Innovations and Challenges in Renal Cancer

A Continuing Medical Education Activity sponsored by InforMEDical Communications, Inc.
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CONTINUING MEDICAL EDUCATION INFORMATION

Release Date: November 24, 2010
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Target Audience
This program is designed for physicians and other medical professionals involved in the clinical care of patients with renal cancer.

Program Overview
This activity provides topical updates and discussion of recent progress by eleven national leaders in renal cancer.

Learning Objectives
After completing this activity, the participant will be able to:

• Assess recent clinical data for immunotherapies, targeted therapies and anti-angiogenic therapies in renal cancer treatment

Statement of Need
Oncologists and other medical professionals who treat patients with renal cancer need to be able to assess important new clinical data and recommendations related to current and novel immunotherapies, targeted therapies and anti-angiogenic therapies, patient assessment and patient selection in this leading area of cancer research so that they can more effectively counsel and treat patients.

Over the past five years several new treatment approaches have been approved for renal cell cancer, raising questions for practitioners regarding the relative value of each agent, who to treat, when to use which agent, and the appropriate role of surgery and immunotherapy in the era of targeted agents. A review of recent data, discussion of recent research and current practice, and discussion of best practice in the context of pertinent case studies by a multidisciplinary panel of experts in renal cancer will assist practitioners in assessing and counseling patients and making better informed treatment decisions.

Companion CME Activity
A companion CME activity, **Innovations and Challenges in Renal Cancer Case Studies**, is available as a webcast discussion of four challenging case studies moderated by Michael B. Atkins, MD, and discussed by the same program faculty. The activity offers up to 1.0 additional *AMA PRA Category 1 Credits™*.

Renal Cancer Case Studies

Receiving FREE CME Credit
To receive CME credit for this activity, read the full activity then click below to complete the post-test and evaluation hosted on *free*CME.com. You will be able to print your CME certificate immediately following completion of the post-test and evaluation.

CME Test and Evaluation

Sponsor
This activity is sponsored by InforMEDical Communications, Inc., an independent medical education company providing up-to-date medical information to healthcare professionals who care for patients with cancer.

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Innovations and Challenges in Renal Cancer

Table of Contents

Overview

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Introduction

In 2010, an estimated 58,000 new cases of renal cell cancer (RCC) will be diagnosed in the United States and 13,000 people will die of this disease.[1] Renal cancer is made up of several different types of cancer, each of which has distinct molecular underpinnings and a different response to treatment. For clear cell carcinoma, the most common variant, three potentially distinct targets and related therapeutic approaches are currently available: immunotherapy, vascular endothelial growth factor (VEGF) pathway blockade, (antiangiogenic therapy), and mTOR inhibition (targeted therapy). Application of these therapies has improved the median survival for patients with advanced RCC from a median of 10 months in 1999[2] to in excess of 2 years.[3] Nonetheless, for patients who either present with or develop metastatic disease the expected 5-year survival rate is still only approximately 10%. Recent research is helping to clarify what can be expected from each treatment approach, which patients are most likely to benefit from particular therapies and opportunities for enhancing the efficacy of each approach. Additional research is beginning to elucidate mechanisms of resistance to various treatment approaches, strategies for treatment sequencing, opportunities and obstacles to combination therapy, as well as potential novel therapeutic targets. It is envisioned that these efforts will enable rational treatment selection and lead to additional improvements in not just median overall survival but also in the proportion of future long term disease free survivors with this disease.

Molecularly Biology of RCC

Approximately 60-80% of clear cell RCC lacks a functional von Hippel-Lindau (VHL) gene as a result of biallelic loss from mutation and/or hypermethylation.[4] The loss of VHL results in the accumulation of HIF (even in normoxic conditions) leading to increased expression of hypoxia regulated genes such as VEGF. This feature is thought to account for the unique initial sensitivity of RCC to VEGF pathway antagonists. One approach to improving therapy is to identify genetic alterations that cooperate with VHL loss in clear cell kidney cancer. Mounting evidence suggests that one of the relevant genetic alterations might be the inactivation of HIF1α on chromosome 14q. Recent data suggests that clear cell tumors with HIF1α loss (those that are HIF2 only) are more aggressive.[4] This, together with earlier work from the Kaelin Laboratory,[5] suggests that HIF2 is the major oncoprotein in RCC while HIF1 actually functions as a tumor suppressor. The view that HIF1 is a tumor suppressor in RCC is supported by other experiments showing that downregulation of HIF1, using shRNA, promotes VHL-/- RCC growth in vitro and in vivo, whereas reintroduction of HIF1 into the approximately 40% of VHL-/- RCC that do not otherwise express HIF1 suppresses their growth in vitro and in vivo (Shen, Kaelin et al personal communication). Thus efforts to target HIF2α appear particularly warranted in this disease.

Other potential targets exist on chromosomes 5 and 8 where chromosomal amplifications have frequently been noted.[6] An integrated analysis aimed at identifying genes in amplification peaks that are consistently over-expressed in tumors with amplifications, confirmed MYC as a potential target of 8q amplification and identified candidate oncogenes in the other regions. Similarly other potentially relevant genes have been identified within the 5q amplification region. Thus, clear cell RCC may soon be further subdivided into multiple subsets based on sophisticated molecular profiling which have prognostic and therapeutic relevance. Understanding the relevance of various subclasses with regard to current and future therapies will require more robust approaches to integrating molecular profiling into current and future clinical trials.

Immunotherapy

Immunotherapy with high dose interleukin 2 (HD IL-2) was first approved for the treatment of patients with RCC in 1992 due to its ability to produce durable complete responses in a subset of patients. However, the toxicity, complexity and unpredictable efficacy of this therapy has limited its use over the years to patients with excellent functional status and access to facilities with expertise and comfort in administering this treatment. In light of the multiple new treatment options for patients with RCC, efforts to update the current efficacy of HD IL-2 and better define those patient populations most likely to benefit have become a priority. Recent studies suggest that IL2 remains active in the era of anti-angiogenic and molecularly targeted therapy with response rates close to double that reported in the original studies leading to its FDA approval, and that opportunities for improving patient selection still exist.[7]

Given the toxicity and limited efficacy associated with HD IL-2, exploration of novel immunotherapy approaches has also become a priority. Better understanding of immune regulation and mechanisms of tumor induced immune suppression have led to more targeted immunotherapy approaches. Studies with CTLA4 antibodies that block immune regulation and PD1 antibodies that block tumor induced immune suppression have shown considerable promise in the treatment of patients with advanced RCC.[8, 9] Additional development is warranted in both single agent and combinations. It will be very important to select the appropriate patient characteristics and include biomarker studies in those trials in order to identify the most responsive populations.

Anti-angiogenic and Molecularly Targeted Therapies

Therapy targeted at the vascular endothelial growth factor (VEGF) and mammalian target of rapamycin (mTOR) pathways has revolutionized the treatment of patients with advanced RCC. Six agents, including four VEGF pathway inhibitors (sorafenib, sunitinib, pazopanib and bevacizumab) and two mTOR inhibitors (temsirolimus and everolimus) are now approved in the United States for treatment of patients with RCC.

Although the VEGF blockers have different properties, all have been shown to produce tumor shrinkage in 50-70% of patients with cytokine refractory RCC. Sunitinib and bevacizumab plus interferon alpha (IFN) have shown significant improvement in response rate and progression free survival (PFS) relative to IFN alone in treatment naive patients.[10, 11] By contrast, sorafenib and pazopanib have shown PFS benefit relative to placebo in either cytokine refractory patients (sorafenib) or a mixture of treatment naive or cytokine refractory patients (pazopanib).[12, 13] An overall survival benefit has not been firmly established for these agents. However, this is

thought to be related to crossover of IFN or placebo treated patients in the clinical trials to VEGF pathway inhibitors after disease progression, as the median overall survival for the study population as a whole has increased from the previous standard of 10-13 months with IFN to 18-24 months.

More selective and potent VEGFR tyrosine kinase inhibitors such as axitinib and tivozanib are currently under investigation and may offer opportunities for enhanced efficacy with reduced "off target" toxicities relative to currently approved agents.[14, 15] In addition, current data suggests that the VEGF pathway inhibitors can exhibit substantial activity when used in sequence perhaps highlighting the potential distinct effects of these agents. However, other data suggests that even the same agent can be active if re-administered after a "drug holiday" supporting the reversibility of resistance mechanisms and the likely pre-eminence of the VEGF pathway in driving RCC progression.

The mechanisms underlying the activity of mTOR inhibitors in RCC is less certain. mTOR inhibition has been shown to down regulate HIF within VHL-defective tumor cells as well as blunt VEGF signaling. In addition, VHL-defective cells are known to be highly sensitive to drugs, such as the mTOR inhibitors, that induce autophagy.[16] This latter effect may explain why temsirolimus has shown its greatest benefit, prolonging overall survival relative to IFN, in patients with poor prognostic features,[17] while everolimus has shown its principal benefit, delaying PFS relative to placebo, in patients whose disease has progressed following sunitinib, sorafenib or both.[18] However, other data suggest that mTOR inhibitors may exhibit their most profound effects in non-clear cell tumors (presumably with wild-type VHL) [19] and in tumors with upregulation of pS6, a marker of mTOR activation.[20] Thus, the activity of the mTOR inhibitors in these other settings or relative to VEGF inhibitors in either general or selected patient populations requires further exploration.

Additional data suggest that rapalogs, such as everolimus and temsirolimus, primarily inhibit TORC1. This isolated inhibition can allow for feedback upregulation of AKT and TORC2 perhaps promoting resistance and possibly even accelerating disease progression. This may be particularly relevant in RCC as TORC2 activity can lead to upregulation of HIF2α. Preclinical studies in RCC xenografts have documented superiority anti-tumor activity of dual TORC1, TORC2 and PI3kinase inhibitors such as BEZ235 relative to rapamycin as well the its ability to block HIF2α.[21] Thus, BEZ235 and other agents that inhibit both TORC1 and TORC2 (and possibly PI3 kinase further upstream in the pathway) may have significant activity in patients with RCC and deserve further exploration.

Efforts to combine VEGF pathway inhibitors have so far produced extra toxicity necessitating considerable dose reductions in one or both agents.[22, 23] Although more selective agents such as temsirolimus and bevacizumab can be combined at full doses, recent data suggest that the activity and toxicity of the combination may be worse than seen with single agent sunitinib.[24] Thus, realization of the promise of combination therapy may require identification of agents that hit truly non-cross resistant targets, particularly those that are relevant to the development of resistance to VEGF pathway inhibition. Promising non-VEGF targets include MET, FGF and angiopoietin 2 and agents that block these pathways are currently under investigation in patients with RCC. Until these or similar agents in combination with VEGF pathway blockade are able to establish an efficacy and safety benefit relative to VEGF pathway inhibition alone, the management of patients with RCC will likely remain sequential single agents.

Translational Research Studies
Although the impact of VEGF and mTOR inhibitor in RCC has been substantial, several limitations have emerged. Complete and/or durable responses have been only rarely observed, necessitating chronic therapy for the vast majority of patients often with attendant side effects. Furthermore, treatment resistance typically develops after a median of 6-11 months for VEGF inhibitors (< 6 months for mTOR inhibitors) prompting treatment decisions for which little guidance is available. Thus, further research is needed to determine how best to use these agents as well as further improve patient outcome.

Several translational research approaches are attempting to accomplish this goal. Considerable effort has focused on identifying clinical, tissue, blood and imaging based biomarkers that can help inform treatment decisions. To date, progress has been made in identifying clinical and pathologic based prognostic biomarkers[25, 26] and in developing imaging and blood-based pharmacodynamic biomarkers; however, despite good hypotheses, the identification of predictive biomarkers that can help select patients for particular therapies has proven elusive. Current research using treatments in the "neoadjuvant" setting provides a means of understanding mechanisms of action for specific agents as well as validating and/or extending biomarker research.[27, 28] While such studies are touted as offering the potential to determine the activity of a particular treatment before embarking on cytoreductive surgery, determination of the clinical utility of such approaches will be difficult. Nonetheless, it is hoped that evaluation of tissue and imaging correlates from these studies will foster biomarker development and rational development of novel therapies and therapeutic combinations. Current research has suggested that the mechanism of resistance to VEGF targeted therapy is at least in part "angiogenic escape".[29] This angiogenic escape appears to be mediated by increases in a variety of proangiogenic and potentially decreases in angiostatic factors,[30-33] some of which have the potential for being targeted therapeutically. It is hoped that understanding of these mechanisms of resistance will inform efforts to extend to benefit of current treatment approaches.

Conclusion
The treatment of patients with advanced RCC has evolved rapidly over the last decade. Eight agents are now approved for use in the US and many other countries, and patient outlook is considerably brighter. Nonetheless, considerable hurdles still exist before the goal of providing long term survival for the majority of patients can be reached. Promising areas of current investigation include: 1) the identification of more potent inhibitors of existing targets, 2) the identification of novel targets and/or therapeutic approaches, 3) the use of active agents in sequence, in combination or in novel settings, 4) the identification of predictive biomarkers that can help select optimal patients for particular treatments and 5) the discovery of mechanisms of resistance to established therapies and a means of overcoming them. Making progress in these areas will be necessary in order to significantly reduce morbidity and mortality related to advanced RCC

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RENAL CANCER BIOLOGY AND NOVEL TARGETS:
The von Hippel-Lindau Tumor Suppressor Protein and
Kidney Cancer Biology

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Introduction

Kidney cancer is one of the 10 most common forms of cancer and is responsible for over 10,000 deaths in the United States each year. The most common form of kidney cancer is clear cell renal carcinoma. Kidney cancer can be cured by nephrectomy if detected at an early stage. Treatment of recurrent or metastatic disease, however, is largely palliative. A minority of patients with advanced disease achievable durable remissions with high-dose interleukin 2. Unfortunately, this therapy is very toxic, must be administered in specialized care centers, and one cannot yet reliably predict which patients will benefit from this therapy. In the past decade, however, new therapies that modulate molecular pathways that are deregulated in clear cell carcinoma by virtue of mutations affecting the von Hippel-Lindau tumor suppressor gene (*VHL*) have been shown to significantly delay disease progression and/or to improve survival in patients with metastatic kidney cancer. This review will provide a brief update on the functions of the *VHL* encoded protein, pVHL, as they relate to kidney cancer therapeutics.

VHL tumor suppressor gene

Individuals who inherit a defective copy of the *VHL* tumor suppressor gene are predisposed to a variety of tumors including vascular tumors of the central nervous system and retina called hemangio-blastomas, adrenal gland tumors (pheochromo-cytomas), and clear cell renal carcinomas. Tumor development in this setting is linked to inactivation of the remaining wild-type *VHL* allele, thus depriving the cell of the wild-type pVHL. Biallelic *VHL* inactivation, either due to mutation or hypermethylation, is also very common (>50%) in sporadic (non-hereditary) clear cell renal carcinomas, especially if one eliminates tumors having variable or mixed histologies.

It is clear that *VHL* inactivation, although a common event in clear cell renal carcinoma, is not sufficient to cause this disease. Indeed, a number of non-random genomic alterations, including amplification of a region of chromosome 5q and loss of most or all of chromosome 14q, are frequently observed in clear cell renal carcinomas and are presumed to conspire with *VHL* loss to cause this disease.[1-5] Exon resequencing efforts recently identified mutations affecting the chromatin modifying enzymes in kidney cancer as additional culprits in this disease.[6]

pVHL has multiple functions but the most thoroughly studied, and the one that appears most tightly related to the suppression of kidney cancer, relates to its ability to inhibit a heterodimeric transcription factor called HIF (hypoxia-inducible factor), consisting of a labile alpha subunit and a stable beta subunit.[7] When oxygen is present pVHL binds directly to HIFα and targets it for polyubiquitination and proteasomal degradation. Under low oxygen conditions (hypoxia) HIFα is not recognized by pVHL and so is free to dimerize with its partner protein, HIFβ (also called aryl hydrocarbon receptor nuclear translocator or ARNT). The HIF heterodimer translocates to the nucleus, binds to specific DNA sequences (hypoxia response elements) and increases the rate of transcription of ~100-200 genes, many of which promote survival under hypoxic conditions. Included amongst these genes are genes that promote the shift from oxidative metabolism to glycolysis (that is, can promote the Warburg effect),[8] autophagy, erythropoiesis, and angiogenesis. The latter two classes of genes can account for two clinical features of kidney cancer, namely, its ability to produce paraneoplastic erythrocytosis and its propensity to induce angio-genesis.

pVHL has a number of other functions that, although incompletely understood at the biochemical level, appear to be at least partly HIF-independent7. For example, loss of pVHL leads to the loss of a specialized epithelial structure called the primary cilium as well as altered microtubule dynamics.[7] Notably, the development of visceral cysts, including renal cysts, is a feature that is shared between a number of other so-called ciliopathies and von Hippel-Lindau disease.[9,10] pVHL also modulates apoptosis in response to nerve growth factor withdrawal, which might account for its role in pheochromocytoma development,[11,12] and also appears to suppress senescence in some contexts.[13,14] Nonetheless, deregulation of HIF appears to be a driving force in the development of pVHL-defective kidney cancers for the reasons outlined below.

VHL link to kidney cancer

Genotype-phenotype correlations in VHL families suggest that the risk of developing kidney cancer is linearly related to the degree to which different VHL alleles deregulate HIF. In short, the VHL alleles linked to the highest risk of kidney cancer are also those that result in the highest levels of HIF.[15] This is in contrast to, for example, the risk of developing pheo-chromocytoma.[16,17] In preclinical models forced activation of HIF target genes is sufficient to override pVHL's tumor suppressor activity,[18] whereas suppression of HIF target genes in pVHL-defective renal carcinoma cells is sufficient to prevent tumor formation.[19,20]

Role of HIF

There are 3 HIFα genes in the human genome and hence "HIF" is actually a generic term. HIF1α is the ubiquitously expressed, canonical, member of the family whereas the expression of HIF2α is more restricted and HIF2α has been less intensively studied. Both HIF1α and HIF2α are capable of activating transcription, while at least some HIF3α isoforms appear to block HIF-dependent transcription.

