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Review

Building on a foundation of VEGF and mTOR targeted agents in renal cell carcinoma

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ABSTRACT

Since late 2005 six new drugs have been approved by the Food and Drug Administration (FDA) for the treatment of metastatic renal cell carcinoma (RCC). However, the similarity of these agents with regard to mechanism of action makes it unclear if each agent has unique clinical utility. This flurry of drug development activity stems from the understanding of the central role that loss of von Hippel Lindau (VHL) gene function plays in the pathophysiology of clear cell RCC. The first agent to establish the therapeutic value of targeting the downstream consequences of VHL loss of function was a vascular endothelial growth factor (VEGF) directed monoclonal antibody, bevacizumab. Following the initial observations with bevacizumab, three VEGF receptor (VEGFR) tyrosine kinase inhibitors, with varied spectra beyond VEGFR, have been successfully developed clinically. Unanticipated clinical activity was observed with inhibitors of mTOR, a central component of the nutrient-sensing PI3 kinase pathway, in RCC. Subsequent work identified that mTOR also regulates the expression of hypoxia inducible factor (HIF), which is regulated by VHL outside of the setting of inactivating mutations or deletions. This appears to tie all of the six approved therapies to the direct consequences of loss of VHL function in clear cell RCC. It remains poorly understood to what extent these therapies differ from one another. Although the outcome of patients with metastatic RCC has been substantially altered with administration of the currently available therapies, the proper selection of currently available therapy, rational development of agents with novel mechanism of action and development of predictive biomarkers of response remains a challenge.

Contents

1. Molecular pathophysiology of renal cell carcinoma

Seventy-five percent of all cases of renal cell carcinoma have clear cell histology. In clear cell renal cell carcinoma (RCC), mutation, deletion or silencing by hypermethylation of the promoter of the VHL gene is the defining somatic genetic event and is found in 90% of cases [1]. Recent investigations have sought to identify additional genetic alterations in RCC and have been largely unsuccessful in doing so [2]. Overall, clear cell RCC has fewer mutations than other common solid tumors. The next two most common mutations in protein encoding genes, following von Hippel Lindau (VHL), were present in 3% of cases each, in a series of

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nearly 500 primary human tumors. While additional genetic alterations will undoubtedly be found with "deeper" sequencing efforts in RCC, VHL remains the only known instigator of malignancy in clear cell RCC. When VHL is inactivated by mutation or by deletion of both alleles, hypoxia inducible factor (HIF) signaling is released from the direct inactivator of HIF function.

HIF has three known isoforms (1, 2, and 3) and each of them has both an α and β subunit [1]. When complexed together in the nucleus, HIFα/β complexes are degraded and HIF signaling does not take place. HIFα subunits can bind to VHL in the cytoplasm and, when this occurs, VHL targets HIFα isoforms for degradation in the proteasome. Under conditions of hypoxia, VHL itself is degraded and this allows free HIFα to translocate to the nucleus and bind to the promoter region of numerous target genes. At this level, HIF1 and HIF2 appear to diverge with regard to the genes that each regulates. Dozens of HIF target genes have been identified, but the best described are growth factors that promote angiogenesis, for HIF1, and proliferation, for HIF2. There is recent evidence that HIF2α can, but does not always, inhibit HIF1α expression and, as a consequence, there are appears to be subclass of clear cell RCCs that predominantly express HIF2α, whereas the other larger subset expresses both HIF1α and HIF2α [3]. Numerous additional pathways have been linked to regulation of HIF expression, including mTOR, as mentioned above. Ultimately, expression and degradation are the predominant mode of regulation of HIF, as opposed to post-translational modification or phosphorylation, common modes of regulation for other signaling molecules.

While the list of pro-angiogenic HIF1α target genes is long, there are several that have been implicated as having the greatest ability to drive angiogenesis by inducing endothelial proliferation and migration into the tumor microenvironment. Vascular endothelial growth factor (VEGF) has been the best studied [4]. But others, such as platelet derived growth factor (PDGF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), angiopoetins, neuropilins, ephrins, and transforming growth factor β (TGFβ), have each been shown to have an independent or complimentary role in tumor angiogenesis [5–11]. Thus, while four drugs that block VEGF signaling have demonstrated utility in the treatment of RCC, a significant amount of unexplored therapeutic opportunity exists in blockade of these other HIF target genes that stimulate angiogenesis. As there are so many HIF target genes, attention has also been focused on blocking the activity of HIF directly or indirectly. Like other transcription factors, the activity of HIFα isoforms is directly proportional to their expression level. It is now thought that inhibition of HIF expression occurring as a consequence of mTOR inhibition is the predominant reason that two rapamycin-analog mTOR inhibitors have shown clinical activity and joined VEGF targeted therapies as validated therapeutics for RCC [12].

