hypoxia-inducible factor (HIF) itself in kidney cancer, rather than the individual HIF-responsive gene products, like VEGF. Genotype-phenotype correlations and preclinical models suggest that downregulation of HIF2α, a subunit of HIF,[6,7] is both necessary and sufficient for the VHL protein to suppress renal carcinoma growth, thus validating HIF2α as a potential therapeutic target in this disease. Unfortunately, transcription factors such as HIF2α are historically difficult to inhibit with drug-like small organic molecules.

Recent studies suggest that small interfering RNAs (siRNAs) can be effectively delivered in vivo when encapsulated in nanoparticles targeted to the transferrin receptor.[8] A first in-human phase I clinical trial involving the systemic administration of siRNAs to patients with solid cancers showed that siRNAs administered systemically can produce a specific gene inhibition (reduction in mRNA and protein) by an RNA interference mechanism of action.[9] The potential for using this approach for targeting HIF2α in patients with mRCC is intriguing, although such studies have yet to be initiated.

Novel targets against angiogenesis and resistance
The exact mechanism of resistance to available targeted therapies in RCC remains largely unknown. Nevertheless, mounting evidence from RCC xenograft models suggests that even with continued VEGF suppression, there is restoration of vasculature visible at histopathologic examination and radiographic tumor perfusion studies.[10] This observation can translate into many therapeutic approaches, such as combinatorial approaches of different VEGF-targeted agents, in an effort to further suppress the VEGF pathway. At this stage, this approach has proven to result in significant toxicity. The combinations of bevacizumab with sorafenib or sunitinib are two examples where despite a high tumor response rates, the combinations were poorly tolerated and required dose reductions or discontinuation in a significant number of patients.[11,12]

Another potential resistance mechanism is upregulation of non-VEGF proteins involved in angiogenesis and tumor growth. Preclinical studies have identified multiple proteins potentially responsible for resistance to VEGF-targeted agents. Examples include the angiopoietin family[13], interleukin-8 (IL-8)[14], fibroblast growth factor (FGF)[15], and MET[16]. In the following, we will focus on the angiopoietin and MET targets, as clinical trials in patients with RCC targeting these pathways are emerging.

Targeting the angiopoietin/Tie-2 axis in RCC.
Angiopoietin-2 (Ang2) modulates angiogenesis in a cooperative manner with VEGF and overexpression of Ang2 in human tumors has been shown to correlate with more advanced disease and poorer outcome.[17] AMG 386 is an investigational, first-in-class recombinant peptibody that inhibits angiogenesis by selectively neutralizing Ang1 and Ang2, thus blocking their interaction with the Tie2 receptor, a key pathway for angiogenesis. In a phase I study[18] combining sorafenib with AMG-386,

the combination was well tolerated and 5/17 (29%) of RCC patients had tumor responses leading to a randomized phase II of sorafenib +/- AMG-386 (clinicaltrials.gov ID: NCT00467025) of 150 VEGF-naive RCC patients. This trial finished accrual and results are eagerly awaited. A phase II study of sunitinib +AMG-386 (clinicaltrials.gov ID: NCT00853372) is well underway and a phase Ib/II study with sunitinib+/- CVX-060 (clinicaltrials.gov ID: NCT00982657) a recombinant humanized monoclonal antibody fused to two Ang-2 binding peptides, has recently begun accrual.

Targeting the MET pathway in clear cell and papillary RCC.
MET dysregulation is common in cancer with several known biological consequences such as invasion, cellular morphogenesis, motility, metastasis, and immortalization. In addition, MET signaling enhances tumor angiogenesis mediated by the VEGF axis. Furthermore, in response to hypoxia, hepatocyte growth factor (HGF), the ligand for MET, is released and its being to MET may enhance metastasis in untreated tumors and contribute to resistance to VEGF-targeted agents.[16] In one study involving a large screen of 88 kinases, multiple short hairpins RNAs (shRNAs) against MET preferentially inhibited the viability of RCC VHL -/- cells.[19] A study of XL-184, a dual VEGFR and MET kinase inhibitor, in patients with advanced VEGF-refractory clear-cell RCC was recently initiated.