There is solid evidence that HIF2α acts as an oncoprotein in pVHL-defective kidney cancers and growing evidence that HIF1α may, in fact, serve as tumor suppressor. For example, pVHL-defective tumors produce either both HIF1α and HIF2α together or exclusively HIF2α.[21,22] Moreover, the appearance of HIF2α in early renal lesions in the kidneys of VHL patients heralds malignant transformation.[23] Interestingly, HIF1α resides on chromosome 14q, which is frequently deleted in kidney cancers. While HIF2α can override pVHL's tumor suppressor activity in vivo, HIF1α cannot.[24] Indeed, HIF1α appears to suppress kidney cancer proliferation in vitro and in vivo.[21,25]
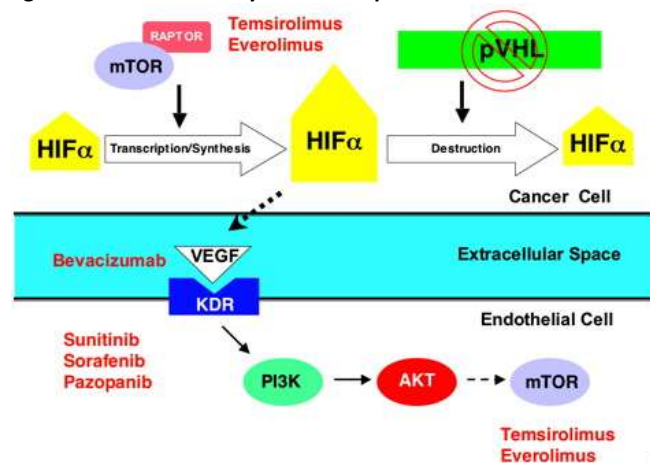
Why HIF1α and HIF2α would have opposite effects with respect to kidney carcinogenesis is not clear. It is clear, however, that the genes that are regulated by these two proteins are overlapping but not entirely congruent. For example, many glycolytic genes, as well

as the proapoptotic/proautophagy gene BNIP3,[26] are primarily controlled by HIF1α while the stem cell factor Oct4 is primarily under the control of HIF2α.[27] It is also clear that HIF1α and HIF2α can have opposing effects on the c-Myc oncoprotein, with the former antagonizing c-Myc function and the latter cooper-ating with c-Myc in certain settings.[28] In addition to such qualitative differences, there are likely to be quantitative differences as well. Specifically, pVHL leads to the accumulation of both HIF1α and HIF2α, for the reasons outlined above. Once stabilized, however, HIF1α remains enfeebled as a transcriptional activator by virtue of the FIH-1 asparaginyl hydroxylase, which hydroxylates a key asparaginyl residue within one of HIF1α's two transactivation domains.[29,30] HIF2α largely escapes this modification.[31,32] As a result, occupancy of a HRE by HIF1α would, at least for certain HIF targets, lead to diminished transcriptional activation relative to occupancy by HIF2α. In other words, HIF1α could act to blunt transcriptional activation by HIF2α in such a scenario.

Treating pVHL-defective kidney cancers
The above considerations provide a conceptual framework for treating pVHL-defective kidney cancers with drugs that inhibit HIF (especially HIF2α) or HIF-target genes linked to tumorigenesis. With respect to the latter, kidney cancers have the highest levels of the angiogenic growth factor VEGF, which is a HIF-responsive gene product, of any solid tumor examined. Four drugs that inhibit VEGF (bevacizumab) or its receptor KDR (sorafenib, sunitinib, pazopanib) have now been approved for the treatment of metastatic kidney cancer based on positive clinical trial data. Although the objective response rates (measured by RECIST criteria) differ amongst these agents the percentage of patients experiencing any tumor shrinkage/disease stabilization (as measured in "waterfall plots") is remarkably similar at about 75%. Indeed, kidney cancer is arguably the most sensitive solid tumor with respect to monotherapy with VEGF inhibitors. This presumably reflects the frequent inactivation of pVHL in this setting as well as the intimate relationship between pVHL and the control of HIF-dependent genes, including VEGF (**Figure 1**).
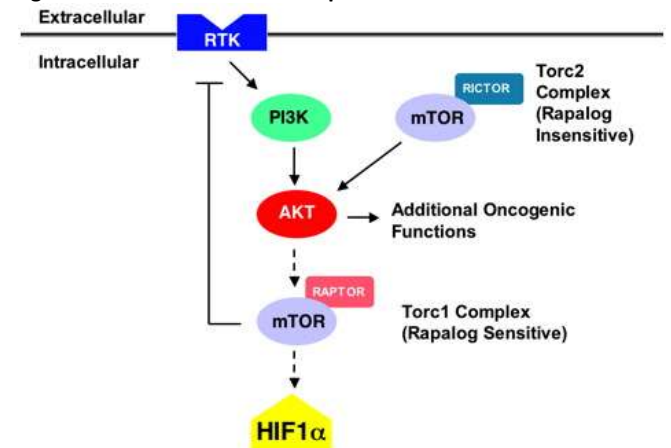
thereby promotes endothelial cell proliferation and survival. KDR signaling leads to mTOR activation.

VHL mutational status does not appear to be a highly robust predictor of response to VEGF blockade although there is a trend toward better responses amongst patients with VHL mutations.[33,34] It is likely, however, that many VHL "wild-type" tumors are phenotypically pVHL-defective, either because VHL hypermethylation, alterations in other pathways that phenocopy pVHL loss, or false-negative mutational readouts.

The steady-state levels of HIFα are determined by its rate of synthesis and by its rate of destruction (Figure 1). HIFα has a very high metabolic turnover rate. Accordingly, HIFα species are amongst the first proteins to disappear when transcription or translation are impaired. This caveat should be borne in mind when analyzing many of the "HIF1 inhibitors" described in the literature. It is very clear, however, that the transcription and translation of HIF is extremely sensitive to changes in the activity of the mTOR kinase,[36] which participates in two complexes called TORC1 and TORC2[36] (Figure 2) The former is under the control of the PI3K, AKT, TSC pathway[35] (**Figure 2**). Mutations affecting this pathway have been linked to the development of hamartomas.[35] TORC1 can be inhibited with rapamycin-like drugs. Notably, mTOR also plays a role downstream of KDR in endothelial cells (**Figure 1**). In short, inhibition of mTOR might downregulate HIF within pVHL-defective tumor cells as well as blunt VEGF signaling (**Figure 1**). It has also been shown that pVHL-defective cells are highly sensitive to drugs, including rapamycin, that induce autophagy.[37,38]

Some or all of these considerations likely relate to the fact that two rapamycin-like drugs, temsirolimus and everolimus,[39,40] have proven to be beneficial in kidney cancer patients who have high risk-features or who have failed KDR inhibitors, respectively.

Figure 1 Control of HIF by mTOR and pVHL

The steady state levels of HIFα, particularly HIF1α, are positively regulated by a complex containing mTOR and Raptor (TORC1 complex), which can be inhibited with rapamycin-like drugs. pVHL targets HIFα for proteasomal degradation and its loss, as a consequence, leads to the HIFα accumulation and activation of HIF target genes such as VEGF. VEGF is a secreted angiogenic polypeptide that engages the KDR receptor on endothelial cells and

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Figure 2 TORC1 and TORC2 complexes

mTOR exists in two complexes, called TORC1 and TORC2. The former contains the protein Raptor and can be inhibited with rapamycin-like drugs. The latter contains the protein Rictor and is relatively insensitive to rapamycin-like drugs. The Raptor complex feedback inhibits signaling by particular receptor tyrosine kinases (RTK). Accordingly, rapamycin-like drugs can lead to paradoxical increases in RTK signaling, including signals flowing through the AKT kinase.

Two factors might conspire to limit the overall effectiveness of rapamycin-like inhibitors for the treatment of kidney cancer. First, in other settings blockade of TORC1 has caused a paradoxical increase in upstream receptor tyrosine kinase signaling due to a loss of TORC1-dependent negative feedback loop (Figure 2).[41,42] Our preliminary evidence indicates that this might occur in kidney cancer cells as well (Sungwoo Lee and W.G.K-unpublished data). Second, TORC1 inhibition seems to preferentially downregulate HIF1 rather than HIF2α 43. Instead, HIF2 appears to be more sensitive to loss of TORC2, which is largely (but not completely), inured to rapamycin-like drugs.[43] A number of dual TORC1/2 inhibitors are now being developed and preliminary data in preclinical kidney cancer are encouraging.[44] As an alternative, Iliopoulos and colleagues have identified small molecules that suppress HIF2α translation in an mTOR-independent manner.[45,46] Whether these compounds can be converted into therapeutics remains to be determined.

Conclusion

It is assumed that more complete inhibition of VEGF signaling will translate into enhanced clinical activity in patients with kidney cancer. In this regard, a number of more potent and more selective VEGF inhibitors are in clinical development and might eventually replace the existing VEGF inhibitors by virtue of superior VEGF blockade, decreased toxicity and/or enhanced ability to be combined with other agents. A caveat, however, is that on-target toxicities, such as microangiopathy[47] and cardiomyopathy,[48,49] will likely limit the degree to which VEGF signaling can be safely blocked in patients. Indeed, a recent trial of combined bevacizumab and sunitinib was halted because of such microangiopathic changes (http://www.cancernetwork.com/rcc/content/article/10165/126529 5).

Virtually all kidney cancer patients eventually develop resistance to VEGF inhibitors, although the underlying resistance mechanism(s) remain poorly understood. Fortunately, some forms of resistance to VEGF blockade can be circumvented by simply changing the choice of inhibitor. Clearly, however, a more detailed understanding of the molecular circuits that allow kidney cancers to escape VEGF inhibition is needed. In one recent study, which awaits confirmation, enhanced secretion of interleukin-8 was implicated as a potential resistance mechanism [50]. Interestingly, interleukin-8 has been shown to conspire with VEGF before to enhance angiogenesis.[51]

Clearly additional targets, and the agents with which to attack them, are needed in kidney cancer so as to build more effective combinations moving forward. It is anticipated that such targets will emerge from a variety of sources including cancer genome resequencing projects, unbiased chemical and genetic screens aimed at identifying vulnerabilities created upon VHL loss, and identification of the genetic alterations, including copy number changes that, together with VHL loss, are responsible for this disease.

Discussion

Dr. Atkins: In many tumors HIF-1 alpha is believed to be associated with poor prognosis, it is hypoxia driven. This situation seems to be different in kidney cancer. To what extent are we looking at HIF-1 alpha as a tumor suppressor in a context-dependent fashion where it is a tumor suppressor if Hif-2 alpha is up or if VHL is lost and the resultant downstream target genes are up? To what extent is that also related to other potential genetic changes in RCC?

Dr. Kaelin: This brings up the point of correlation versus causation. When people in Biotechs ask where they should test a HIF-1 alpha inhibitor, I say I do not know because almost all of the data is guilt by association. You may have an aggressive tumor that is growing rapidly, outgrows its blood supply, gets hypoxic, up-regulates Hif-1 alpha and ergo Hif-1 alpha is associated with a bad prognosis. But of course that does not mean that Hif-1 alpha is causing the bad behavior; it could be the result of the bad behavior. I do not know of a solid tumor today where I can say definitively Hif-1 alpha is a driver. Now, there are some animal models where you can make a case that Hif-1 alpha is acting as a driver in a particular cell line growing subcutaneously in a nude mouse, but I think the data are pretty soft at the moment in terms of Hif-1 alpha.

Dr. Atkins: Might there be effects of Hif-1 alpha upregulation that interact with the stroma such as increases in LDH, or decreases in immune function, so that it may be associated with poor prognosis by creating an environment that allows the tumor to grow? You might not see it when you are only testing at the tumor cell level.

Dr. Kaelin: Well, yes. I think Hif-1 alpha has plausibility on its side and correlations on its side, but I do not know that in all cases we can say definitively that it is the driver. And this is not the first time Hif-1 alpha has paradoxically scored as a tumor suppressor. There are other models now where knocking out Hif-1 alpha promotes tumor growth.

Dr. Rathmell: There was a paper in the past year suggesting that histone demethylases were mutated in many RCCs. Can you comment on their potential role as therapeutic targets in this disease?

Dr. Kaelin: The nice thing about working with histone-methylation is that both the methyltransferases and the demethylases are potentially drug-able, in contrast to, for example, the situation with kinases and phosphatases where if you lose the kinase you cannot develop a drug that targets the phosphatase. We have done an experiment recently where in tumors that lack the MEN1 methyltransferase complex when we block the corresponding demethylase, tumor growth is diminished. So first of all I think we have to figure out whether all of these mutations that were just reported were all loss of function or whether some are gain of function. But then I think to your point I think potentially we can play games on both sides of the equation. So if it is a gain of function methyltransferase mutation then we can target the methyltransferase with a drug. If it is a loss of function methyltransferase mutation then you want to drug the demethylase that acts as the counterbalance to that.

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The von Hippel-Lindau tumor suppressor protein and kidney cancer biology

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RENAL CANCER BIOLOGY AND NOVEL TARGETS:
Genetically Defined Groups in Renal Cancer
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Introduction

Much of our understanding of how to delineate types of renal cancers has come from studies of inherited cancer susceptibility syndromes. While such syndromes are estimated to account for <3% of all renal cancers, they have contributed greatly to our knowledge of the biological basis of sporadic disease. Cancer susceptibility syndromes with a high risk of renal cancer include:

- von Hippel Lindau disease (vHL)
- hereditary papillary renal cancer (HPRCC)
- hereditary leiomyomatosis and renal cancer (HLRCC), and
- Birt-Hogg-Dube (BHD)

Each of these inherited diseases is associated with a predominant type of renal cancer – clear cell (ccRCC), papillary type 1, papillary type 2 and hybrid chromophobe/oncocytoma cancers, respectively. The study of inherited disease has enabled the development and use of targeted therapeutics for all patients with renal cancer. In addition, genetic changes may serve as predictive or prognostic biomarkers for treatment efficacy, which has been most thoroughly explored in relationship to VHL mutation status in ccRCC.

Von Hippel Lindau disease and ccRCC

vHL is an autosomal dominant cancer susceptibility syndrome in which patients develop hemangioblastomas of the brain, spine and retina, clear cell renal cancer, pancreatic cysts, pancreatic neuroendocrine tumors, endolymphatic sac tumors and pheochromocytomas.[1] The gene responsible for inherited susceptibility to vHL, VHL, was found through the study of multiple case families.[2] VHL is mutated not only in inherited ccRCC, but also in the vast majority of sporadic ccRCCs, with both copies lost in 86% and genetic or epigenetic changes of one allele found in 96%.[3] The VHL protein comprises part

of a complex, the main function of which is to ubiquitate the alpha regulatory subunits of the hypoxia inducible factor (HIF) family and target them for degradation.[4] The HIFs are transcription factors that regulate adaptation to tissue hypoxia, and loss of VHL allows chronic activation of the hypoxic response, including upregulation of the vascular endothelial growth factors (VEGFs), even under normoxic conditions.[5] The link between VHL and HIF has provided the basis for development of VEGF-targeted therapies for ccRCC.

Mechanisms of VHL mutation as biomarkers in ccRCC

VHL can be altered through point mutation (either truncating or missense), promoter methylation and larger genomic deletions or rearrangements. The different types of mutations, as well as their location, have been studied as potential prognostic or predictive markers associated with response to VEGF-inhibitors in ccRCC. Many of the studies of VHL mutations as biomarkers are limited due to sample size, incomplete genetic characterization or as in studies of predictive markers by inclusion of multiple VEGF inhibitors. The studies of VHL mutational status as a prognostic marker have been inconsistent, with some suggesting that loss is associated with a

worse, and others a better, prognosis. The largest study by Choueiri et al. examining VHL mutational status as a predictive biomarker in

123 patients treated with a variety of VEGF-inhibitors suggested that loss of function mutations in VHL were associated with treatment response.[6] However, VHL mutation status did not appear to associate with progression-free and overall survival. In order to fully evaluate the potential role of VHL mutation status as predictive or prognostic biomarker, it needs to be a component of large scale prospective clinical trials with thorough genetic evaluation.

Hereditary papillary renal cancer

HPRCC is an autosomal dominant syndrome characterized by multifocal, bilateral type I papillary renal cell carcinomas without extra-renal manifestations.[7,8] The responsible mutated gene is MET.[9] However, MET is mutated in less than 10% of sporadic type papillary renal cancers. Clinical trials of MET inhibitors for type 1 papillary renal cancers are underway.[10]

Hereditary leiomyomatosis and renal cancer

HLRCC is an autosomal syndrome characterized by the development of cutaneous and uterine leiomyomas and renal cancer.[11,12] Papillary type 2 is the predominant pathological type associated with HLRCC and tends to be early onset, high grade and aggressive.[13] The mean age of diagnosis is 40; metastatic renal cancer has been observed in individuals as young as 17. The mutated gene in HLRCC is fumarate hydratase (FH), which encodes the enzyme that converts fumarate to malate in the Kreb's cycle.[14] Consistent with a postulated role as a tumor suppressor gene, loss of the wild type allele is observed in renal cancer from individuals with FH mutations. However, mutations have not been observed in patients with sporadic RCC, but in part the lack of this observation may arise due to the limited number of papillary type 2 tumors included in the screening series.[15,16]

Birt Hogg Dube syndrome

BHD is an autosomal dominant syndrome characterized by the development of fibrofolliculomas (dysplastic hair follicles), lung cysts and pneumothoracies, and renal cancer, predominantly hybrid oncocytic tumors.[17,18] The gene in which mutations cause BHD is named folliculin (FLCN).[19] The FLCN protein has no homology to previously identified proteins, and its function is still largely unknown. A wide spectrum of renal cancers has been observed in patients with BHD, even within the same kidney.[20] The most common type of tumor is an unusual hybrid oncocytic tumor (mixed oncocytoma and chromophobe). Observation of a hybrid oncocytic tumor in any patient should prompt an evaluation for BHD, as it is so characteristic of this disease. In BHD, FCLN functions as a tumor suppressor gene, and, unusually, the second allele of FCLN is most frequently inactivated by point mutation rather than loss.[21] However, mutations in FLCN are rarely identified in sporadic renal cancers, most commonly in chromophobe tumors.[22,23]

Molecular profiling to define sub-groups of renal cancers

Both DNA and RNA-based molecular profiling in renal cancers has been done as proof of concept to demonstrate that these methods can differentiate between different types, such as clear cell and papillary renal cancers. However, more recent studies have focused on delineating sub-types within genetically defined groups of renal cancers, with most studies focusing on ccRCCs.

Gordan et al. recently demonstrated that within the group of ccRCCs with pVHL loss caused by mutation or methylation, two sub-types exist, those expressing HIF1α and HIF2α (termed 'H1H2') and expressing HIF2α only (H2).[24] Whereas H1H2 tumors show increased activation of Akt/mTOR and MAPK signaling pathways, H2 tumors have greater c-Myc activity. H2 tumors demonstrate increased expression of genes involved in double strand break repair, such as BRCA1 and BARD1, and consequently decreased levels of DNA damage, as measured by γH2AX and genomic copy number changes. In addition, they have higher levels of proliferation, and H2-only expressing cell lines progress more quickly through S-phase. Additional studies are necessary to delineate whether these sub-types of ccRCC are of prognostic or predictive significance in relationship to treatment with VEGF inhibitors.