Two essential questions remain unanswered in the field of systemic therapy for metastatic RCC: to what extent each agents exerts a unique mechanism of action in RCC and which mechanisms underlie primary or secondary resistance to therapy. Consideration of these areas is necessary to inform the rational development of the next generation of therapies for RCC.

2. Mechanism of action for currently available therapies

2.1. Bevacizumab

A complete understanding of the mechanism of action is lacking even for the most selective targeted therapy developed to date for RCC, bevacizumab. The immediate molecular action is clear. Bevacizumab was designed to bind one of the VEGF isoforms, VEGF-A, with high affinity and specificity [13] (Fig. 1). This strategy was pursued because of the central role that has been described for VEGF-A in tumor angiogenesis. VEGF-A binds to VEGF receptors -1 and -2. The signaling function of VEGFR-1 is poorly understood, but VEGFR-2 clearly stimulates downstream signal transduction in the mitogen activated protein (MAP) kinase pathway and phopshoinosytol-3-kinase (PI3K) pathway via RAS activation. These pathways regulate survival and proliferation of endothelial cells. Furthermore, VEGF-A acts as a chemo-attractant for proliferating endothelial cells. Thus, VEGF-A has been implicated in the

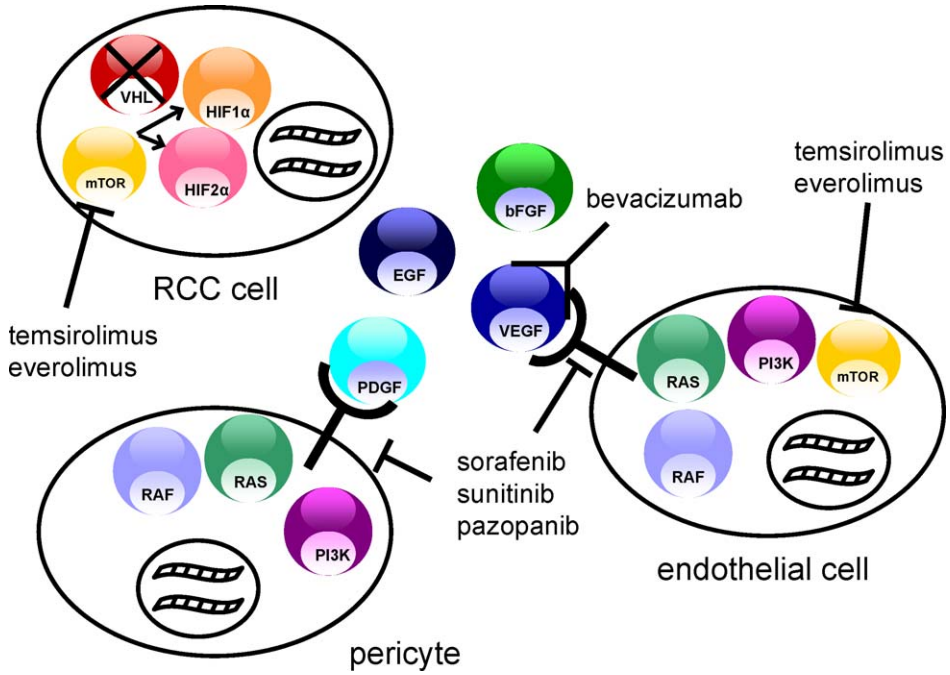

Fig. 1. Molecular and cellular mediators of angiogenesis in clear cell renal cell carcinoma. VHL: von Hippel Lindau gene product; HIF: hypoxia inducible factor; mTOR: mammalian target of rapamycin; VEGF: vascular endothelial growth factor; PDGF: platelet-derived growth factor; EGF: epidermal growth factor; bFGF: basic fibroblast growth factor; PI3K: phosphoinositol-3 kinase.