It is also important to note that MET carries a particularly important role in the less common RCC histological subtype: papillary RCC (PRCC). One familial form of PRCC is associated with germline activating mutations of MET, while amplification and overexpression of MET is also seen in the more common sporadic forms .[20] GSK1363089 is a novel inhibitor of receptor tyrosine kinases targeting MET and VEGFR. In a phase I study, partial responses were noted in 3 of 4 patients with PRCC, leading to the initiation of a multi-center phase II study of GSK1363089 in patients with histologically confirmed PRCC. Preliminary data showed that the drug was well tolerated and that 9 of 53 evaluable patients (17%) achieved tumor responses.[21]

Inhibition of targets upstream of mTOR
The rapamycin analogues temsirolimus and everolimus have demonstrated efficacy in the treating metastatic RCC. However, the growing understanding of the PI3K/Akt/mTOR signaling cascade provides insight into potential means to improve on the outcomes achieved by rapamycin analogue treatment.[22] For example, rapamycin analogues inhibit the mTORC1 complex, a key regulator of protein synthesis and cell cycle entry. However, rapamycin is not an effective inhibitor of mTORC2[23], and thus its use can paradoxically result in Akt activation through loss of mTORC1-mediated negative feedback upstream of PI3K. ATP competitive inhibitors that can effectively block both mTORC1 and mTORC2 may therefore have improved clinical utility.[24] Direct inhibitors of PI3K, or dual PI3K/mTOR inhibitors, may have more activity than mTORC1 inhibitors[25] and are being evaluated in early-phase clinical trials.[22] Additionally, perifosine, a heterocyclic alkylphospholipid that alters Akt signaling by disrupting the interaction with membrane phospholipids, has demonstrated some activity in patients with RCC. In 2 phase II studies (N=44[26] and N=24[27]) involving patients who had received prior VEGF inhibitors, responses were seen in <5% of patients in both trials. However, over 40% of patients experienced stable disease lasting more than 12 weeks and the therapy was well tolerated with few major toxicities.

Conclusion
Despite major advances in treating RCC, durable tumor responses from targeted therapies remain uncommon while toxicities associated with these treatments are common. Agents that block novel targets look to improve upon the successes realized with existing antiangiogenic therapy with the goal of increasing the rate of clinically significant responses and diminishing the severity of side effects.

Discussion
Dr. McDermott: If you start treatment when a patient walks in the door, could you be shortening their survival by putting them on targeted therapy versus watching them?
Dr. Atkins: Well, when you look at the clinical results it is hard to believe that that is the case compared to no treatment. But comparing treating at once to waiting to start until there is clinical progression–I do not know.
Dr. McDermott: It is one thing when you are comparing your new drug to interferon, but when you are comparing it to another TKI you could be shortening survival by starting right away. Should we be exploring initial observation? Should we be exploring treatment breaks? In some ways doing nothing might accomplish as much as doing something if our goal is to lengthen survival and improve quality of life.
Dr. Choueiri: When the ECOG adjuvant study has results, if you found out that the people who are treated with sunitinib or sorafenib had a worse PFS that would be major.
Dr. Cho: I think you can prolong PFS, but you may not necessarily prolong survival in the ECOG adjuvant study.
Dr. Atkins: Of course we are all worried about that with the adjuvant study. But we think it was important to do the study because, clearly, it could dramatically improve PFS and maybe treating in the minimal residual disease state is the only way that one could eliminate all disease and actually cure some patients with these agents. I do not believe so, but it is certainly possible and I have an open mind about it. But what is really going to be interesting in the adjuvant studies is to see what happens in the three or six months after the treatment stops. Is there an acceleration of progression on the treatment arms compared to the placebo arms such that the relapse rates on the treatment arms begin to approach that of the placebo treated population? We are hopeful that that will not be the case, but I think and we will learn something from that data.
Dr. Sznol: Has there ever been an analysis of certain good prognosis patients treated with sunitinib for survival? Because my impression is that when you give sunitinib, the overall survival is not all that much better than interferon in that population.
Dr. Atkins: The overall survivals in the Phase III trials of bevacizumab plus interferon vs interferon, or sunitinib vs interferon, in the good prognosis patients, is identical whether you start on interferon or whether you start with a VEGF pathway inhibitor. But almost all the people on the interferon arm get VEGF pathway inhibitors, and their survival is almost certainly better than it would be if those drugs were not available.
Dr. Sznol: But you probably don't adversely impact survival by just waiting until they develop clinically significant progression before starting the VEGFr inhibitors. We are only going to get so much out of additional disruption of vascular pathways. We need to branch out to other areas.
Dr. Kaelin: There is pretty good evidence that the WNT pathway is important in kidney cancer. I do not know that anybody knows how to drug the WNT pathway yet. I also think MET should be explored. I think your anti-PD1 — I mean, the low-lying fruit now is the PD1 combination with the VEGF inhibitor–I think has to be done like yesterday.
Dr. Atkins: Yes, unfortunately it is going to be in the future, but a trial that would make a lot of sense is bevacizumab and the PD1 antibody. They are two specific drugs with clearly identifiable targets. We know that interferon and bevacizumab do not interfere with each others' activity. Both antibodies are IV drugs so they could be given together. That combination might produce a really interesting result in patients