Recent copy number analyses of ccRCCs, sporadic and associated with VHL disease, showed a similar profile between both groups, although the sporadic tumors were more heterogeneous with more events per tumor.[25] Unsupervised clustering of expression profiles could not distinguish between the two groups. Standard karotyping has been performed in 282 ccRCCs in patients with nephrectomies to examine whether cytogenetic changes were prognostic.[26] Deletion of 3p was associated with a better prognosis (p=0.03), whereas 4p (p<0.001), 9p (p<0.01) and 14q (p<0.01) loss were associated with a worse prognosis. In multivariate analysis, loss of 9p emerged, along with stage and grade as associated with poor survival.

Expression profiling has been used to delineate sub groups of ccRCC. In 177 tumors obtained at the time of nephrectomy using an array of 3,674 genes, Zhao at al. identified two major sub-groups, which encompassed two and three smaller groups, respectively.[27] These groups were associated with significant survival differences, and the activation of distinct pathways. More recently, two studies by Skubitz et al. and Brannon et al. have been performd on smaller sample sets of ccRCCs (16 and 48), but using much larger gene sets.[28, 29] Both analyses also identify two groups of ccRCCs, one of which is dominated by metabolism genes, the other by wound healing and epithelial to mesenchymal transition genes; the former group appears to be associated with a significant survival advantage. Expression analysis in 75 ccRCCs, a sub-set of 101 that underwent whole exome sequencing, also showed two groups – hypoxic and non-hypoxic.[30] In the former group, most (65%) carried a point mutation in VHL; the latter group was associated with NF2 mutations. JARID1C, SETD2 and UTX, histone modification genes, were each mutated in 3% of ccRCCs. A signature expression profile was associated with JARID1C and SETD2 mutations, but each of these account for a very small percentage of ccRCCs overall.

Conclusion
These different approaches all suggest that there are distinct molecularly defined sub-types of ccRCC, however additional work needs to be done to integrate them together. Future studies should combine HIF status, copy number, mutational data and expression profiling for optimal sub-grouping of ccRCC.

Discussion
Dr. Atkins: An interesting question is whether these tumors evolve. As we talk about immune therapy and then angiogenic therapy and then TOR inhibition therapy, is the mechanism of resistance or escape in those settings somehow related to selection of a different subset of tumors that may have profiles different from the primary tumor? Or is resistance related to a physiologic adaptation that can

reverse once the selective pressure is removed? I do not think we know the answer, but I think that it is clinically relevant.

Dr. Stadler: I am convinced that we are underestimating the complexity. We call one disease renal cancer, but we know that it is not one disease to start with. We have a couple of different histologic subtypes starting out, and then we have a couple of different clear-cell subtypes. And then we have selective pressure with regard to metastases, that allow certain things to grow out, and then we introduce selective pressures of therapy. Furthermore, we have not even talked about the complexities within the stroma.

Dr. Kaelin: I would argue that as bad as it is in kidney cancer it is worse in many other tumors. To a first approximation kidney cancer is a disease caused by VHL loss. You can assume that and you will be right 90-percent of the time.

Dr. Stadler: I want to know what makes a clear cell a clear cell. I mean, clearly VHL loss is a critical step, but I think we need to put some names to some of the other lesions seen in these tumors, and then we will have the "Vogel-gram" for clear cell, and then we can ask these more sophisticated questions of, well, if the tumor evolves or is put under drug selection, what comes out?

Dr. Choueiri: In essence we don't know the reason we have all this heterogeneity in RCC including that 10-percent of clear cell RCC has wild type for VHL. Some of this may be technical and they may be clear cell, some of them may be some other cancer. It becomes even more complex when you start talking about other changes such as sarcomatoid differentiation because the tumor might have been clear cell to start with or it may have been something else completely different.

Dr. Kaelin: It looks to me that about 70–75-percent of clear cell patients get at least some benefit from VEGF pathway inhibition, which is consistent with having about 75-percent of clear cell as VHL-defective tumors. Now, I do not know that anyone has gone back to look to see whether people who are not getting any tumor shrinkage whatsoever are in fact molecularly clear-cell carcinomas.

Dr. Choueiri: We had 100-140 VHL patients and looked at CAIX status but we did not have a strong correlation with either response or progression free survival. VHL mutation was independently associated with response but not with survival or progression free survival. But the real issue is much, larger. What has happened in the past 7-10 years in this field was that all the large clinical studies were not required to collect tissue, and we as investigators did not push enough on industry to require tissue and to fund those studies. So here we are with drugs that we don't understand which population they work best in. The next generation of studies absolutely needs funding allocated for tissue collection to get these analyses going.

Dr. Atkins: Yes. The cooperative groups may be our opportunity to ask biology questions if industry does not fund them. But there is one study which I am hoping will help us address this question, and that is the RECORD 3 Study. This study looks at sunitinib versus everolimus first line with a switch to the alternative drug at the time of progression. I believe there will be extensive tumor tissue collection and hopefully we will learn something about who responds to an mTOR inhibitor versus a VEGF pathway inhibitor from that study.

Dr. Nathanson: Phospho-proteomic arrays, particularly RPPA, where you want to actually look at big proteomic efforts, really need fresh tissue and I think that the importance of fresh tissue has been really undervalued. We need to push for fresh tissue that can be used for a variety of studies that we just cannot do adequately on paraffin. Paraffin embedded tissue is really second best. This is an important issue for the kidney cancer community.

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Genetically Defined Groups in Renal Cancer

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IMMUNOTHERAPY IN RENAL CANER:
Update on the Role of IL-2 for Metastatic Kidney Cancer

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Introduction

The ability of some renal tumors to evoke an immune response and the lack of benefit seen with standard chemotherapy and radiation led to the application of immunotherapy for patients with metastatic renal cell carcinoma (RCC).[1-3] In an attempt to reproduce or accentuate this response, various immunotherapeutic strategies have been used, including nonspecific stimulators of the immune system, specific antitumor immunotherapy, adoptive immunotherapy, the induction of a graft-vs-tumor response via allogeneic hematopoietic stem cell transplantation, and the administration of partially purified or recombinant cytokines.[4-14] Although immunotherapy was once the standard of care, the advent of novel therapies that target angiogenesis and signal transduction pathways has produced significant clinical benefits and prompted a reassessment of the role of immunotherapy.[15-18] Recent insights into how the immune response to a tumor is regulated may allow patients to obtain a durable response to immunotherapy without the need for chronic treatment typically required of anti-angionenic and tumor targeted approaches. This review describes how improvements in patient selection, combination therapy, and investigational agents might expand and better define the role of IL-2 in metastatic RCC.

Cytokine therapy

Although a number of cytokines have shown antitumor activity in RCC, the most consistent results have been reported with interleukin 2 (IL-2) and interferon alfa (IFN-α). In contrast to the results seen with VEGFR targeted therapies (eg, sorafenib, sunitinib), which lead to tumor shrinkage in most treated patients but do not produce responses that persist following discontinuation of therapy, the administration of high-dose bolus IL-2 has consistently produced durable responses in a small percentage of patients with advanced RCC.[19-21] However, the substantial toxicity and limited efficacy that are associated with IL-2 have narrowed its application to highly selected patients treated at specialized centers.[22,23] Although IFN-α has produced modest benefits in unselected patients, randomized clinical trials have revealed a small survival benefit with manageable toxic effects when compared with non–IFN-α control arms.[24-31] As it became the de facto standard of care worldwide, regulatory agencies have supported the use of IFN-α as the control arm for randomized trials with molecularly targeted therapies that are described elsewhere in this issue.[15-18] The results of these investigations have, in general, established the superiority of VEGF pathway and mTOR targeted agents in previously untreated patients, thereby narrowing the future use of IFN-α as a single agent in this setting.

In recent years, the relative merits of these low- and high-dose cytokine regimens have been clarified by the results of 4 randomized trials (Table 1).[32-35] In the most consequential trial, the French Immunotherapy Group randomized patients with an intermediate likelihood of response to IL-2 and IFN-α to receive medroxyprogesterone (control group), subcutaneous IFN-α, subcutaneous IL-2, or the combination of IFN-α and IL-2.[35] Although significant toxicity was more common in the IL-2 and IFN-α arm, median overall survival did not differ between the arms. The investigators concluded that subcutaneous IFN-α and IL-2 should no longer be recommended in patients with metastatic renal cell carcinoma and intermediate prognosis.

Table 1. Select randomized trials of cytokine therapy in metastatic renal cell cancer

Trial	Treatment Regimens	N	Response Rate %	Durable Complete Response (%)	Overall Survival (mo)[*]
French Immunotherapy Group[32]	CIV IL-2	138	6.5	1	12
	LD SC IFN-?	147	7.5	2	13
	CIV IL-2 + IFN-?	140	18.6	5	17
	MPA	123	2.5	1	14.9
French Immunotherapy Group[35]	LD SC IFN-?	122	4.4	3	15.2
	LD SC IL-2	125	4.1	0	15.3
	SC IL-2 + IFN	122	10.9	0	16.8
National Cancer Institute Surgery Branch[33]	HD IV IL-2	156	21	8	NR
	LD IV IL-2	150	13	3	NR
	HD IV IL-2	95	23	7	17.5
Cytokine Working Group[34]	LD SC IL-2/ IFN-?	91	10	NR	13
	HD IV IL-2	95	23	NR	17.5

Abbreviations: CIV, continuous IV infusion; CR, complete response; HD, high dose; IFN-α, interferon alfa; IL-2, interleukin 2; IV, intravenous; LD, low dose; MPA, medroxyprogesterone acetate;; NR, not reported; RR, response rate; SC, subcutaneous.
[*] The overall survival difference was not statistically significant in all cases.

Taken together, these studies suggest that high-dose intravenous (IV) bolus IL-2 is superior in terms of response rate and possibly response quality to regimens that involve low-dose IL-2 and IFN-α, intermediate- or low-dose IL-2 alone, or low-dose IFN-α alone. Consequently, although low-dose single cytokine therapy has a limited role in patients with metastatic RCC, high-dose IV IL-2 remains a reasonable option for appropriately selected patients with access to such therapy. More significantly, correlative biomarker investigations associated with these trials suggest that the potential exists for identifying predictors of response (or resistance) and thus limiting IL-2 therapy to those most likely to benefit.

Pathologic and molecular predictors of response to IL-2

Influence of Histologic Subtype.

Responses to immunotherapy are most frequently seen in patients with clear cell RCC.[36-38] This observation was detailed in a retrospective analysis of pathology specimens obtained from 231 patients (163 primary and 68 metastatic tumor specimens) who had received IL-2 therapy in Cytokine Working Group (CWG) clinical trials.[38] For patients with primary tumor specimens available for review, the response rate to IL-2 was 21% (30 of 146) for patients with clear cell histologic primary tumors compared with 6% for patients with non–clear cell histologic tumors (1 responder in 17 patients). Among the patients with clear cell carcinoma, response to IL-2 was also associated with the presence of good predictive features (eg, more than 50% alveolar and no granular or papillary features) and the absence of poor predictive features (eg, more than 50% granular or any papillary features). As a result of these data, it may be appropriate for patients whose primary tumor is of non–clear cell histologic type or of clear cell histologic type but with poor predictive features to forgo IL-2–based treatment altogether.

Immunohistochemical markers.

Carbonic anhydrase IX (CAIX) has been identified as an immunohistochemical marker that might predict the outcomes of patients with RCC. In an analysis by Bui et al, CAIX expression in more than 85% of tumor cells (high CAIX expression) has been associated with improved survival and a higher objective response rate in IL-2–treated patients.[39] Building on this work, Atkins et al developed a 2-component model that combined pathology analysis and immunohistochemical staining for CAIX.[40] In a retrospective analysis, this model was able to identifya good risk group that contained 26 (96%) of 27 responders to IL-2 compared with only 18 (46%) of 39 nonresponders (odds ratio, 30; P<.01). A significant survival benefit was also seen for this group (P< .01).

Molecular markers.

Through gene expression profiling of tumor specimens, Pantuck et al were able to identify a set of 73 genes whose expression distinguished complete responders from nonresponders after IL-2 therapy.[41] In their hands, complete responders to IL-2 have a signature gene and protein expression pattern that includes CAIX, PTEN, and CXCR4. A similar analysis identified loss of chromosome 4, 9, and 17p as possible predictors of IL-2 nonresponsiveness.[42] Further investigation into these regions may improve our understanding of the molecular basis of an effective immune response in RCC. Although these approaches require prospective validation, it may become a powerful aid for clinicians in selecting appropriate treatment options for patients with advanced RCC.

Current investigation in patient selection The CWG conducted the high-dose IL-2 "Select" Trial to determine, in a prospective fashion, if the predictive model proposed by Atkins et al could identify a group of patients with advanced RCC who are significantly more likely to respond to high-dose IL-2–based therapy (good risk) than a historical, unselected patient population.[40] The preliminary clinical results of this trial revealed a response rate (28%) that was significantly higher that the historical experience with high-dose IL-2.[43] Analysis of tumor (central pathology review and staining for CAIX) and blood based predictive markers is ongoing to further improve the selection criteria for IL-2 and limit its application to those patients most likely to benefit. As the list of effective therapies for metastatic RCC grows, improvements in patient selection will be necessary to ensure that patients who might attain a durable remission with IL-2 will not miss this opportunity.

IL-2 therapy after VEGF pathway–directed therapy The emergence of molecularly targeted therapies has offered hope for improved clinical outcome for patients with RCC. Vascular endothelial growth factor (VEGF) pathway–directed therapy has been recommended for frontline use in patients with good or intermediate prognosis with other treatments reserved for patients with poor prognostic features or at time of disease progression. However, a retrospective analysis suggests that the toxicity of IL-2 therapy may be higher in patients who have received prior VEGF-targeted therapy, particularly sunitinib, and antitumor activity may be diminished.[44] Although the mechanism for the observed increased incidence of cardiovascular complications remains speculative, the assumption that IL-2 can be given safely after VEGF pathway–targeted therapy may not be valid.

Combination of immunotherapy and targeted/antiangiogenic therapy Although the role of low-dose single-agent cytokines is limited, combinations of cytokines with targeted therapy may have merit. Bevacizumab was combined with high dose IL-2 in a CWG trial. Preliminary results suggest that these two agents can be given safely in combination and produce efficacy improvements that are additive but not synergistic.[45] Two recently completed large phase III trials of interferon plus bevacizumab vs interferon alone have demonstrated superior efficacy with the combination regimen compared with cytokine monotherapy and suggest the potential of an additive effect.[18, 46] Confirmation of the benefit of combination therapy will require a randomized trial comparing the combination to bevacizumab alone.

Investigational immunotherapy

Metastatic RCC has long been a testing ground for novel immunotherapies. Several such approaches, including vaccination and allogeneic bone marrow transplantation, have been tested during the past 2 decades. The initial reports of applying allogeneic bone marrow transplantation were encouraging, but further clinical trials have highlighted the potential toxicity and limited applicability of this approach.[10-12] Vaccination therapy has shown the ability to induce potentially relevant immune responses, although clinical benefit and objective responses have not been consistently observed.[47-49] Avigan et al have conducted a series of clinical trials with a dendritic cell/tumor fusion vaccine approach that have shown encouraging clinical responses in patients with a variety of malignancies, including RCC.[47] To realize the full potential of a vaccine approach in RCC, combinations with immune stimulants (eg, granulocyte-macrophage colony-stimulating factor) and inhibitors of natural T-cell regulation pathways (eg, CTLA4 blockade, T-regulatory cell depletion) may be necessary.

An improved understanding of the molecular mechanisms that govern the interaction between a tumor and host immune response have led to the development of several novel immunotherapies that have recently entered the clinic (Table 2). Obstacles to effective immunotherapy for RCC likely include the physiologic down-modulation of the immune response through the increased expression of molecules such as CTLA4 on the surface of activated T cells. Mechanisms identified as leading to tumor-induced immune suppression have included RCC expression of B7H1 (PDL1), which serves to restrict the cytolytic function of tumor-infiltrating T lymphocytes and stimulation of T-regulatory cell (CD4+ CD25+) production, which limits T-cell receptor signaling.

Table 2. Investigational immunotherapeutic approaches to the treatment of metastatic renal cell cancer

Target	Drug	Class	Development Phase
Blockade of T-cell regulation			
CTLA4[50]	Ipilimumab	Fully human IgG1 mAb	Phase III
PD1[51,52]	MDX-1106	Fully human mAb	Phase I
Inhibition of tumor-induced T-cell function			
TGF-β[53]	GC1008	Fully human mAb	Phase I
TGF-β2	AP12009	Fully human mAb	Phase I
T-cell activation			
CD137[54]	BMS-663513	mAb	Phase II (melanoma)
Cytokines[55]	Interleukin-21	Recombinant molecule	Phase I
Dendritic cell activation			
Toll-like receptor[56]	HYB2055	TLR9 agonist	Phase II

Abbreviations: mAb, monoclonal antibody; TGF, transforming growth factor.

The list of novel agents currently being pursued includes agents that block T-cell regulation (eg, CTLA-4 and PD1 antibodies),[50-52] inhibit tumor-induced immunosuppression (eg, transforming growth factor β antibody, PDL1 antibody),[53] and activate T cells (eg, CD-137 antibody, IL-21)[54,55] and dendritic cells (eg, toll-like receptor agonists).[56] Several of these agents have shown encouraging efficacy signals in early trials. Immune related adverse events associated with CTLA-4 antibodies, including enteritis, skin rash and hypophysitis, have occasionally been life threatening and have also been associated with tumor response.[50] Combination of cytokines and agents that block immune downregulation may prove particularly effective in selected patients. A recent report of high-dose IL-2 and ipilimumab (CTLA4 antibody) in patients with metastatic melanoma revealed manageable toxicity with a complete response rate of 17% suggesting a potential role for this combination in RCC patients.[57] However, the development of targeted immunotherapy for RCC is complicated by the increasing array of other treatment options and their potential impact on the immune system.

Conclusion
RCC has long been considered an immunologically influenced malignancy and thus served as a platform for the clinical testing of anticancer immunotherapy. The nonspecific cytokines, IL-2 and IFN-α, have undergone the most testing and produced only modest benefits for unselected patients. High-dose IL-2 remains the only approach to produce durable responses in patients with metastatic RCC and can thus be considered in appropriately selected patients. For patients unlikely to benefit from, unable to receive, or who progress after IL-2, the emergence of molecularly targeted therapies offers hope for improved clinical outcome.15-18 Additional molecular and pathologic selection opportunities exist for cytokines, but considerable validation work is needed before these selection features can be used clinically. Cytokine therapy optimally should be given in the context of a clinical trial investigating combination therapy and/or patient selection to maximize the benefit of this approach. Targeted immunotherapeutic strategies have been tested in patients with metastatic RCC, but definitive evidence of clinical benefit is only emerging.

In recent years, the list of effective therapies (eg, angiogenesis inhibition; signal transduction inhibition and immunotherapy) for patients with metastatic RCC has increased substantially. The advent of targeted therapy in RCC does not eliminate the potential utility of immunotherapy but rather necessitates efforts to rationally refine this treatment approach through patient selection, combination

regimens, and novel agents that together may extend overall survival and increase the cure rate for patients with this disease.