initiating steps of angiogenesis, in normal development, response to hypoxia and tumor angiogenesis. Many tumors highly overexpress VEGF-A, but only in RCC is there an oncogenic event that directly regulates VEGF production. In other tumors, VEGF is expressed as a consequence of hypoxia in the core of growing tumors that have outstripped their blood supply [14]. This results in physiologic degradation of VHL and release of HIF to drive the transcription of VEGF and other HIF target genes. Levels of VEGF-A production are far higher in RCC than in other tumors [15], likely due to the fact that every cell in the tumor has the underlying molecular drive to do so, as opposed to other tumor types where only those cells in hypoxic regions of the tumor produce it.

Bevacizumab binds to VEGF-A with high affinity and, at the doses typically administered, very little free VEGF-A remains in all tissue compartments which a molecule as large as an antibody can reach [4]. Either because of loss of negative feedback regulation in normal tissues or due to the induction of greater degrees of hypoxia in tumors, or both, VEGF-A levels significantly rise in the setting of bevacizumab therapy. However, the amount of antibody administered with the standard dose of 10 mg/kg every two weeks is sufficient to bind this increased number of VEGF-A molecules [16–18]. Ultimately, VEGF-A/bevacizumab complexes are degraded by the reticuloendothelial system. The half-life of bevacizumab is approximately two weeks [19]. Thus, repeated doses given in every two to three week intervals result in drug accumulation.

One indicator of complete or nearly complete VEGF-A depletion in the setting of bevacizumab therapy is the development of hypertension during therapy [16]. The precise mechanism of hypertension in this setting is not known, but it is clear that numerous circulating mediators of blood vessel are not altered by VEGF targeted therapy [20]. Furthermore, treatment-emergent hypertension is quickly reversible with drug withdrawal, suggesting that hypertension does not result from a diminution in the number or caliber of vessels in the systemic vascular bed. The most likely remaining explanation, which is extremely difficult to prove in patients, is the regulation of the smooth muscle relaxant nitric oxide by VEGF. Under normal physiologic circumstances, high VEGF levels result in increased expression of inducible nitric oxide synthase and increased production of nitric oxide [21]. Nitric oxide ultimately induces a decrease in smooth muscle tone in vasculature, dilation of arterioles and a drop in systemic vascular resistance. Measuring changes in nitric oxide in tissues is exceedingly difficult due to the extremely short half-life of nitric oxide. Thus, there is only indirect evidence that this critical blood pressure homeostatic mechanism is the unintended target of VEGF depletion with VEGF targeted therapy. The fact that blood pressure increases are more severe in some patients than others, and absent in some, is without an adequate explanation. It is hypothesized that variation in free VEGF levels or germ-line genetic variation in the VEGF-A or VEGFR-1 or VEGFR-2 could account for these differences [22,23]. If differences in unbound VEGF were to account for this variability in hypertension, that might indicate the need for individualization in dosing of bevacizumab or the need for another therapeutic approach to more completely block the action of VEGF.

There is evidence that VEGF receptors are expressed on a subset of clear cell RCCs, raising the possibility that VEGF targeted therapies might be directly cytotoxic to tumor in some circumstances [24,25]. This hypothesis has not been directly tested, but the observation warrants evaluation of VEGFR expression on tumor cells as a predictive marker of response to VEGF targeted therapies.

It should be noted that other VEGF isoforms, namely VEGF-C and VEGF-D, have been implicated in the pathophysiology of RCC [26–29]. Bevacizumab does not bind these isoforms. We will address the development of novel, broader spectrum VEGF targeted therapies in the section regarding novel therapies in developments.