with kidney cancer, but I think it is a ways off before BMS will consider studying such a combination.

Dr. Kaelin: We all think VEGF blockade is going to be important in the future, but who thinks sunitinib and sorafenib are going to be the drugs that we are going to build into future combinations? If I was the NCI Director, I would have taken a flamethrower to half of those clinical trials because they do not matter and they are not getting us to where we have to get. We have to get more active agents. We have PD1 and maybe MET done properly, and a few of these other new targets. We should be focusing our resources on testing these agents.

Dr. Atkins: Part of the development of the new VEGF pathway inhibitors is not just cleaner inhibition of the VEGF pathway, but the concept that they are oral and thus can allow for combination studies to be done a little more easily than they were with sunitinib or sorafenib. But to me, the issue in the combination approach is: should you be just taking two drugs that are both active, but maybe active in different tumor populations, and put them together in hope that you see a higher response rate? Can you maybe do the same by just giving the right population the right drug? Or – can you add a drug to another drug and produce actual synergy? Can the combination lead to tumor cell death, via a mechanism that is not seen with either drug alone?

Dr. Kaelin: Or, can you decrease the probability of resistance by combining them? That is the classical reason for bringing the two drugs together, right? Just decrease the probability that any one cell is going to figure out a way around both drug mechanisms.

Dr. Atkins: Which is why some of these combination approaches are focused on the vasculature because that is what is getting hurt the most with the VEGF pathway inhibitors and if there is some way you can prevent the vasculature from surviving or escaping, you might enhance the benefit of the therapy.