Discussion
Dr. Atkins: The IL-2 Select study represents an important contribution and a well done study. I think we have to assume that there is a reason why some people respond and others don't. You have tissue, blood, DNA, plenty or responders and non-responders and hypotheses to be tested. How do you optimally use these tools to find an answer to why some patients respond and others don't?
Dr McDermott: While we were unable to confirm our primary hypothesis that CA-9 staining predicts for benefit to HD IL-2 we have several other hypotheses that we hope to confirm. If we are successful in this effort, a new model of selection for HD IL-2 will emerge for patients with mRCC.
Dr. Nathanson: Do you have access to a source of material that won't change, which is DNA from the patient? If you think that there might be a phenotype that is predictive of response to immunotherapy that may be inherited you could test for this in your study. You could compare those patients who had excellent responses to those who didn't respond at all and assess whether various inherited factors are affecting outcome. You don't need a big sample–even 50 and 50. I've seen very interesting data come out of small studies with well defined phenotype. Like secondary malignancies, hearing loss after cisplatin; the key is to have a very well defined phenotype.
Dr McDermott: We do have access to DNA for almost all of the patients as we have collected and stored PBMCs on this cohort. If we could obtain the funding for the studies you suggest, we would be glad to collaborate with you on this effort. What you're talking about now, doing genome-wide studies, was not as feasible when this trial was designed but certainly could be pursued in the future.
Dr. Nathanson: As food for thought, if you were giving other immunotherapies IL-2 for renal cancer, do you think the same factors would predict for response?
Dr. McDermott: I would like to think that, but that hypothesis remains to be investigated. Our goal for this study was not to find a predictive marker that was limited to IL-2, but to help identify factors that might help select patients with RCC for immunotherapy. I think this is, ultimately, the way we are going to cure are larger percentage of patients with metastatic disease.
Dr. Stadler: In regard to the endpoint, I wonder whether response is the right metric, or whether it ought to be something else – durable response or 90% response– and I would consider reanalyzing this data using that metric and incorporate some of the other markers you propose to look at.

Dr. McDermott: I agree. Even if the initial result suggests CAIX doesn't predict for response to IL-2, we can still examine the data as you suggest in 2-3 years and report on factors that predict or don't predict for durable response to therapy which is the most important endpoint following HD IL-2 therapy.

Dr. Stadler: OK as long as you're honest that this was not a pre-specified endpoint, its hypothesis generating and it's interesting.

Dr. George: The point is, are people still going to use HD IL-2 off protocol? So, it is still clinically relevant to understand, if nothing else, who is NOT responding and who is, in fact, responding to HD IL-2. Your 6% CR may not be different from a historical number, and your 23 month median PFS may be worse than we've seen. It may just be technique– RECIST vs WHO, but at the end of the day, we do need to understand who we should be selecting, by marker or by clinical parameters, for this treatment.

Dr. Rathmell: I think that we need to bear in mind that a 28% PR rate in a highly selected group of patients, even though it includes a few high risk people, is not as good as sunitinib. A 23 month median survival is about the same as good risk patients achieve with sunitinib. So, we have not achieved a benefit for the majority of patients. What we need to focus on is increasing CRs and very durable PRs.

Dr. McDermott: In my mind there is no comparison between IL-2, good and bad, with any other FDA approved therapy for mRCC. It is more toxic and less likely to produce tumor shrinkage, but it is the only agent that can provide durable benefit. There will be a group, 10-15% of initial cohort that will have durable benefit: people who have responded and have yet to progress. So in the era of targeted therapy, HD IL-2 can still offer a durable benefit and achieve the primary goal of any patient. This is not to say that IL-2 is great. Its weaknesses persist and there are definitely some people who should not get it. However, in the short term, the only mRCC patients who are going to get cured of their disease are the ones who can respond to immunotherapy. Therefore, efforts to understand which patients benefit from this therapy and which do not should be pursued. And therapies that offer durable benefit with less toxicity than HD IL-2 (e.g. PD-1 antibodies) should be aggressively investigated.

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IMMUNOTHERAPY IN RENAL CANCER:
Immune Checkpoint Modulators for the Treatment of Metastatic Renal Cancer
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Introduction

The responsiveness of metastatic renal cancer (mRCC) to immune stimulating agents has been known for many years. Low rates of objective tumor regression have been reported consistently in clinical trials of cancer vaccines and various cytokines. Of the cytokines, interferon-alpha and interleukin-2 (IL-2) appear to demonstrate the highest response rates, in the range of 5-20%, and therefore have been studied extensively alone and in combination with other agents. As discussed above, high dose IL-2 in particular produces very durable complete remissions in approximately 5% of patients with mRCC , including patients with large tumor burdens, and thus provides important proof-of-concept for the therapeutic potential of immunotherapy in this disease.[1]

The immunologic mechanisms by which IL-2 produces tumor regression in mRCC are not fully understood. Nevertheless, it seems reasonable to assume that IL-2 and other immune therapies are activating or expanding T-lymphocytes that specifically recognize antigens expressed by renal carcinoma. Further promoting the development, expansion, and effector function of these tumor-specific lymphocytes could lead to even better anti-tumor responses. In line with this hypothesis, several groups have attempted to immunize patients against their tumor. Only a limited number of broadly expressed defined cancer-associated antigens have been identified in renal carcinoma, therefore several cancer vaccines have used allogeneic or autologous tumor cells as the source of antigen, and have relied on advances in immunology (for example, derivation of autologous heat shock protein containing potential peptide antigens, or fusions of dendritic cells with tumor cells) to produce more effective T-cell responses to the vaccine antigens.[2-5]

The minimal to modest success of cancer vaccines and cytokine therapy to date is not surprising, when viewed in the context of a more modern understanding of the extensive and complex regulation of immune responses, and the immune inhibitory influences within the tumor microenvironment. The identification of

multiple positive and negative regulators of T-cell activation and function provides new opportunities for effectively modulating anti-tumor immune responses in mRCC. These new agents may provide key agonist and survival signals to T-cells, or more importantly block regulatory checkpoints for T-cell expansion and function. Although a logical use of the checkpoint modulators is in combination with cancer vaccines, many patients may already have ongoing antigen presentation and immune responses against their cancer, thus the checkpoint modulators alone may be sufficient to induce tumor regression, similar to and perhaps more effectively than interferon or IL-2.

Three immune checkpoint modulators have received limited evaluation in metastatic renal cancer, including:
- antibodies to CTLA-4 (ipilimumab)
- agonist antibody to CD137
- PD1 blockade

Because these molecules are found on more than one immune cell type, and because expression may be time and context dependent, the exact mechanisms contributing to their anti-tumor activity in animal models or patients will be difficult to define. Consequently, selection of patients most likely to respond to any individual agent may also prove challenging.

Antibodies to CTLA4

CTLA4 is brought to the surface of activated T-cells, and upon binding to its ligands CD80 or CD86 on antigen-presenting cells, inhibits further lymphocyte proliferation.[6] CTLA4 is also expressed on T-regulatory cells.[7] Two blocking antibodies to CTLA4, ipilimumab and tremelimumab, were advanced into clinical development, mostly focused on metastatic melanoma.
Ipilimumab. The largest experience with ipilimumab in mRCC was published from the Surgery Branch, NCI.[8] The first cohort of 21 patients had all received prior high dose IL-2 and received a loading dose of 3 mg/kg, followed by 1 mg/kg every 3 weeks. One patient (4.7%) developed a partial response (PR) in lung and adrenal metastases that lasted 18 months, but progressed in a single bone site. The second cohort of 40 patients received 3 mg/kg every 3 weeks. Three of 14 (21.4%) patients without prior high dose IL-2 and 2 of 26 (7.7%) with prior high dose

IL-2 achieved PR. Responding sites included liver, bone, and lung. Response durations at the time of publication were 7, 8, 12, 17 and 21+ months. Two of the partial responders progressed only in a single new site. Similar to the experience with ipilimumab in patients with melanoma, one of the partial responders initially showed disease progression after 2 doses, before lesions began to regress. Autoimmune adverse events including colitis, hypophysitis, rash, and adrenal insufficiency were observed similar to those reported for ipilimumab administration in patients with melanoma. Three patients developed a bowel perforation. There was strong correlation in this study between occurrence of autoimmune adverse events and tumor response.

The results of the ipilimumab trial in patients with mRCC can now be placed in context of mature and extensive data generated in patients with metastatic melanoma.[9-13] The overall objective response rate to ipilimumab in the small phase II trial in patients with mRCC is similar to response rates observed in patients with metastatic melanoma. In the NCI trial, although no patient achieved a complete response and only

one patient had an ongoing response at the time of publication, three of the patients progressed only at a single site that could be managed with radiation or surgery. The study reported from NCI also did not describe patients who may have had mixed responses or regression that did not meet partial response criteria, although the investigators alluded to at least one patient with a mixed response. In data generated for ipilimumab in patients with metastatic melanoma, those achieving mixed responses, or developing progression in a single site after a good response, were felt to derive survival benefit from treatment. In addition, overall survival in patients with metastatic melanoma is increased by ipilimumab despite low objective response rates and minimal to no effect on median time to progression.[9] Surprisingly, no other trials of ipilimumab or tremelimumab in patients mRCC are reported in the literature. The results of the NCI trial and the similarities in activity of ipilimumab in melanoma and mRCC support further study of ipilimumab in patients with mRCC.

Antibody to CD137

CD137 (4-1BB) is expressed after activation of several different types of immune cells.[14,15] An agonist signal through CD137 can provide co-stimulation for T-cells, increase T-cell survival, promote cytokine production and increase T-cell cytotoxicity. A phase I trial of a fully human IgG4 agonist antibody to CD137 administered every 3 weeks was conducted, followed by randomization of 30 metastatic renal cancer patients to the 1, 3 and 10 mg/kg dose levels.[16] At the time of data presentation, none of 22 patients with mRCC had achieved an objective response. Despite the lack of clear activity in this small trial, because of the important role of CD137 signaling in T-cell activation and survival, additional studies of anti-CD137 in patients with mRCC should be considered, possibly in combination with other agents.

PD1 blockade

PD1 is expressed by activated T-cells, memory T-cells and regulatory T-cells, and downregulates T-cell function upon binding to its ligands.[17] Blockade of PD1 in vitro enhances T-cell proliferation and cytokine production in response to a challenge by specific antigen targets or by allogeneic cells in mixed lymphocyte reactions. Thompson et al reported that one ligand for PD1, (PD-L1 or B7-H1) was expressed on tumor cells or on tumor-infiltrating T-cells in 44% of clear cell renal cancers, and was associated with worse survival, regional node involvement, distant metastases, and advanced nuclear grade.[18] In a subsequent analysis of 306 nephrectomy specimens of clear cell cancer, 23.9 % expressed B7-H1 in tumor cells by immunohistochemistry staining, and similar to the prior study, expression correlated with worse survival and higher nuclear grade.[19] The same group also studied 267 nephrectomy specimens of clear cell renal cancer for both T-cell infiltration and PD1 expression by the tumor infiltrating lymphocytes.[20] Immune cell infiltrates were absent in 49% of patients. In the other 51%, PD1 expression was correlated with the extent of tumor immune cell infiltration. These preclinical studies provided a compelling rationale to study blocking antibodies against PD1 or PD-L1 in patients with metastatic renal cancer.

MDX1106.

The initial phase I trial of a blocking antibody to PD1 (MDX 1106, BMS 936558, ONO4538) demonstrated that single doses of 0.3 to 10 mg/kg were well tolerated and associated with a low rate of adverse events.[21] Limited re-treatment was allowed in this trial, given as 2 doses spaced 4 weeks apart at intervals of 3 months. The single patient with mRCC enrolled to the study, with disease in multiple sites, and previously treated with sunitinib, sorafenib, and an HDAC inhibitor, achieved an unmaintained ongoing PR that now exceeds 24 months. The pre-treatment tumor specimen from this patient demonstrated substantial expression of B7-H1. In a subsequent phase I trial, doses of 1, 3, and 10 mg/kg administered every 2 weeks were evaluated, and similar to the initial study, anti-PD1 was well tolerated at all dose levels with a low incidence of grade 3 or grade 4 adverse events.[22]

All patients enrolled were required to demonstrate disease progression on or after a prior treatment. At the time of the latest data analysis, 16 patients with clear cell mRCC were evaluable for response, 2 treated at 1 mg/kg and 14 at 10 mg/kg. At the 1 mg/kg level, one patient achieved a complete response of lung, pleural-based, and lymph node metastases. Four of the 14 evaluable patients at 10 mg/kg achieved confirmed or unconfirmed PR. Overall, 5/16 (31%) achieved objective responses. Regression was observed in large lesions, including a large intact primary tumor. All

of the responders (confirmed and unconfirmed) remain progression-free from 7+ to 17+ months since beginning treatment. Although the analysis is not fully complete, activity was also observed in some of the patients with mRCC not meeting criteria for PR; for example, tumor regression in one patient treated at 1 mg/kg only met criteria for stable disease but he remains progression-free 20+ months from first dose on trial. Similar to ipilimumab, patients demonstrated varying kinetics of tumor response, including initial mixed responses subsequently followed by reduction in size of the growing lesions.

Conclusion

Overall, results from the limited studies of the checkpoint inhibitors ipilimumab and anti-PD1 in patients with mRCC suggest clinically important anti-tumor activity. The value of these agents, similar to IL-2, is likely to be in the induction of very durable responses and possibly cure of metastatic disease, in contrast to the small molecule targeted agents. Many questions remain, for example, the activity of the agents in different subtypes of clear cell cancer and other histologic types of renal carcinoma is not yet known. Identification of predictive biomarkers for response will be an important component of the clinical development of the agents. Although finding predictive biomarkers may prove difficult for ipilimumab, tumor expression of the ligand B7-H1 may be associated with response to anti-PD1 or anti-PD-L1.

For subsequent development and ultimately in clinical practice, we will need to address when and how to use these agents in patients with metastatic disease, and how to integrate their use with the approved anti-angiogenesis and mTOR inhibitors. Future studies will also likely be initiated to determine the activity of combinations, for example of anti-PD1 with anti-CTLA4, or of either of these agents with IL-2.[23] Similarly, combinations of anti-PD1 or ipilimumab with agents such as sunitinib or bevacizumab that have high rates of tumor regression may lead to synergistic clinical activity. Other immune checkpoint modulators will enter clinical development in the near future, and should be studied in patients with mRCC. Because of the potential for these therapies to improve outcomes for patients with metastatic renal cancer, clinical trials should be considered for appropriate patients with metastatic disease ahead of standard treatment with approved non-curative agents.

Discussion

Dr. Atkins: So, let us just fantasize for a second that you are in charge of the development of this drug and you have the ability to do what you want. What would you do?

Dr. Sznol: I would try to get this in on the market for kidney cancer as quickly as possible. I think we could propose a single-arm study third-line for patients whose disease failed VEGF pathway and mTOR pathway inhibitors. If you observe a 15 or 20-percent durable remission rate in 100 patients, there is no reason why this agent should not be approved, especially with a toxicity profile that we have seen so far. It depends on how you define durable response, but if you define a durable remission as six plus months or more, or even a year or more, you may see a 15 to 20-percent rate in that population. Some of the responses have been observed in patients who have progressed on prior sunitinib or both sunitinib and sorafenib therapy.

Dr. Atkins: If you are sitting on ODAC, would you consider this an unmet need, in which case you could get an accelerated approval for response rate alone? Or would this drug have to show an improved survival in a randomized trial?

Dr. Hutson: There is going to be a shifting in ODAC's interpretation of new drugs for kidney cancer. Hopefully Pfizer, who has the largest database now with temsirolimus, axitinib and

sunitinib, can prospectively validate the most rigorous way of defining the impact of PFS on overall survival. We do not have enough patients to do the trials,
so there are going to have to be well-defined endpoints.

Dr. Sznol: I understand that argument. But everolimus does not have a proven survival benefit. Sunitinib seems to have a survival advantage, but not definitively proven because of the crossover. So neither the front-line nor the second-line agents have clearly defined survival advantages in Phase 3 trials. If you go to a third-line setting for which there is no approved agent, and you have a drug with a reasonable durable response rate with a good risk-to-benefit ratio that includes improvement in symptoms, I think it would be difficult for a regulatory body to turn the drug down. Now if the real response rate was 5-percent, it would be more difficult to use this strategy.

Dr. McDermott: But it is just 16 patients so far.

Dr. Stadler: We are getting ahead of ourselves. We need to get some more experience with this drug in renal cancer.

Dr. Sznol: I agree completely that a lot more Phase 2 work needs to be done—and that dosing schedule and looking at the different types of histology, all are really important. But concurrently with that, I would begin a 100-150 patient Phase 2 study in a previously treated group in the hope that if significant activity is seen similar to the very impressive preliminary experience, it might be a short track to drug approval and getting this agent available to patients in need.

Dr. Atkins: Since this is an agent that has a target, I think it is a good opportunity to also think about the biology of tumor response Why are more aggressive looking tumors potentially more likely to benefit from this approach? For example, PDL1 expression on the cell surface can inhibit the PI3 kinase/ AKT pathway. It is essentially a TOR inhibitor. So does that mean that expression of PDL1 by tumor cells creates a profoundly immune-suppressive environment that can be reversed with the PD1Ab? In addition, we should consider what is known about the causes of upregulation of PDL1 on tumor cells.

Dr. Sznol: It is possible that the PD1-PDL1 interaction makes the cell more resistant to apoptosis. If you block the interaction you also might make the cell more sensitive to, for example, cell death from other agents, chemotherapy or targeted agents, whatever the case might be. What causes PDL1 upregulation is not completely clear. It may actually result from T-cells infiltrating the tumor, and intra-tumoral production of interferon-gamma. All of that needs to be worked out. Selecting out for responders may be difficult. The easy guess is that the patients who respond will have T-cell infiltrates and tumors over-expressing PDL1, and possibly poorly differentiated tumors, but I would evaluate these biomarkers retrospectively in a Phase 2 trial.

Dr. George: Is it is worth building in mandatory biopsies of metastatic disease? Patients will want to get on these studies, and these are the opportunities that we typically miss because we do not want to slow accrual.

Dr. Sznol: Absolutely.

Dr. Stadler: The complexity of doing these biopsies correctly is completely underappreciated by the clinical researcher.

Dr. Atkins: Once a drug is approved, it is hard to get patients to agree to go on a study that requires a biopsy when they can get the drug without the biopsy. So, this is a time to do it so we can learn what we need to learn to use this drug optimally.

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ANTI-ANGIOGENIC AND TARGETED THERAPY IN RENAL CANCER:
VEGF Inhibition in Renal Cell Carcinoma—What have we learned, what are we missing?
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Introduction

Whether by rational design, serendipity, or a combination of both, the last decade of targeted therapy has brought to clinical practice several treatments based on inhibiting vascular endothelial growth factor (VEGF) or its signaling receptors for patients with metastatic renal cell carcinoma (RCC). This concept has been predicated on several important differentiating features of RCC tumors, particularly those with predominantly clear cell or conventional type histology.