2.2. VEGFR inhibitors: sorafenib, sunitinib, and pazopanib

Sorafenib, sunitinib, and pazopanib are kinase inhibitors with binding VEGF receptors -1, -2, and -3 (Fig. 1) [30–32]. While these may be the most clearly relevant targets in clear cell RCC, the additional activity against PDGF receptor β (PDGFRβ) inherent with these agents is likely a valuable property when targeting tumor angiogenesis [33,34]. PDGF has been shown to be critical in recruitment of pericytes to sprouting tumor vessels and pericyte-endowed vessels are more resistant to anti-angiogenic therapy than those that lack pericytes [35,36]. Each drug inhibits additional kinases, and the spectrum of kinases beyond VEGF and PDGF receptors differs significantly [37]. Despite demonstrating clinical benefit in clear cell RCC, the possibility remains that many of the kinases inhibited by each agent may contribute to toxicity and not to efficacy. For that reason, the therapeutic index of VEGF receptor inhibitors with more narrow spectrum of inhibition could be greater. One of newer VEGFR inhibitors in development, axitinib, may illustrate this point [38]. This agent has, perhaps, the greatest potency against VEGF receptors relative to other kinases [39], and is associated with the highest response rate reported to date with any of the VEGF signaling inhibitors in patients who have not previously received similar agents.

Not all kinases inhibited beyond VEGFR and PDGFR are thought to be a liability. Sorafenib, for example, is a potent inhibitor of RAF1 [30]. A key constituent of the MAP kinase pathway, RAF1 has been shown to be essential for VEGF stimulated proliferation of endothelial cells [40,41]. In preclinical models, RAF1 antagonism results in endothelial cell apoptosis and inhibition of angiogenesis [42–44]. Thus, RAF inhibition could complement VEGF receptor inhibition by blocking multiple points in endothelial cell signaling.

There are differences in the spectrum of kinases inhibited by sorafenib, sunitinib and pazopanib and the relative potency of each against well-described mediators of angiogenesis as well as other kinases [37,45]. These differences were perhaps best illustrated in a study which employed a novel method of assaying kinase activity. Sorafenib and sunitinib, amongst other kinase inhibitors competed with kinase domains of 119 kinases fused to bacteriophages [37]. Bound bacteriophage bound to the ligand could then analyzed by quantitative PCR. This method allows for a more definitive ranking of potency with which targets are inhibited compared to independently conducted isolated kinase assays. In this system, sunitinib bound 73 kinases in addition to VEGFR-2 while sorafenib bound 40 additional kinases. The potency of each agent for VEGFR-2 was 0.23 nM for sunitinib versus 93 nM for sorafenib. Similarly, sunitinib exhibited greater potency against PDGFRβ (0.21 nM) than sorafenib (41 nM). While this might immediately suggest that sunitinib achieves far greater inhibition of these targets in clinical use, one must also factor in the fact that the standard daily dose of sorafenib is 16-fold higher than sunitinib in terms of milligrams administered. The clinical significance of inhibiting "off target" kinases such as JAK1, KIT, and the calcium/calmodulin-dependent protein kinases is not yet known. The possibilities are three-fold: either non-VEGFR effects augment efficacy positively or negatively, or they contribute only to toxicity. Toxicity driven by such off-target effects is not only a matter of negatively impacting quality of life while on therapy, but, in some cases, could limit escalation of dosing of a given agent and thereby limit the degree of inhibition that can be achieved against the primary therapeutic targets of the agent.

2.3. mTOR inhibitors: temsirolimus and everolimus

mTOR inhibition was added to the list of successful molecular interventions in RCC by showing improved disease control compared to the historical standard, interferon and compared to best supportive care in patients who had progressed while

receiving sorafenib or sunitinib [46,47]. Angiogenesis inhibition had been observed with mTOR inhibitors in preclinical models [48]. It was subsequently found that mTOR regulates HIF expression in several tumor models [49,50]. This provided a plausible mechanistic link between mTOR and efficacy in RCC, given the known role of VHL and HIF in this disease (Fig. 1). However, it is also known that the phosphoinositol-3-phosphate kinase pathway (PI3 kinase pathway), in which mTOR plays a critical role, is responsible for a significant element of VEGF receptor and other growth factor receptor signaling in endothelial cells [51,52]. Thus, it remains possible that temsirolimus exerts a significant effect on both the tumor cell and endothelial cells and either or both could result in inhibition of angiogenesis.