References

Novel Targets in Metastatic Renal Cell Carcinoma

1. Courtney KD, Choueiri TK. Optimizing recent advances in metastatic renal cell carcinoma. Current Oncology Reports. 2009; 11: 218-26.
2. Choueiri TK. Axitinib, a novel anti-angiogenic drug with promising activity in various solid tumors. Curr Opin Investig Drugs. 2008; 9: 658-71.
3. Rixe O, Bukowski RM, Michaelson MD, et al: Axitinib treatment in patients with cytokine-refractory metastatic renal-cell cancer: a phase II study. The lancet oncology 2007;8(11):975-84.
4. 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.
5. 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). J Clin Oncol. 2010; 28: 15s, Abstract 4599.
6. Raval RR, Lau KW, Tran MG, et al: Contrasting properties of hypoxia-inducible factor 1 (HIF-1) and HIF-2 in von Hippel-Lindau-associated renal cell carcinoma. Mol Cell Biol. 2005; 25: 675-86.
7. Kondo K, Klco J, Nakamura E, Lechpammer M, Kaelin WG, Jr. Inhibition of HIF is necessary for tumor suppression by the von Hippel-Lindau protein. Cancer Cell. 2002; 1: 237-46.
8. Heidel JD, Yu Z, Liu JY, et al: Administration in non-human primates of escalating intravenous doses of targeted nanoparticles containing ribonucleotide reductase subunit M2 siRNA. Proc Natl Acad Sci USA. 2007; 104: 5715-21.
9. Davis ME, Zuckerman JE, Choi CH, et al: Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. Nature. 2010; 464: 1067-70.
10. Schor-Bardach R, Alsop DC, Pedrosa I, et al: Does arterial spin-labeling MR imaging-measured tumor perfusion correlate with renal cell cancer response to antiangiogenic therapy in a mouse model? Radiology. 2009; 51: 731-42.
11. Feldman DR, Baum MS, Ginsberg MS, et al: Phase I trial of bevacizumab plus escalated doses of sunitinib in patients with metastatic renal cell carcinoma. J Clin Oncol. 2009; 27: 1432-9.
12. Sosman JA, Flaherty KT, Atkins MB, et al: Updated results of phase I trial of sorafenib (S) and bevacizumab (B) in patients with metastatic renal cell cancer (mRCC). J Clin Oncol. 2008; 26: Abstract 5011.
13. Shibuya M. Vascular endothelial growth factor-dependent and -independent regulation of angiogenesis. BMB Reports. 2008; 41: 278-86.
14. Huang D, Ding Y, Zhou M, et al: Interleukin-8 mediates resistance to antiangiogenic agent sunitinib in renal cell carcinoma. Cancer Res. 2010; 70: 1063-71.
15. Alessi P, Leali D, Camozzi M, Cantelmo A, Albini A, Presta M. Anti-FGF2 approaches as a strategy to compensate resistance to anti-VEGF therapy: long-pentraxin 3 as a novel antiangiogenic FGF2-antagonist. European Cytokine Network. 2009; 20: 225-34.
16. Eder JP, Vande Woude GF, et al: Novel therapeutic inhibitors of the c-Met signaling pathway in cancer. Clin Cancer Res. 2009; 15: 2207-14.
17. Sfiligoi C, de Luca A, Cascone I, et al: Angiopoietin-2 expression in breast cancer correlates with lymph node invasion and short survival. International Journal of Cancer. 2003; 103: 466-74.
18. Hong D, Gordon M, Appleman L, et al: Interim Results From a Phase 1b Study of Safety, Pharmacokinetics and Tumor Response of the Angiopoietin1/2-Neutralizing Peptibody AMG 386 in Combination With AMG 706 (Motesanib), Bevacizumab or Sorafenib in Advanced Solid Tumors. 33rd European Society for Medical Oncology (ESMO) Congress. September 12–16, 2008; Stockholm, Sweden. Abstract 462PD.
19. Bommi-Reddy A, Almeciga I, Sawyer J, et al: Kinase requirements in human cells: III. Altered kinase requirements in VHL-/- cancer cells detected in a pilot synthetic lethal screen. Proc Natl Acad Sci USA. 2008; 105: 16484-9.
20. Chowdhury S, Choueiri TK. Recent advances in the systemic treatment of metastatic papillary renal cancer. Expert Rev Anticancer Ther. 2009; 9: 373-9.
21. Srinivasan R, Linehan WM, Vaishampayan U, et al: A phase II study of two dosing regimens of GSK 1363089 (GSK089), a dual MET/VEGFR2 inhibitor, in patients (pts) with papillary renal carcinoma (PRC). J Clin Oncol. 2009; 27: 15s Abstract 5103.
22. Courtney KD, Corcoran RB, Engelman JA. The PI3K pathway as drug target in human cancer. J Clin Oncol. 2010; 28: 1075-83.
23. Le Tourneau C, Faivre S, Serova M, Raymond E. mTORC1 inhibitors: is temsirolimus in renal cancer telling us how they really work? Br J Cancer. 2008;99:1197-203.
24. Meric-Bernstam F, Gonzalez-Angulo AM. Targeting the mTOR signaling network for cancer therapy. J Clin Oncol. 2009; 27: 2278-87.
25. Cho DC, Cohen MB, Panka DJ, et al: The Efficacy of the Novel Dual PI3-Kinase/mTOR Inhibitor NVP-BEZ235 Compared with Rapamycin in Renal Cell Carcinoma. Clin Cancer Res. 2010 Jul 15;16(14):3628-38.
26. Vogelzang NJ, Hutson TE, Samlowski W, et al: Phase II study of perifosine in metastatic renal cell carcinoma (RCC) progressing after prior therapy (Rx) with a VEGF receptor inhibitor. J Clin Oncol. 2009; 27: 15s Abstract 5034.
27. Cho DC, Figlin RA, Flaherty KT, et al: A phase II trial of perifosine in patients with advanced renal cell carcinoma (RCC) who have failed tyrosine kinase inhibitors (TKI). J Clin Oncol. 2009; 27: 15s Abstract 5101.