- First is the clinical observation that RCC tumors routinely invade and grow within vascular spaces;
- Second, that primary tumors typically, but not always, grow much larger than metastatic sites and that debulking these primary tumors improves long term survival;[1,2]
- Third, these tumors are relatively hypervascular and are commonly associated with both spontaneous central necrosis and bleeding risks;
- Most critically, that genetic alterations in the von Hippel Lindau (VHL) tumor suppressor gene are seen in the vast majority of clear cell RCC tumors.[3].

All of these features support the hypothesis that RCC tumors are unusually dependent upon their tumor microenvironment and in particular, on pro-angiogenic growth factors, most notably VEGF, in order to expand and progress. Also recognizing that, until recently, there were few reliable systemic treatment options for this patient population, it is perhaps clear why VEGF-targeted therapies have been successfully developed for this disease. This short review will focus on reported clinical data and observations for VEGF inhibition in RCC, what we have learned, and what we still need to determine in order to fully capitalize on the significant progress made to date.

Today there are two general classes of VEGF-targeted therapy that have been successfully developed for treatment of RCC patients:
- tyrosine kinase inhibitors of the VEGF receptors (TKIs) and
- bevacizumab the neutralizing monoclonal antibody to VEGF A.

While bevacizumab is specific to VEGF A isoforms, the TKIs range in their level of specificity, from one or more of the VEGF receptors and a few additional class three receptor tyrosine kinases, to multiple receptor tyrosine kinase receptors across several classes (see Table 1 for examples). However, it is difficult to assess to what extent the differences in "off target" profiles and affinities as well as pharmacokinetics explain the variations seen in clinical benefit or adverse event profile of each of these TKI agents.

Progression-free survival

Phase III clinical studies of VEGF-targeted therapies in RCC thus far have primarily demonstrated an improvement in progression-free survival (PFS). The first approvals used either a historical standard of subcutaneous interferon alpha or placebo.[4,5] For example, sunitinib demonstrated a median 11 month PFS versus 5 months for interferon alpha in untreated patients with metastatic RCC (mRCC) , while sorafenib showed a doubling of PFS (5.5 months versus 2.8 months) compared to placebo in a largely cytokine-refractory mRCC population

(4, 5). Following this, two Phase III studies investigating interferon alpha with or without bevacizumab demonstrated a significant improvement in PFS in favor of the bevacizumab arms (median PFS 10.2 versus 5.4 months for the European AVOREN study and median 8.5 months versus 5.2 months for the CALGB 90206 study).[6,7] Most recently pazopanib also demonstrated a significant PFS compared to placebo in an untreated, mRCC population (9.2 versus 4.2 months).[8] Ongoing Phase III studies of axitinib, tivozanib, and dovitinib are using sorafenib as a control arm but are still primarily focused on demonstrating an improvement in PFS (clinicaltrials.gov).

Through all of the above referenced studies, the differences seen in PFS have been robust and backed up by secondary endpoints. In terms of objective response rates (ORR), sunitinib, pazopanib, and bevacizumab plus interferon alpha have all demonstrated significant ORR > 20% (4,6-8). In addition, the duration of these responses have been statistically longer than those seen for interferon alpha alone. Across all subgroup analyses in all of these

Overall survival

Despite the robust and consistent pattern of PFS benefit demonstrated for VEGF-targeted therapies in patients with RCC, an overall survival advantage seen has not been clearly seen. All four of the first-line VEGF-targeted Phase III studies reported to date have demonstrated a trend towards an improvement in overall survival, but none have reached statistical significance. In large part this is thought to be due to subsequent treatment with other available VEGF targeted therapy.[7-10] Historically, the median survival of broadly defined patients with mRCC treated with interferon has ranged from 12 to 16 months; however, in the current studies median survival for the interferon control arms have ranged from 17.4 to 21.8 months.[9,10] Nevertheless, some secondary analyses suggest that patients who receive multiple VEGF-targeted therapies may in fact derive a much greater improvement in survival. For instance, in the AVOREN trial, patients treated with sunitinib subsequent to bevacizumab and interferon alpha had a median survival of 43.6 months, and 31.6 months for any second-line treatment in the CALGB 90206 study.[7,10]

Adverse events

Adverse events have been well documented from all these studies and affect several important organ systems including gastrointestinal, cardiovascular, dermatologic, hematologic, renal, respiratory, musculoskeletal and psychiatric, as well as constitutional symptoms. Here the route and class of VEGF-targeted therapy seem to matter. In particular, for orally administered multi-targeted TKIs the most common toxicities include gastrointestinal (diarrhea, nausea, vomiting, mucositis and dyspepsia) dermatologic (including hand foot syndrome, rash), fatigue/asthenia, hypertension, minor bleeding, elevated creatinine, liver function test abnormalities, as well as decreases in white blood cells, platelets and anemia. Some ongoing Phase III studies comparing two TKIs will help determine if one is better tolerated than another. With regards to bevacizumab toxicity in patients with RCC, it is impossible to discern completely from the AVOREN and CALGB 90206 studies how much of the toxicity profile is from bevacizumab versus the combination with interferon; however, phase II studies of bevacizumab alone suggest common toxicities are more limited to fatigue/asthenia, hypertension and proteinuria.[11,12]

Table 1. Tyrosine kinase inhibitors

Agent	Class	Route of Administration	Targets
Bevacizumab	Monoclonal antibody	intravenous	VEGF A isoforms
Sunitinib [17]	Multitargeted TKI	Oral	VEGFR 1-3 PDGFR a,b C-Kit Flt-3 RET
Sorafenib [18]	Multitargeted TKI	Oral	VEGFR 2,3 PDGFR b C-Kit Flt-3 C-RAF B-RAF
Pazopanib [19]	Multitargeted TKI	Oral	VEGFR 1-3 PDGF a,b C-Kit
Axitinib [20]	Multitargeted TKI	Oral	VEGFR 1-3 PDGFR b C-Kit
Tivozanib [21]	Multitargeted TKI	Oral	VEGFR 1-3 FMS-like tyrosine kinase
Dovitinib [22]	Multitargeted TKI	Oral	VEGFR 1-3 PDGFR b C-Kit FGFR 1-3 CSF receptor

Less common but more concerning for this class of therapy are the serious adverse events that have been seen, including potentially life threatening toxicities. Spontaneous, tumor-related and wound-related (dehiscence) bowel perforations, myocardial infarctions (MI), cerebrovascular accidents (CVA), reversible leukoencephalopathy syndrome (RPLS) and life-threatening infections have all been associated with VEGF-targeted therapies.[4-8] Thankfully the event rate for each of these is low (around 1 %) but potentially could be greater with sequential or concomitant treatment. These risks will need to be balanced as we attempt to expand the use of VEGF-targeted therapies into adjuvant settings and combination strategies.

Conclusion
VEGF-targeted therapies are effective individually at delaying disease progression and in all likelihood, at collectively extending survival. Toxicities are broad based and significant but rarely life threatening. However, despite all of the approved therapies, we see surprisingly few complete responses, and the vast majority of patients have disease progression within 2 years. At present, there is no evidence we have cured any additional patients through the use of VEGF-targeted therapy, although there are three Phase III adjuvant studies ongoing with VEGF-targeted therapy in patients with RCC to test this possibility. Despite an enormous effort in development, there is surprisingly little clinical data to help us understand mechanisms of progression in this disease.

In several other cancer types, it is clear that inhibition of a pathway activated by a dominant genetic alteration not only results in improved clinical benefit but also in clear mechanisms of resistance that are selected for within that same signaling pathway. Termed "oncogenic addiction" these cancer types appear to depend upon this critical path not just for primary progression, but for secondary progression even in the setting of prolonged inhibition. Some of these mechanisms have

been clearly elucidated, as in the case of c-Kit mutations in gastrointestinal stromal tumors (GIST) or bcr-Abl translocations in

chronic myelogenous leukemia (CML); however, other circumstances may be more subtle, but no less addicted. In the case of prostate cancer, the androgen receptor (AR) signaling pathway is frequently activated in castrate resistant prostate cancer and appears to be important to cancer progression, as evidenced by the clinical effects of secondary inhibition of androgen biosynthesis or potent AR inhibition.[15,16]

To what extent VEGF inhibition in RCC results in some of these same patterns of resistance is not known. However, early observations that patients may derive clinical benefit from sequential approaches to VEGF pathway inhibition suggests that either RCC has some elements of VEGF addiction or, at the least, an incomplete mechanism of resistance. What is missing is data with regard the molecular and genomic profiles of resistance to VEGF inhibition and the effect of combined VEGF blockade. Early efforts to combine VEGF-targeted therapy have been associated with unacceptable toxicity (reviewed below) but these efforts should not be disregarded. Strategies evaluating complete VEGF blockade are needed test whether we can achieve durable, complete responses in patients with RCC with VEGF-targeted therapies.

Discussion
Dr. McDermott: Should sorafenib be a drug that experts advocate for use in RCC if all these trials prove that second-generation drugs are more active?
Dr. George: Well, interferon is still FDA approved.
Dr. McDermott: I am not saying remove it from the market, but a general oncologist could think it does not really matter what you use

or when you use it because it is hard to predict benefit and toxicity with these drugs, so you could try whatever you like and if something doesn't work you just go to the next one.

Dr. George: I think we need to begin to understand that RCC can be thought of as a number of different subsets, then begin to figure out profiles for each subset to move forward in a rational manner. Otherwise we are stuck with the NCCN guidelines of just a sheet of recommendations and then lots of Level 2, 3 recommendations to follow.

Dr. McDermott: Right. Recent ASCO data indicate that some groups should perhaps be on a warning list for referral – those people with rapid progression. You should be thinking about clinical trials in those groups of people up front.

Dr. George: We need to think of that subset a little differently They are difficult to study because they do have a very short survival. Identifying those patients even before they fail sunitinib or other front-line therapy would be ideal for planning second line therapy, because if you try to capture them after the fact, you are likely dealing with serious clinical issues such a cord compression and brain metastases that make it difficult to get them eligible for a clinical trial. So that is where I think knowing upfront what their prognosis is and what drug would work best for them would be really helpful.

We have said you cannot combine MTOR with VEGF inhibitors. People have said this is dangerous, and there is no added benefit to the combination. But have we really explored all the combinations? Could the more selective VEGF pathway inhibitors combine better with mTOR inhibitors? What about the dual Tor inhibitors? So maybe we are close and we just have not done the right two combinations on the first pass.

Dr. McDermott: I think we need to think about the biology in order to rationally develop combinations.

Dr. Rathmell: Yes, I agree we are very close, but we are need to understand what causes resistance because ultimately everybody gets resistance to these drugs.

Dr. Atkins: I just want to clarify your statement that people whose disease progresses rapidly on VEGF pathway inhibitors tend to also exhibit rapid disease progression on an mTOR inhibitor. That has not been our experience. I think there is a subset of people who progress rapidly VEGF pathway inhibitors whose disease can respond extremely well to a TOR inhibitor. Thus, this represents an opportunity to possibly tease out at least two populations who might get a different targeted therapy first.

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VEGF Inhibition in Renal Cell Carcinoma—What have we learned, what are we missing?

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ANTI-ANGIOGENIC AND TARGETED THERAPY IN RENAL CANCER:
Targeting the mTOR Pathway in Renal Cell Carcinoma
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Introduction

The mammalian Target of Rapamycin (mTOR) is the second molecular target (after VEGF signaling) for which small molecule inhibitors have been developed and shown to have significant clinical activity in patients with advanced renal cell carcinoma (RCC). The mTOR inhibitor temsirolimus is now FDA-approved for the first-line treatment of patients with RCC. It's structural analogue everolimus is similarly approved as second line therapy for RCC patients who have failed treatment with sunitinib and/or sorafenib.[1,2] Responses to these agents, however, remain limited to a subset of patients and all patients treated with these drugs eventually develop progressive disease. In this article, we will review the clinical experience with mTOR inhibitors in RCC, relevant class-specific toxicities, and future clinical directions. We will also discuss possible novel strategies to target this signaling pathway.

Clinical experience with mTOR inhibitors in patients with RCC

Temsirolimus and everolimus have both demonstrated clinical efficacy in large randomized phase III trials in patients with advanced RCC. After showing promising activity in a phase II trial randomizing patients with metastatic RCC to three different doses,[3] temsirolimus was assessed in a randomized three-arm Phase III trial comparing temsirolimus alone versus interferon-α (IFN-α) alone versus the combination of both.[1] As the phase II study suggested potentially unique efficacy in patients with poor prognostic features, the phase III study chose to focus on patients with metastatic RCC and ≥3 of 6 risk factors; (5 MSKCC risk factors + >1 metastatic site). Overall, 626 previously untreated patients were enrolled and randomized in a 1:1:1 fashion to receive IFN-α alone, temsirolimus alone, or the combination. The overall survival of patients treated with temsirolimus alone was statistically longer than those treated with IFN-α alone (7.3 versus 10.9 months; 0.73 hazard ratio, p=0.0069). There was no statistical difference between patients treated with IFN-α alone and the combination of IFN-α and temsirolimus. Temsirolimus was thus the first molecularly targeted agent to demonstrate a statistically significant survival benefit in first-line therapy of patients with metastatic RCC. Accordingly, temsirolimus was approved by the FDA for therapy in advanced RCC on May 30, 2007 and is now considered a standard first-line therapeutic option for patients with poor prognostic features.

While temsirolimus was assessed in untreated patients, everolimus was assessed in a randomized, double-blind, placebo-controlled phase III in patients with advanced RCC who had failed prior treatment with either sorafenib, sunitinib, or both within the preceding 6 months (REnal Cell cancer treatment with Oral RAD001 given Daily-1 [RECORD-1]).[2] Overall, 416 patients were enrolled and randomized in a 2:1 fashion to receive either everolimus (n=277) or placebo (n=139) each together with best supportive care. The primary endpoint was PFS as randomization was unblinded at time of progression and patients on placebo were allowed to crossover to open-label everolimus, confounding any potential differences in overall survival. The trial was halted at the second interim analysis after 191 progression events had been observed. At the final central radiology assessment the median PFS for patients treated with everolimus was 4.88 months as compared with 1.87 months in the placebo group (hazard ratio 0.33, [95% CI 0.25-0.43] p < 0.0001).[4] Five patients (2%) in the everolimus group experienced partial responses vs. none in the placebo group. Based on these results, everolimus was approved the FDA in March, 2009 for the treatment of patients with advanced RCC who failed either sorafenib, sunitinib or both.

Important toxicities of mTOR inhibitors

Although in general well tolerated, treatment with either everolimus or temsirolimus can be associated with many of the same side-effects observed with the VEGF-targeted TKIs. These most commonly include rash, nausea, diarrhea, stomatitis/mucositis, cytopenias, and fever. However the rapalogues can also induce toxicities which are distinct from those seen with VEGF pathway targeted therapies in RCC and are worthy of specific discussion. These toxicities include pneumonitis, endocrine abnormalities and the possibility of immunosuppression.

Pneumonitis.

Pneumonitis has been observed with all the rapalogues and appears to be a class effect of the allosteric inhibitors of mTOR.[5] The exact incidence of this toxicity seems to vary widely from study to study. In the phase III trials of temsirolimus and everolimus discussed above, the incidence of pneumonitis was fairly low, with incidences of only 2% and 8%, respectively, of any grade event.[2,6] However, in a retrospective study in patients with non-small cell lung cancer, White et al reported that 16 (25%) of 64 patients examined showed radiographic evidence of pneumonitis which was felt to be attributable to everolimus.[7] Other studies have suggested that pneumonitis from TORC1 inhibitors may be more common in patients with pre-existing pulmonary conditions.[8] Pneumonitis may be more commonly appreciated radiographically, where it most frequently presents as ground glass-opacity and occasionally as parenchymal consolidations and pleural effusion, than clinically. When symptoms are present, most patients experience dypsnea on exertion and cough, occasionally accompanied by fever, malaise, and hypoxia. While many mechanisms have been proposed, including cell-mediated auto-immunity and T-cell-mediated delayed-type hypersensitivity,[4,9] the exact molecular basis for this toxicity remains unknown. Although there are currently no specific guidelines to the management of rapalogue-associated pneumonitis, other etiologies, particularly infectious, should be first excluded. Most investigators appear to agree that treatment should be held in patients with overt symptoms attributable to pneumonitis and a brief course of steroids may be considered. Treatment resumption, usually at a lower dose, may be considered following resolution of symptoms. There does not appear to be consensus for patients with only radiographic findings of pneumonitis, but continuing therapy with careful observation or lowering the dose appear to be common interventions.

Endocrine side effects.

Treatment with rapalogues has also been associated with several endocrine abnormalities, namely hyperlipidemia and hyperglycemia. These toxicities appear quite common in patients with RCC treated with either temsirolimus or everolimus. In the phase III trial of temsirolimus, the incidence of hypercholesterolemia, hyperlipidemia and hyperglycemia in patients treated with temsirolimus alone was 24%, 27%, and 26% respectively.[1] In the RECORD-1 study, the incidence of hypercholesterolemia, hypertriglyceridemia, and hyperglycemia in patients treated with everolimus was 76%, 71%, and 50%, respectively.[2] Studies with rapamycin suggest that the

hyperlipidemia (observed as elevations in HDL, LDL, cholesterol, and triglycerides) induced by rapalogues is due to reduced catabolism of lipoprotein particles.[10] While this toxicity is quite common and therefore requires continuous monitoring, rapalogue-induced hyperlipidemia is usually manageable with statins or gemfibrozil (for hypertriglyceridemia) and typically does not require treatment cessation. Similarly, animal studies with rapamcyin have shown that hyperglycemia is a direct side effect of treatment with rapalogues due to enhancement of insulin resistance and reduction of β-islet cell mass and function.[11] Therefore, monitoring of fasting glucose levels is recommended for all patients treated with mTOR inhibitors, particularly those with pre-existing diabetes. Therefore initiation of oral anti-glycemic agents or escalation of current diabetic regimen may be indicated.

Immunosuppression.
As the rapalogues were developed first as immunosuppressive agents in the transplant setting, treatment with these agents has always raised concerns regarding the potential for immunosuppression in cancer patients. Recent studies have suggested that rapamycin may actually enhance the immune response to infections by both enhancing the CD8+ T-cell response and by increasing the differentiation of effector cells into potent memory T-cells.[12,13] Nonetheless, the reported incidence of infection in patients treated with either temsirolimus or everolimus in phase III trials was higher than that for their respective control arms (27% in patients treated with temsirolimus alone versus 14% in those treated with IFN; 10% in patients treated with everolimus versus 2% in those treated with placebo).[1,2] Therefore, the issue of whether rapalogues may be immunosuppressive cannot be considered to be completely resolved. Although current data does not support the use of antibiotic prophylaxis, clinical vigilance is recommended to the possibility of increased frequency of infections, particularly in those patients with pre-existing chronic viral infections or immunosuppressive conditions. In particular, recent reports filed through Medwatch, have indicated that treatment with everolimus may trigger the activation of hepatitis B in patients with a history of resolved or inactive hepatitis B.[14] In these patients, initiation of anti-hepatitis medication such as lamuvidine is recommended prior to the initiation of everolimus.

Future clinical directions
Although both temsirolimus and everolimus are approved by the FDA for the treatment of patients with advanced RCC, the role of these TORC1 inhibitors will likely continue to evolve as many questions regarding their efficacy in specific therapeutic situations are addressed. Both agents are being studied or considered in multiple other clinical scenarios and therapeutic strategies including sequential therapy with VEGF pathway inhibitors, combinational regimens the adjuvant setting, and in patients with non-clear cell histology.

Sequential therapy.
 Multiple retrospective analyses have suggested that there is no true cross-resistance for VEGF pathway and mTOR inhibitors given in sequence.[15,16] Investigators have therefore proposed to examine specific sequences of novel agents given as single agents in an effort to identify a particular sequence of agents that may result in maximal duration of disease control while perhaps also minimizing toxicity. With respect to everolimus, this is specifically being examined in the RECORD-3 trial, a large phase II trial in which previously untreated patients with metastatic clear cell RCC will be randomized to receive either first-line everolimus followed by second-line sunitinib or first-line sunitinib followed by second-line everolimus. Similarly,

temsirolimus is currently being investigated in a phase III trial versus sorafenib in patients who have failed initial therapy with sunitinib.

Combinational therapy.
Given the distinct targets of recently approved treatments for patients with RCC (i.e. inhibition of VEGF signaling vs. inhibition of mTOR), there has been considerable interest in whether combinations of these two classes of agents may lead to additional therapeutic efficacy. Perhaps the most studied approach thus far involves the combination of TORC1 inhibitors with bevacizumab. The combination of temsirolimus and bevacizumab showed encouraging efficacy in a phase II trial in patients with advanced RCC who have failed VEGF-targeted TKI therapy with an overall response rate of 16%.[17] However, in a separate randomized phase II study in which untreated RCC patients were randomized to receive either the combination of temsirolimus and bevacizumab, sunitinib, or the combination of bevacizumab and IFN-α, the response rates in the individual arms were 25%, 24%, and 34%, respectively.[18] These results, combined with the observation of significant premature treatment stoppage in the temsirolimus-bevacizumab arm (43%), have raised questions about both the additive efficacy and the toxicity of this combination. Nonetheless, the combination is also actively being assessed in multiple larger trials including in an arm the Eastern Cooperative Oncology Group (ECOG) Trial 2804 and in a large phase III trial in which patients are randomized to the combination of either temsirolimus and bevacizumab or bevacizumab and IFN-α (INTORACT Trial), so more information should be forthcoming.

Similar combinational studies with everolimus are also underway. A phase II trial of the combination of everolimus and bevacizumab produced five partial responses (17%) and a median progression-free survival of 11 months in 29 patients who had received prior VEGF receptor TKI therapy.[19] This data, plus the desire to examine the role of maintained VEGF pathway blockade following sunitinib or sorafenib resistance, has led the CALGB to propose an intergroup phase III trial randomizing patients whose disease has progressed following sorafenib and/or sunitinib to either everolimus alone or the combination of everolimus and bevacizumab.

Adjuvant therapy.
Although there are no therapies approved for the adjuvant treatment of patients with high-risk RCC, the recent approval of multiple therapies in the metastatic setting has prompted the assessment of these agents in the adjuvant setting. Studies involving sorafenib and/or sunitinib are currently underway and anticipated to reach accrual goals in the near future, but mature results are not envisioned for several years. In particular, the efficacy of everolimus in patients with metastatic RCC, together with its novel mechanism of action, favorable toxicity profile and oral administration make it an attractive agent to also test in the adjuvant setting. Accordingly, a large randomized placebo controlled phase III trial is being planned within the U.S. Intergroup mechanism to formally assess the role of adjuvant everolimus in patients with resected high-risk RCC.

Non-clear cell RCC.
Although the efficacy of TORC1 inhibitors has primarily been established in clear cell RCC, further analysis of the pivotal phase III trial leading to the FDA approval of temsirolimus suggested this TORC1 inhibitor may be even more effective compared with interferon in patients with non-clear cell RCC than clear cell RCC.[20] The median overall survival of temsirolimus versus interferon was 11.6 vs. 4.3 months in patients with non-clear cell histology (75% of which were of papillary sub-type) compared with 10.7 vs. 8.2 months

in patients with clear cell RCC. The possibility that TORC1 inhibitors in general may have unique efficacy in non-clear cell RCC has prompted the initiation of a randomized phase II trial of temsirolimus versus sunitinib in European patients with metastatic non-clear cell RCC. Likewise, everolimus will also be studied in a phase II trial in 60 European patients with metastatic papillary RCC (RAPTOR Trial [RAD001 in Advanced Papillary Tumor Program in Europe]). These two phase II trials should provide critical information regarding the efficacy of TORC1 inhibitors in patients with non-clear cell histology RCC.

Beyond first generation mTOR inhibitors
Despite the established efficacy of temsirolimus and everolimus in RCC, only a subset of patients with advanced RCC experience substantial clinical responses from treatment with these agents. Furthermore, these clinical responses are neither complete nor durable off therapy and all patients will eventually experience disease progression. The efficacy of these allosteric inhibitors of mTOR may be limited in part because they primarily inhibit the function of TORC1, the complex including mTOR and raptor, and have less activity against TORC2, the complex including mTOR and rictor (rapamycin insensitive companion of TOR). Recent studies have suggested that the expression of Hypoxia Inducible Factor (HIF)-2α, argued by many to be the more relevant HIF in RCC, is dependent almost completely upon TORC2 and largely independent of TORC1 function.[21] Furthermore, some pre-clinical studies have suggested that inhibition of TORC1 can lead to activation of signaling pathways upstream of mTOR including those mediated by phophatidy-linositol 3-kinasee (PI3-K) and Akt (Protein Kinase B).[22,23] As PI3-K and Akt activate numerous kinases, transcription factors and other proteins associated with cell growth and survival in addition to mTOR, persistent activation of these pathways might undermine the efficacy of TORC1 inhibition.

Not surprisingly, a new generation of agents targeting the PI3-K/Akt/mTOR pathway is in active clinical development. Inhibitors which directly inhibit the kinase function of mTOR, and thereby suppress the activity of both TORC1 and TORC2, are now entering clinical assessment. Given the aforementioned dependence of HIF-2α expression on TORC2, these direct mTOR kinase inhibitors would have the advantage of inhibiting the expression of both HIF-1α and HIF-2α. The possibility of rapalogue-induced feedback activation of PI3-K/Akt has made the dev-elopment of inhibitors of these upstream kinases an attractive strategy and many such agents are now in clinical development. Whether the many theoretical advantages of PI3-K/Akt or direct mTOR kinase inhibitors translates into superior clinical efficacy in patients with advanced RCC, however, remains to be seen.

Conclusion
With the recent FDA approvals of both temsirolimus and everolimus for the treatment of patients with advanced RCC, TORC1 inhibitors have now joined the antagonists of VEGF signaling and non-specific immune-therapies in a crowded therapeutic field in RCC. Despite these dev-elopments, however, only a subset of patients with RCC experience substantial clinical responses following treatment with TORC1 inhibitors. Therefore, efforts must continue to explore mechanisms of resistance to these agents to aid in the development of more effective agents directed against this critical pathway. Efforts must also focus on identifying predictive biomarkers of response to the rapalogues in order to develop more effective patient selection strategies. Through these efforts, the role of mTOR inhibitors in RCC therapy will almost certainly continue to evolve as it enters clinical assessment in a multitude of clinical settings including sequential,

combinational, and adjuvant therapy as well as in patients with non-clear cell RCC

Discussion
Dr. Atkins: Are the dual TOR inhibitors or PI3-kinase inhibitors that you discussed working by a different mechanism than the VEGF inhibitors in treating the kidney tumors?
Dr. Cho: Our preclinical work suggests they are working by a different mechanism; we do not see any evidence of an anti-angiogenic effect and yet we see diminished proliferation and diminished tumor growth.
Dr. Kaelin: Well, frankly I will be surprised if they are not working in part by blocking angiogenesis. Certainly many models, many of which are based on preclinical experiments including using genetically defined mice and zebra fish, would suggest that the PI3Kinase, mTOR pathway should be important for angiogenesis.
Dr. Stadler: We have focused on the tumor. I mean on the cancer cell itself. We cannot forget that the mTOR pathway, especial-ly the TOR-1 pathway, is critically important in the immune system as well and that these–at least the rapalogues–are potent immune suppressive agents. So what do you know about the TOR-1 /TOR-2 inhibitors and their affect on various components of the immune system?
Dr. Cho: From Phase 1 trials we have not observed significant opportunistic infections, but those are things that we will keep an eye out for.
Dr. Stadler: But it is clearly known that these drugs produce fairly profound immune suppression.
Dr. Atkins: Right. By the way, it is interesting that we are seeing benefits with mTOR inhibitors in some patients with aggressive tumors. That fact that an approach associated with immunosuppression works against these tumors, perhaps changes the discussion about where you would want to test immunotherapies such as the PD1 antibody.
Dr. Choueiri: It looked like your interpretation of MTOR inhibition was that it was really primarily through S-6 kinase down-regulation that you are suggesting this mechanism.
Dr. Cho: I think clinically you could argue that those who benefit are have sufficiently high blood levels to also inhibit TORC-2 to some extent.
Dr. Atkins: Once again, the concept that different tumors respond better to different treatments puts a premium on biomarker studies.
Dr. Kaelin: I think one other thing we could think about going forward is whether autophagy plays a role here. Just to spice things up, another gene on 5Q is a gene involved in autophagy

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Targeting the mTOR Pathway in Renal Cell Carcinoma
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ANTI-ANGIOGENIC AND TARGETED THERAPY IN RENAL CANCER:
Molecularly Targeted Therapy in Renal Cell Carcinoma: Sequential versus Combination-based Therapies
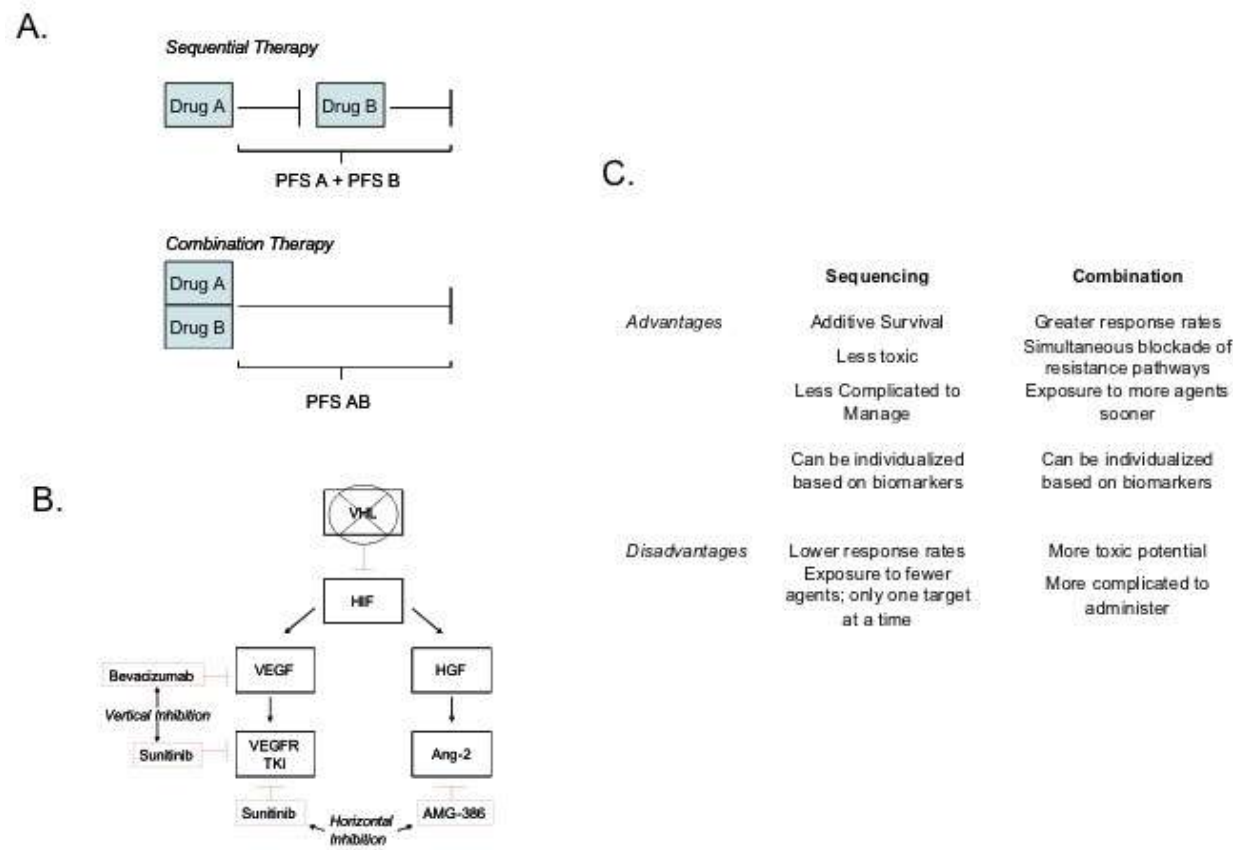
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Introduction
Metastatic clear cell renal cell carcinoma (mRCC) is a cancer with a complex molecular pathogenesis that has been exploited for drug development.[1] Inhibition of tumor angiogenesis, through targeting the VEGF pathway, has been an effective approach in mRCC.[2] Four VEGF pathway inhibitors, sunitinib, pazopanib, sorafenib, and
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bevacizumab, have been approved.[3-10] Blocking the mTOR pathway, which plays a role in tumor cell survival and proliferation, has also been an effective therapeutic approach and mTOR inhibitors currently approved for patients with RCC include, temsirolimus and everolimus.[11-13] All of these agents have improved survival endpoints (PFS or OS), however, complete responses are rare and patients still succumb to their cancer. In an effort to improve upon clinical outcomes, two trial design strategies are being explored: sequential therapy and combination therapy (Figure 1A, B). Select prospective data and a highlight of ongoing trials evaluating these methods will be presented in this review

Figure 1

Clinical Trials Involving Sequential Use Targeted Agents
Multiple studies have been published evaluating the use of sequential targeted agents for the treatment of mRCC (Table 1). Given the available classes of agents, two approaches have been examined, sequential use of VEGF pathway inhibitors and sequential use of VEGF and mTOR inhibitors.

VEGF inhibitor → VEGF inhibitor. Cross resistance between VEGF inhibitors appears uncommon. This has been shown in several retrospective studies, and validated in other prospective trials. A recently published study explored sorafenib therapy following prior front-line sunitinib.[14] This study showed continued benefit of VEGFR tyrosine kinase inhibition in this cohort (ORR, 9.6%, median TTP 16

weeks, OS 32 weeks). Another trial examining the use of sorafenib following either front-line bevacizumab or sunitinib, showed similar results (tumor shrinkage rate 38%, PFS 3.8 months).[15] Sunitinib also has been evaluated in the second-line setting following bevacizumab. In this phase II study, sixty-two patients were treated with second-line sunitinib therapy with a ORR of 23% and a PFS of 7.0 months.[16] Axitinib, a highly potent and selective VEGF inhibitor, has been explored following sorafenib failure. Sixty-two patients were treated in this manner and an ORR of 22.6%, PFS of 74%, and OS of 13.6 months were noted.[17] In summary, numerous trials have shown that sequential use of VEGF inhibitors can result in meaningful responses and continued clinical benefit.

Table 1. Trials of Molecularly Targeted Agents in Sequence or Combination

Sequencing or Combination	Authors/Trial Name	No.	Comparator Arm	Results or Primary Endpoint
Sequencing Trials				
Sorafenib->Axitinib	Rini, et al [17]	62	Single arm	ORR 22.6%, PFS 7.4 mos., OS 13.6 mos.
Sorafenib->Sunitinib	Zimmerman, et al [24]	22	Single arm	ORR 18%, PFS 4.8 mos.
Sunitinib->Sorafenib	DiLorenzo, et al [14]	52	Single arm	ORR 9.6%, TTP 16 wks, OS 32 wks
Sunitinib->ABT869(VEGF TKI)	Tannir, et al [25]	53	Single arm	ORR 18.1%, PFS 4.9 mos.
Bevacizumab->sunitinib	Rini, et al [16]	61	Single arm	ORR 23%, PFS 7 mos
Bevacizumab or sunitinib ->sorafenib	Shepard, et al [15]	31	Single arm	ORR 0%, PFS 3.8 mos.
VEGF TKI ->everolimus	Motzer, et al/ RECORD-1 study [12,13]	410	placebo	PFS 4.9 v. 1.9 mos.
VEGF inhibitor +/-mTOR i -> perifosine	Vogelzang, et al [26]	45	Single arm	ORR 9%, PFS 15 wks
Upcoming or Ongoing Sequencing Trials				
Sunitinib ->everolimus	RECORD-3 study	390	Everolimus->sunitinib	PFS, noninferiority
Sunitinib, bevacizumab, temsirolimus, or cytokine ->axitinib	Axis study	650	Sorafenib	PFS
Sunitinib->sorafenib	Switch study	540	Sorafenib->sunitinib	total PFS
Sunitinib->Temsirolimus	Torisel 404 study	480	Sunitinib->sorafenib	PFS
Combination Trials				
Bevacizumab + sorafenib	Sosman, et al [19]	47	Single arm	ORR 46%, TTP 11.2 mos.
Bevacizumab + temsirolimus	Merchan, et al [27]	35	single arm	ORR 16%
Bevacizumab + erlotinib	Hainsworth, et al [22]	59	Single arm	ORR 25%, PFS 11 mos.
Bevacizumab + erlotinib + imatinib	Hainsworth, et al [23]	88	Single arm	ORR 17%
Bevacizumab + everolimus	Hainsworth, et al [20]	80	Single arm	ORR 28%, PFS 8.1 mos.
Bevacizumab + temsirolimus	Escudier, et al [21]	88	Sunitinib	NPR at 48wks: 30.7%(BT), 40.5%(S), 65.9%(BI)
			Bevacizumab/ interferon	PFS: 8.2 mos (BT), 8.2 mos. (S), 16.8 mos. (BI)
Bevacizumab + sunitinib	Feldman, et al [18]	26	Single arm	ORR 52%
Bevacizumab + IL-2	Dandamudi, et al [28]	51	Single arm	ORR 28%, PFS 9 mos.
Upcoming or Ongoing Combination Trials				
Bevacizumab + sorafenib	BeST trial	360	bevacizumab	PFS
Bevacizumab + temsirolimus				
Temsirolimus + sorafenib				
Bevacizumab + temsirolimus	INTORACT trial	800	bevacizumab/interferon	ORR and Survival
AMG 386 + sorafenib	Amgen	150	Sorafenib	PFS
AMG 386 + sunitinib	Amgen	80	Phase II	Safety and tolerability
BNC105P + everolimus	Bionomics	152	everolimus	Phase I: MTD Phase II: 6-mo PFS

Table 1: Prospective studies evaluating sequencing and combination targeted therapies. Legend: BI, bevacizumab/interferon; BT, bevacizumab+temsirolimus; MTD, maximum tolerated dose; NPR, non-progression rate; ORR, overall response rate; OS, median overall survival; PFS, median progression-free survival; S, sunitinib; TTP, median time-to-progression.

VEGF inhibitor → mTOR inhibitor.
The benefit of using mTOR inhibitors following VEGFR TKIs has also been demonstrated. In the randomized phase III RECORD-1 trial, 410 mRCC patients who had failed at least one prior VEGF TKI were randomized to everolimus or placebo. Everolimus demonstrated a superior PFS compared to placebo (4.9 v. 1.9 months).[12] The findings from this trial led to the approval of everolimus for patients with mRCC who have failed prior VEGFRTKI therapy. Although inhibition of the mTOR pathway in patients who have progressed on VEGFR TKIs is a reasonable approach, the optimal sequencing approach, VEGF inhibitor → VEGF inhibitor or VEGF inhibitor → mTOR inhibitor, currently remains to be seen.

Ongoing or Upcoming Sequencing Trials.
There are several ongoing trials which are focusing on the question of sequencing of targeted agents in mRCC (Table 1). The RECORD3 trial is a randomized, open-label, multicenter phase II trial that will evaluate the efficacy of everolimus followed by sunitinib compared to sunitinib followed by everolimus in treatment naive mRCC patients. Another study, known as the AXIS trial, will compare axitinib versus sorafenib for patients with mRCC who have received either sunitinib, bevacizumab (plus interferon), temsirolimus, or cytokine therapy in the front-line setting. In a trial evaluating the optimal sequencing of sunitinib and sorafenib, the SWITCH trial will randomize patients with treatment naive mRCC to sorafenib followed by sunitinib compared with sunitinib followed by sorafenib. Finally, the TORISEL 404 is comparing second-line sorafenib compared to second-line temsirolimus in patients with mRCC who have progressed on first-line sunitinib. The results of these studies will help to further delineate the optimal sequencing of targeted agents for patients with mRCC.

Clinical Trials involving Combination Targeted Therapies
Unlike the sequencing approach, the goal of combination therapy is to provide an additive or synergistic anti tumor effects including enhanced tumor shrinkage or a more durable response. Several studies have been performed evaluating combination regimens for patients with mRCC patients (Table 1). Although several trials have shown promising clinical activity, this has often been offset by increased toxicity or low clinical activity.

Vertical Inhibition.
Efforts to target the same pathway at two different points have focused on the VEGF pathway, which appears to be the most critical, targetable pathway in RCC to date. A phase I study of bevacizumab and sunitinib was recently reported. In this study 26 patients were treated, with an ORR of 52% which is higher than that expected with either agent alone.[18] The combination resulted in an increased frequency of grade 3 or 4 hypertension, proteinuria and thrombocytopenia. Many patients (48%) had to come off of the trial due to adverse events and several patients developed microangiopathic hemolytic anemia or reversible posterior leukoencephalopathy syndrome. A similar phase I trial combining bevacizumab and sorafenib (nG) demonstrated an ORR of 46% and a median time to progression of 11.8 months.[19] Of note, in order for the combination to be tolerable, lower doses of both agents were required.Although the combination of VEGF inhibitors has provided some of the highest response rates seen in studies, the accumulation of toxicity outweighs the benefit.

Horizontal Inhibition.
Horizontal inhibition has the potential advantage of combining agents with non-overlapping toxicities with a goal of an additive or synergistic effect. This method has been employed in several prospective studies (Table). Two phase II studies evaluating the combination of VEGF inhibition and mTOR inhibitors have recently been reported. In one study, 80 patients with metastatic RCC were treated with a combination of bevacizumab and everolimus.[20] The ORR was 28% and median PFS of 8.1 months, similar to findings seen with bevacizumab alone. Grade 3 and 4 toxicities were higher than would be anticipated with each agent alone. The phase II TORAVA study compared the combination bevacizumab/temsirolimus (BT, n=88) versus sunitinib (S, n=42) versus bevacizumab/interferon (BI, n=40).[21] The primary endpoint was non-progression rate (NPR) at 48 weeks. Results from this trial showed no benefit from the bevacizumab/temsirolimus combination compared to the other arms (NPR at 48 wks: BT: 30.7%, S: 40.5%, BI: 65.9%), but did show increased grade 3 or 4 toxicities (BT: 38.5%, S: 14.3%, BI: 27.5%). The BT combination arm was associated with three deaths compared to

none on the comparator arms. Other attempts at horizontal inhibition have been made including bevacizumab combinations with other agents such as epidermal growth factor receptor inhibitors or c-kit/pdgfR inhibitors.[22,23] These studies have failed to show clinical benefit over expected outcomes with bevacizumab alone.

Ongoing or Upcoming Combination Trials.
There are a variety of trials which are ongoing with some further exploring the VEGF/mTOR combination (e.g. BeST trial, Intoract trial) while others are evaluating new combinations (Table 1). AMG-386 is a unique agent which inhibits angiopoetin, which is an important pro-angiogenic molecule and a potential escape pathway during VEGF inhibition. AMG-386 is being studied in two different front-line combination trials for patients with RCC (paired with sunitinib or sorafenib). Another unique agent, BNC-105P, is a vascular disrupting agent, which is currently being combined with everolimus in a phase I/II trial for patients who have progressed on prior VEGFR tyrosine kinase inhibitors. At present, it remains to be seen if these novel combinations will produce more meaningful effects than sequential use; however, the benefit of a combination approach will require careful consideration of any additive toxicity impact the regimen produces.

Conclusion
The management of patients with RCC has changed dramatically with the introduction of six active molecularly targeted agents. Current approaches to improve survival endpoints include the serial use of agents or combination approaches. Both methods can potentially curtail escape mechanisms within the tumor and thus further extend anti-tumor effect. Although each approach has its potential advantages and disadvantages, it is unclear which is optimal. (Figure 1C)

At present, the combination of VEGF and mTOR inhibitors appears to compromise tolerability with no additional clinical benefit. The greatest improvement in response rate appears to be in trials which have implemented vertical inhibition of the VEGF pathway; however, this application also has the most toxic profile. For a combination approach to be reasonable, it will need to have a significantly longer PFS than the serial use of the two agents and be tolerable. Clinical trials are underway which will evaluate different sequencing and combination approaches which will hopefully shed light on the best management and further advance survival endpoints for patients.

The future of RCC patient management remains promising; however, as more active agents are identified their optimal application remains an ever growing challenge. It is imperative to identify and incorporate robust molecular biomarkers which will enable individualization of therapy for patients with RCC. With proper identification of patients for unique combinations or serial use of molecularly-targeted agents, greater strides forward in advancing survival outcomes will hopefully be made.

Discussion
Dr. Stadler: Are we doing any trials that really look at adding an agent at the time of progression?
Dr. Hutson: Not that I am aware of.
Dr. Atkins: Companies are reluctant. I think part of it is you need to have a combination that is tolerated first before you can feel comfortable adding an agent at progression. And, if it is a tolerable combination, why not just start it to begin with and compare it to the single agent?

Dr. George: A fundamental question that I do not think that we have addressed is whether continued VEGF pathway inhibition is important in this disease.

Dr. Atkins: Well, that is why that CALGB trial of everolimus with or without bevacizumab in VEGFR TKI resistant patients is so important. It will start before the end of the year.

Dr. George: Let me just ask one thing because this seems like a tremendous amount of work. You look at all the different randomized combination studies, thousands of patients, and are we going to learn anything from any of these using PFS as our only endpoint?

Dr. Hutson: I am worried that we are not going to learn much. I fear that we will enroll thousands of patients, and spend millions of dollars, but at the end of the day these PFS values are not going to be so dramatically different that we are going to conclude anything.

Dr. George: We control the patients. So should we be enrolling patients to these kinds of studies? Or should we say look, we need to do something different that actually addresses a key clinical or scientific question? That actually advances the field?   Dr. Atkins: I think we will learn some things, but not what the pharmaceutical companies want. If we have the tumors, we can learn from the RECORD-3 trial whether different populations respond to TOR inhibitions than to VEGF pathway inhibitors, because it is the only head-to-head first-line comparison of these two strategies. The clinical endpoints may be irrelevant to that question. We may also learn with axitinib versus sorafenib or tivozanib versus sorafenib, to what extent hitting the VEGF pathway harder first-line influences the impact of subsequent therapies. This appeared to be the case in the RECORD -1 study, where everolimus appeared to be less effective in patients who had received prior sunitinib than those who had received prior sorafenib. Also what happens when we start giving therapies that hit other targets in the second line setting? Does the degree of VEGFR inhibition in the front line make a difference? But these are all ancillary questions that need to be looked at in the context of those trials, not the primary aims of those trials, so we will need to be vigilant and persuasive to ensure that they get addressed and not swept under the rug, if the trial does not achieve its primary objective.

Dr. Stadler: Unfortunately, most of these trials are not powered for any of those secondary endpoints, so I worry that they are not likely to provide definitive answers.

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Molecularly Targeted Therapy in Renal Cell Carcinoma: Sequential versus Combination-based Therapies

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ANTI-ANGIOGENIC AND TARGETED THERAPY IN RENAL CANCER:
Targeting the mTOR Pathway in Renal Cell Carcinoma

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Introduction

The mammalian Target of Rapamycin (mTOR) is the second molecular target (after VEGF signaling) for which small molecule inhibitors have been developed and shown to have significant clinical activity in patients with advanced renal cell carcinoma (RCC). The mTOR inhibitor temsirolimus is now FDA-approved for the first-line treatment of patients with RCC. It's structural analogue everolimus is similarly approved as second line therapy for RCC patients who have failed treatment with sunitinib and/or sorafenib.[1,2] Responses to these agents, however, remain limited to a subset of patients and all patients treated with these drugs eventually develop progressive disease. In this article, we will review the clinical experience with mTOR inhibitors in RCC, relevant class-specific toxicities, and future clinical directions. We will also discuss possible novel strategies to target this signaling pathway.

Clinical experience with mTOR inhibitors in patients with RCC

Temsirolimus and everolimus have both demonstrated clinical efficacy in large randomized phase III trials in patients with advanced RCC. After showing promising activity in a phase II trial randomizing patients with metastatic RCC to three different doses,[3] temsirolimus was assessed in a randomized three-arm Phase III trial comparing temsirolimus alone versus interferon-α (IFN-α) alone versus the combination of both.[1] As the phase II study suggested potentially unique efficacy in patients with poor prognostic features, the phase III study chose to focus on patients with metastatic RCC and ≥3 of 6 risk factors; (5 MSKCC risk factors + >1 metastatic site). Overall, 626 previously untreated patients were enrolled and randomized in a 1:1:1 fashion to receive IFN-α alone, temsirolimus alone, or the combination. The overall survival of patients treated with temsirolimus alone was statistically longer than those treated with IFN-α alone (7.3 versus 10.9 months; 0.73 hazard ratio, p=0.0069). There was no statistical difference between patients treated with IFN-α alone and the combination of IFN-α and temsirolimus. Temsirolimus was thus the first molecularly targeted agent to demonstrate a statistically significant survival benefit in first-line therapy of patients with metastatic RCC. Accordingly, temsirolimus was approved by the FDA for therapy in advanced RCC on May 30, 2007 and is now considered a standard first-line therapeutic option for patients with poor prognostic features.

While temsirolimus was assessed in untreated patients, everolimus was assessed in a randomized, double-blind, placebo-controlled phase III in patients with advanced RCC who had failed prior treatment with either sorafenib, sunitinib, or both within the preceding 6 months (REnal Cell cancer treatment with Oral RAD001 given Daily-1 [RECORD-1]).[2] Overall, 416 patients were enrolled and randomized in a 2:1 fashion to receive either everolimus (n=277) or placebo (n=139) each together with best supportive care. The primary endpoint was PFS as randomization was unblinded at time of progression and patients on placebo were allowed to crossover to open-label everolimus, confounding any potential differences in overall survival. The trial was halted at the second interim analysis after 191 progression events had been observed. At the final central radiology assessment the median PFS for patients treated with everolimus was 4.88 months as compared with 1.87 months in the placebo group (hazard ratio 0.33, [95% CI 0.25-0.43] p < 0.0001).[4] Five patients (2%) in the everolimus group experienced partial responses vs. none in the placebo group. Based on these results, everolimus was approved the FDA in March, 2009 for the treatment of patients with advanced RCC who failed either sorafenib, sunitinib or both.

Important toxicities of mTOR inhibitors

Although in general well tolerated, treatment with either everolimus or temsirolimus can be associated with many of the same side-effects observed with the VEGF-targeted TKIs. These most commonly include rash, nausea, diarrhea, stomatitis/mucositis, cytopenias, and fever. However the rapalogues can also induce toxicities which are distinct from those seen with VEGF pathway targeted therapies in RCC and are worthy of specific discussion. These toxicities include pneumonitis, endocrine abnormalities and the possibility of immunosuppression.

Pneumonitis.

Pneumonitis has been observed with all the rapalogues and appears to be a class effect of the allosteric inhibitors of mTOR.[5] The exact incidence of this toxicity seems to vary widely from study to study. In the phase III trials of temsirolimus and everolimus discussed above, the incidence of pneumonitis was fairly low, with incidences of only 2% and 8%, respectively, of any grade event.[2,6] However, in a retrospective study in patients with non-small cell lung cancer, White et al reported that 16 (25%) of 64 patients examined showed radiographic evidence of pneumonitis which was felt to be attributable to everolimus.[7] Other studies have suggested that pneumonitis from TORC1 inhibitors may be more common in patients with pre-existing pulmonary conditions.[8] Pneumonitis may be more commonly appreciated radiographically, where it most frequently presents as ground glass-opacity and occasionally as parenchymal consolidations and pleural effusion, than clinically. When symptoms are present, most patients experience dypsnea on exertion and cough, occasionally accompanied by fever, malaise, and hypoxia. While many mechanisms have been proposed, including cell-mediated auto-immunity and T-cell-mediated delayed-type hypersensitivity,[4,9] the exact molecular basis for this toxicity remains unknown. Although there are currently no specific guidelines to the management of rapalogue-associated pneumonitis, other etiologies, particularly infectious, should be first excluded. Most investigators appear to agree that treatment should be held in patients with overt symptoms attributable to pneumonitis and a brief course of steroids may be considered. Treatment resumption, usually at a lower dose, may be considered following resolution of symptoms. There does not appear to be consensus for patients with only radiographic findings of pneumonitis, but continuing therapy with careful observation or lowering the dose appear to be common interventions.

Endocrine side effects.

Treatment with rapalogues has also been associated with several endocrine abnormalities, namely hyperlipidemia and hyperglycemia. These toxicities appear quite common in patients with RCC treated with either temsirolimus or everolimus. In the phase III trial of temsirolimus, the incidence of hypercholesterolemia, hyperlipidemia and hyperglycemia in patients treated with temsirolimus alone was 24%, 27%, and 26% respectively.[1] In the RECORD-1 study, the incidence of hypercholesterolemia, hypertriglyceridemia, and hyperglycemia in patients treated with everolimus was 76%, 71%, and 50%, respectively.[2] Studies with rapamycin suggest that the

hyperlipidemia (observed as elevations in HDL, LDL, cholesterol, and triglycerides) induced by rapalogues is due to reduced catabolism of lipoprotein particles.[10] While this toxicity is quite common and therefore requires continuous monitoring, rapalogue-induced hyperlipidemia is usually manageable with statins or gemfibrozil (for hypertriglyceridemia) and typically does not require treatment cessation. Similarly, animal studies with rapamcyin have shown that hyperglycemia is a direct side effect of treatment with rapalogues due to enhancement of insulin resistance and reduction of β-islet cell mass and function.[11] Therefore, monitoring of fasting glucose levels is recommended for all patients treated with mTOR inhibitors, particularly those with pre-existing diabetes. Therefore initiation of oral anti-glycemic agents or escalation of current diabetic regimen may be indicated.

Immunosuppression.
As the rapalogues were developed first as immunosuppressive agents in the transplant setting, treatment with these agents has always raised concerns regarding the potential for immunosuppression in cancer patients. Recent studies have suggested that rapamycin may actually enhance the immune response to infections by both enhancing the CD8+ T-cell response and by increasing the differentiation of effector cells into potent memory T-cells.[12,13] Nonetheless, the reported incidence of infection in patients treated with either temsirolimus or everolimus in phase III trials was higher than that for their respective control arms (27% in patients treated with temsirolimus alone versus 14% in those treated with IFN; 10% in patients treated with everolimus versus 2% in those treated with placebo).[1,2] Therefore, the issue of whether rapalogues may be immunosuppressive cannot be considered to be completely resolved. Although current data does not support the use of antibiotic prophylaxis, clinical vigilance is recommended to the possibility of increased frequency of infections, particularly in those patients with pre-existing chronic viral infections or immunosuppressive conditions. In particular, recent reports filed through Medwatch, have indicated that treatment with everolimus may trigger the activation of hepatitis B in patients with a history of resolved or inactive hepatitis B.[14] In these patients, initiation of anti-hepatitis medication such as lamuvidine is recommended prior to the initiation of everolimus.

Future clinical directions
Although both temsirolimus and everolimus are approved by the FDA for the treatment of patients with advanced RCC, the role of these TORC1 inhibitors will likely continue to evolve as many questions regarding their efficacy in specific therapeutic situations are addressed. Both agents are being studied or considered in multiple other clinical scenarios and therapeutic strategies including sequential therapy with VEGF pathway inhibitors, combinational regimens the adjuvant setting, and in patients with non-clear cell histology.

Sequential therapy.
 Multiple retrospective analyses have suggested that there is no true cross-resistance for VEGF pathway and mTOR inhibitors given in sequence.[15,16] Investigators have therefore proposed to examine specific sequences of novel agents given as single agents in an effort to identify a particular sequence of agents that may result in maximal duration of disease control while perhaps also minimizing toxicity. With respect to everolimus, this is specifically being examined in the RECORD-3 trial, a large phase II trial in which previously untreated patients with metastatic clear cell RCC will be randomized to receive either first-line everolimus followed by second-line sunitinib or first-line sunitinib followed by second-line everolimus. Similarly,

temsirolimus is currently being investigated in a phase III trial versus sorafenib in patients who have failed initial therapy with sunitinib.

Combinational therapy.
Given the distinct targets of recently approved treatments for patients with RCC (i.e. inhibition of VEGF signaling vs. inhibition of mTOR), there has been considerable interest in whether combinations of these two classes of agents may lead to additional therapeutic efficacy. Perhaps the most studied approach thus far involves the combination of TORC1 inhibitors with bevacizumab. The combination of temsirolimus and bevacizumab showed encouraging efficacy in a phase II trial in patients with advanced RCC who have failed VEGF-targeted TKI therapy with an overall response rate of 16%.[17] However, in a separate randomized phase II study in which untreated RCC patients were randomized to receive either the combination of temsirolimus and bevacizumab, sunitinib, or the combination of bevacizumab and IFN-α, the response rates in the individual arms were 25%, 24%, and 34%, respectively.[18] These results, combined with the observation of significant premature treatment stoppage in the temsirolimus-bevacizumab arm (43%), have raised questions about both the additive efficacy and the toxicity of this combination. Nonetheless, the combination is also actively being assessed in multiple larger trials including in an arm the Eastern Cooperative Oncology Group (ECOG) Trial 2804 and in a large phase III trial in which patients are randomized to the combination of either temsirolimus and bevacizumab or bevacizumab and IFN-α (INTORACT Trial), so more information should be forthcoming.

Similar combinational studies with everolimus are also underway. A phase II trial of the combination of everolimus and bevacizumab produced five partial responses (17%) and a median progression-free survival of 11 months in 29 patients who had received prior VEGF receptor TKI therapy.[19] This data, plus the desire to examine the role of maintained VEGF pathway blockade following sunitinib or sorafenib resistance, has led the CALGB to propose an intergroup phase III trial randomizing patients whose disease has progressed following sorafenib and/or sunitinib to either everolimus alone or the combination of everolimus and bevacizumab.

Adjuvant therapy.
Although there are no therapies approved for the adjuvant treatment of patients with high-risk RCC, the recent approval of multiple therapies in the metastatic setting has prompted the assessment of these agents in the adjuvant setting. Studies involving sorafenib and/or sunitinib are currently underway and anticipated to reach accrual goals in the near future, but mature results are not envisioned for several years. In particular, the efficacy of everolimus in patients with metastatic RCC, together with its novel mechanism of action, favorable toxicity profile and oral administration make it an attractive agent to also test in the adjuvant setting. Accordingly, a large randomized placebo controlled phase III trial is being planned within the U.S. Intergroup mechanism to formally assess the role of adjuvant everolimus in patients with resected high-risk RCC.

Non-clear cell RCC.
Although the efficacy of TORC1 inhibitors has primarily been established in clear cell RCC, further analysis of the pivotal phase III trial leading to the FDA approval of temsirolimus suggested this TORC1 inhibitor may be even more effective compared with interferon in patients with non-clear cell RCC than clear cell RCC.[20] The median overall survival of temsirolimus versus interferon was 11.6 vs. 4.3 months in patients with non-clear cell histology (75% of which were of papillary sub-type) compared with 10.7 vs. 8.2 months

in patients with clear cell RCC. The possibility that TORC1 inhibitors in general may have unique efficacy in non-clear cell RCC has prompted the initiation of a randomized phase II trial of temsirolimus versus sunitinib in European patients with metastatic non-clear cell RCC. Likewise, everolimus will also be studied in a phase II trial in 60 European patients with metastatic papillary RCC (RAPTOR Trial [RAD001 in Advanced Papillary Tumor Program in Europe]). These two phase II trials should provide critical information regarding the efficacy of TORC1 inhibitors in patients with non-clear cell histology RCC.

Beyond first generation mTOR inhibitors
Despite the established efficacy of temsirolimus and everolimus in RCC, only a subset of patients with advanced RCC experience substantial clinical responses from treatment with these agents. Furthermore, these clinical responses are neither complete nor durable off therapy and all patients will eventually experience disease progression. The efficacy of these allosteric inhibitors of mTOR may be limited in part because they primarily inhibit the function of TORC1, the complex including mTOR and raptor, and have less activity against TORC2, the complex including mTOR and rictor (rapamycin insensitive companion of TOR). Recent studies have suggested that the expression of Hypoxia Inducible Factor (HIF)-2α, argued by many to be the more relevant HIF in RCC, is dependent almost completely upon TORC2 and largely independent of TORC1 function.[21] Furthermore, some pre-clinical studies have suggested that inhibition of TORC1 can lead to activation of signaling pathways upstream of mTOR including those mediated by phophatidy-linositol 3-kinasee (PI3-K) and Akt (Protein Kinase B).[22,23] As PI3-K and Akt activate numerous kinases, transcription factors and other proteins associated with cell growth and survival in addition to mTOR, persistent activation of these pathways might undermine the efficacy of TORC1 inhibition.

Not surprisingly, a new generation of agents targeting the PI3-K/Akt/mTOR pathway is in active clinical development. Inhibitors which directly inhibit the kinase function of mTOR, and thereby suppress the activity of both TORC1 and TORC2, are now entering clinical assessment. Given the aforementioned dependence of HIF-2α expression on TORC2, these direct mTOR kinase inhibitors would have the advantage of inhibiting the expression of both HIF-1α and HIF-2α. The possibility of rapalogue-induced feedback activation of PI3-K/Akt has made the dev-elopment of inhibitors of these upstream kinases an attractive strategy and many such agents are now in clinical development. Whether the many theoretical advantages of PI3-K/Akt or direct mTOR kinase inhibitors translates into superior clinical efficacy in patients with advanced RCC, however, remains to be seen.

Conclusion
With the recent FDA approvals of both temsirolimus and everolimus for the treatment of patients with advanced RCC, TORC1 inhibitors have now joined the antagonists of VEGF signaling and non-specific immune-therapies in a crowded therapeutic field in RCC. Despite these dev-elopments, however, only a subset of patients with RCC experience substantial clinical responses following treatment with TORC1 inhibitors. Therefore, efforts must continue to explore mechanisms of resistance to these agents to aid in the development of more effective agents directed against this critical pathway. Efforts must also focus on identifying predictive biomarkers of response to the rapalogues in order to develop more effective patient selection strategies. Through these efforts, the role of mTOR inhibitors in RCC therapy will almost certainly continue to evolve as it enters clinical assessment in a multitude of clinical settings including sequential,

combinational, and adjuvant therapy as well as in patients with non-clear cell RCC

Discussion
Dr. Atkins: Are the dual TOR inhibitors or PI3-kinase inhibitors that you discussed working by a different mechanism than the VEGF inhibitors in treating the kidney tumors?
Dr. Cho: Our preclinical work suggests they are working by a different mechanism; we do not see any evidence of an anti-angiogenic effect and yet we see diminished proliferation and diminished tumor growth.
Dr. Kaelin: Well, frankly I will be surprised if they are not working in part by blocking angiogenesis. Certainly many models, many of which are based on preclinical experiments including using genetically defined mice and zebra fish, would suggest that the PI3Kinase, mTOR pathway should be important for angiogenesis.
Dr. Stadler: We have focused on the tumor. I mean on the cancer cell itself. We cannot forget that the mTOR pathway, especial-ly the TOR-1 pathway, is critically important in the immune system as well and that these–at least the rapalogues–are potent immune suppressive agents. So what do you know about the TOR-1 /TOR-2 inhibitors and their affect on various components of the immune system?
Dr. Cho: From Phase 1 trials we have not observed significant opportunistic infections, but those are things that we will keep an eye out for.
Dr. Stadler: But it is clearly known that these drugs produce fairly profound immune suppression.
Dr. Atkins: Right. By the way, it is interesting that we are seeing benefits with mTOR inhibitors in some patients with aggressive tumors. That fact that an approach associated with immunosuppression works against these tumors, perhaps changes the discussion about where you would want to test immunotherapies such as the PD1 antibody.
Dr. Choueiri: It looked like your interpretation of MTOR inhibition was that it was really primarily through S-6 kinase down-regulation that you are suggesting this mechanism.
Dr. Cho: I think clinically you could argue that those who benefit are have sufficiently high blood levels to also inhibit TORC-2 to some extent.
Dr. Atkins: Once again, the concept that different tumors respond better to different treatments puts a premium on biomarker studies.
Dr. Kaelin: I think one other thing we could think about going forward is whether autophagy plays a role here. Just to spice things up, another gene on 5Q is a gene involved in autophagy

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TRANSLATIONAL RESEARCH STUDIES IN RENAL CANCER:
Pharmacodynamic and Predictive Biomarkers for VEGF and mTOR Directed Therapies

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Introduction

VEGF and mTOR pathway directed inhibitors have become standards of care for treatment of patients with metastatic renal cancer. Despite the paradigm shift these agents have introduced, overall survival benefit is modest, toxicities can be significant, development of clinical resistance is common and the choice of initial or subsequent drug to use in any specific patient remains unclear. As with many modern oncologic therapies, there is hope that judicial use of biomarkers can guide therapy.

In general a biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.[1] Pharmacodynamic biomarkers reflect a pharmacologic effect of the agent on the host or tumor and predictive biomarkers provide suggest the likelihood of benefit or toxicity from a specific agent.[2] An example of the former is neutropenia following paclitaxel therapy and of the latter is expression of the estrogen receptor to predict benefit from tamoxifen. In this section, we will review pharmacodynamic and predictive biomarkers of mTOR and VEGF pathway directed therapy with a focus on those showing the greatest promise for guiding therapeutic selection.

Tumor Characteristics as Biomarkers

The simplest and most common predictive biomarker in oncology is tumor site of origin and histology. For renal cancer, it has been suggested that non-clear cell subtypes may have preferential benefit to mTOR pathway inhibitors.[3] These studies have, however, been limited by the lack of central pathologic review and suggestions that poor prognosis patients, who tend to have more poorly differentiated and less well characterized tumors, may have a preferential benefit to these agents, as well.[4]

In regards to tumor molecular characteristics, VHL mutation status has been evaluated as a predictive biomarker of VEGF pathway targeted therapy. As might be expected from the fact that VHL pathway alteration is pathognomonic for clear cell renal cancer, most studies have not shown significant correlations. One study suggested that loss of function mutations were associated with response but not progression free or overall survival.[5] For mTOR directed therapy, preclinical studies would suggest that HIF upregulation, which is present in essentially all clear cell renal cancers, and alterations of the AKT/PTEN pathway would be associated with treatment benefit.[6,7] High expression of phospho-S6 kinase and p-AKT were modestly associated with objective tumor response in one small study, but no general association between mTOR directed therapy benefit with VHL pathway or PTEN status has yet been demonstrated.[8,9] More recently, it has been suggested that clear cell renal cancer can be divided into subtypes based on HIF-1α specific expression.[10] Whether this has any therapeutic relevance remains to be determined.

Therapy Toxicities as Biomarkers

By definition, the most common toxicities associated with a particular therapy are pharmcodynamic biomarkers. In the context of VEGF pathway directed therapy, the most common on-target toxicity is elevated blood pressure (BP). Although the incidence of hypertension per standard toxicity scales is modest in phase III trials, careful BP measurements suggest that elevation occurs in the majority of patients usually within 24 hours of beginning therapy.[11] Improved progression free and overall survival has been reported with development of systolic BP greater than 140 or diastolic BP greater 90 in retrospective analyses of trials with sunitinib[12] and axitinib,[13] and with grade 2 or 3 hypertension in a trial with bevacizumab.[14] A prospective randomized phase II trial is evaluating the impact of axitinib dose titration to achieve hypentension on therapeutic outcome. (ClinicalTrials.gov NCT00835978).

Fewer studies have evaluated mTOR directed toxicities as biomarkers, but lipid and glucose elevations are target specific effects. Whether these have any predictive value remains to be determined, but careful evaluation suggests that glucose and triglyceride changes occur in the majority of patients and are not associated with each other.[15]

Serum and Plasma Based Biomarkers

A number of studies have demonstrated increased levels of plasma VEGF with VEGF pathway directed therapy, most convincingly with sunitinib,[16] but these changes have not necessarily had any predictive value. Studies evaluating baseline VEGF levels have more generally demonstrated a modest prognostic, but not necessarily predictive value.[16,17] One study suggested that lower levels of soluble VEGFR-3 might have some predictive value for benefit from sunitinib, but this has not been replicated. Retrospective analysis of the phase III temsirolimus data suggested that baseline LDH was not only prognostic, but possibly predictive marker for benefit from temsirolimus.[18]

Imaging Based Biomarkers

The most common imaging based biomarker is change in tumor size or burden with therapy. However, VEGF and mTOR pathway directed agents also slow disease growth and standard RECIST based response rates do not fully capture their anti-tumor activity. VEGF pathway inhibitors, however, target tumor vasculature and thus parameters derived from contrast-enhanced imaging have been evaluated as biomarkers. The simplest incorporation of these observations are the Morphology, Attenuation, Size and Structure (MASS) criteria.[19] Post treatment lesions are evaluated for central necrosis or decreased attenuation as well as size. MASS criteria favorable response is better correlated with progression and disease specific survival than standard RECIST response.

More quantitative vascular parameters can be derived from dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI). DCE-MRI tracks the diffusion of an intravenously administered paramagnetic contrast agent (i.e., gadolinium) into the extravascular tissue over time. Although several parameters can be calculated, the most useful biomarkers for VEGF pathway targeted therapy are K^{trans} and the mathematically related K^{ep}. Derivation of these is beyond the scope of this article, but in essence they reflect a combination of tumor blood flow and permeability. Studies to date have indicated that decrease in K^{trans} is a pharmacodynamic marker for VEGF pathway targeted therapy, but that the changes have little or no predictive value.[20,21] Some data suggests that high baseline values of K^{trans} may be correlated with benefit from such therapy.[21] Similar

vascular parameters can be derived from dynamic contrast-enhanced ultrasonography (DCE-US). Reduction in tumor vascularity can be detected after 1 or 2 weeks of therapy and in preliminary studies is correlated with progression free and overall survival.[22] For mTOR directed therapy, decreased tumor glucose uptake, as demonstrated by FDG-PET imaging is a clear pharmacodynamic marker,[23,24] but is unlikely to be predictive. The preclinical suggestion that baseline FDG-PET uptake is predictive of benefit from mTOR directed therapy is being evaluated in a prospective trial (NCT00529802).

Pharmacologic and Pharmacogenomic Based Biomarkers
The value of pharmacokinetic parameters as predictors of patient outcome has not been well studied in the context of VEGF or mTOR pathway directed therapy for patients with renal cancer. For sunitinib, there has been a suggestion that increased exposure correlates with increased tumor shrinkage, and prolonged progression free and overall survival.[25] Even fewer studies have evaluated potential pharmacogenomic predictors despite the known metabolism of many VEGF and mTOR directed agents by highly polymorphic enzymes. There is some interesting preliminary data suggesting that certain VEGF or VEGFR single nucleotide polymorphisms may correlate with the development of hypertension and patient outcome.[26]

Conclusion
Although the outcome of patients with metastatic RCC has been substantially altered with administration of VEGF and mTOR directed therapies, selection of specific treatments for any individual patient remains challenging. A number of putative pharmacodynamic and predictive biomarkers have been suggested to be helpful. Nevertheless, none have been fully qualified, and substantial work, especially in a prospective manner, remains to be done before they can be recommended for general clinical use.

Discussion
Dr. Atkins: Does hypertension at baseline correlate in any way with benefit for VEGF blocking agents?

Dr. Stadler: No. Whether one has hypertension at baseline does correlate with development of hypertension as a toxicity. However it does not necessarily correlate with whether there is an increase in blood pressure relative to baseline. If you are already hypertensive and you get a delta of 10 then it is a toxicity, whereas if you are normal and get a delta of 10, you do not have toxicity yet.

Dr. Atkins: Is there any data about VEGF polymorphisms and the frequency of hypertension? Is there data from kidney cancer or other cancers about the relationship between VEGF polymorphisms and the frequency of hypertension on VEGF blocking agents?

Dr. Stadler: Yes. There seem to be some SNPs both in the VEGF as well as the VEGFR gene that correlate with development of either hypertension as a toxicity or a change in blood pressure. There are studies that have been proposed in the context of, for example, some prospective CALGB trials to look at that.

Dr. Atkins: It would seem to me that polymorphisms in the receptor would be more relevant than polymorphisms in VEGF in terms of predicting for either hypertension or for response to some of these agents. Have there been studies looking at polymorphisms in the receptor?

Dr. Stadler: There is a study, but it is not the greatest quality.

Dr. Hutson: One of the big problems I have is patients come to me and I try and figure out how often should I do CT scans or chest x-rays. There is no established recommendation for how you should monitor for disease progression like has been created for patients

with melanoma or other cancers. Can you design a blood test with a number of these markers that would be helpful in determining disease progression or directing imaging?

Dr. Stadler: Could one? Maybe. Are these going to do it? Probably not based on what we know about variability within the population. I tell folks who want a blood test for their renal cancer to take a look at the prostate cancer literature and to be careful of what you wish for. Sometimes a sensitive marker of disease can cause more headaches than it can solve problems.

Dr. Hutson: Understood. But when a patient comes in, I honestly do not know how to determine what scanning is appropriate. Should I just not do any scans at all, wait until you become symptomatic? Do a CT once a year? Or should I do it on the basis of risk? If we had an inexpensive blood test we could just send off it would really help.

Dr. Stadler: Well, the biggest problem with that is that we already do not know what to do with patients who have come in with 5 mm tumors. Do you start treatment early or do you start later? If I am presented with a new blood test that lets me detect tumor even earlier, it could create additional problems.

Dr. Atkins: You really want things that you can act on. And we have trouble knowing what action to take with small incidental findings on CT scan that are too small to biopsy. This problem could be exacerbated by a sensitive blood test. On the other hand a blood test might validate a non-specific imaging finding or vice versa. Furthermore, having a blood biomarker might prompt research studies that might elucidate important principles. For example, is there any evidence that VEGF levels are associated with tumor vascularity? Do other cytokine levels correlate with the onset of resistance to VEGF pathway inhibitors? Can such profiles guide therapy selection> d Even though we have a lot of imaging studies, we do not know whether what you see in the images correlates with something we could possibly detect in the blood.

Dr. Stadler: The answer is probably no. Of all the VEGF studies that have been done, VEGF in the blood is probably most closely correlated with platelet level. And if you do it in platelet-free plasma so that you get rid of the platelet problem then the amount of VEGF probably correlates best with tumor burden, sort of an expensive LDH

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ANTI-ANGIOGENIC AND TARGETED THERAPY IN RENAL CANCER:
Novel Targets in Metastatic Renal Cell Carcinoma
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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.

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

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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 armamentarium.[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.

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

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