3. Limitations of currently available therapies for RCC

The clinical utility of bevacizumab, the VEGFR inhibitors (sorafenib, sunitinib, and pazopanib), and the mTOR inhibitors (temsirolimus and everolimus) has been established in patients with metastatic RCC (Table 1) [16,18,31,46,47,53,54]. However, the trials were conducted in varied patient populations with regard to histologic subtype of RCC and prior treatment status. Comparison of their relative efficacy is, therefore, extremely difficult. Reported differences in overall survival and progression-free survival are likely confounded by prognostic factors that can only be controlled in the context of a randomized trial comparing any one of these agents with another. This has not been done to date. Response rate, though dependent on histologic subtype is more easily compared amongst these agents and there appears to be two distinct groups: bevacizumab, sorafenib, temsirolimus and everolimus producing responses in 10% of fewer and sunitinib and pazopanib being associated with response rates of 30% and higher. The distinct features of the drugs themselves or the way in which they are administered that might account for this difference is not understood. Importantly, it is not clear that higher response rates are associated with improvements in overall disease control (progression-free or overall survival) and thus cannot be taken as evidence of superior clinical benefit.

Mechanisms of resistance to VEGF targeted therapy in RCC remain largely undescribed. Under consideration are tumor factors, such as VHL gene status, relative HIF1α versus HIF2α expression, and differential expression of HIF responsive genes (VEGF, PDGF, bFGF, amongst others). Variability in expression of growth factor receptors for these same growth factors on endothelial cells, and downstream activation of signal transduction pathways are also plausible distinguishing features of responsive versus refractory tumors [55,56]. Lastly, host factors that may influence outcome in the setting of these therapies include functional polymorphisms in the genes encoding the direct targets of each agent or the downstream signaling mediators of drug effect, as has been shown in breast cancer patients receiving bevacizumab [22]. Even differences in drug exposure among a population of treated patients may explain differences in outcome, as data from phase II trials with sunitinib suggest [57]. It is likely that multiple factors influence outcome in the therapy of anti-angiogenic therapy for RCC and the best predictive models may contains multiple variables related to signaling pathways and drug metabolism.

Table 1
Anti-angiogenic drugs currently approved in renal cell carcinoma.

Drug	Target	Definitive clinical trial(s)
Bevacizumab	VEGF-A	Yang et al. [16], Escudier et al. [18]
Sorafenib	VEGFR-1, -2, and -3, PDGFRβ, RAF1, KIT	Escudier et al. [53]
Sunitinib	VEGFR-1, -2, and -3, PDGFRβ, KIT	Motzer et al. [54]
Pazopanib	VEGFR-1, -2, and -3, PDGFRβ, KIT	Sternberg et al. [31]
Temsirolimus	mTOR (TORC-1 complex)	Hudes et al. [47]
Everolimus	mTOR (TORC-1 complex)	Motzer et al. [46]

VEGF: vascular endothelial growth factor; VEGFR: vascular endothelial growth factor receptor; PDGFRβ: platelet-derived growth factor β.

The exploration of predictive markers of outcome in the setting of therapy with the available therapies for clear cell RCC is in its infancy. One clear distinction within this histologically defined tumor type is the presence or absence of VHL. While only a small minority of clear cell RCCs have intact VHL (10%), this would plausibly define a unique subset within this disease, and one for which the available therapies are ineffective [58,59]. In a small cohort of RCC patients treated with VEGF targeted therapy the presence of mutations in VHL or hypermethylation of the VHL promoter, which would be expected to result in silencing of gene expression, were weakly predictive of better progression-free survival compared to the small subset of patients with intact VHL [58]. When only those mutations that would result in complete loss of functional VHL protein were considered, along with those tumors with promoter hypermethylation, a stronger association was seen between VHL status and PFS, but still not statistically significant. The implication of these findings is that VEGF targeted therapy may be most beneficial for those patients whose tumors lack functional VHL, and therefore, have unique dependence on HIF activity and subsequent VEGF expression. Even among the patients whose tumors have VHL loss of function there is great range in disease control, indicating that further characterization is required to define the factors that predict outcome in this subgroup, which represents 90% of the clear cell RCC population.

Mechanisms of resistance to VEGF targeted therapy in clear cell RCC with loss of VHL function are not defined. To the same degree that responsiveness to VEGF targeted therapy is highly variable, resistance may be mediated by different factors for patients whose tumors progress early or late in the course of therapy. There are data from animal models of tumor angiogenesis that VEGF or VEGF receptor inhibition leads to increased production of PDGF and bFGF by tumors [60–62]. PDGF upregulation may be addressed by the cross reactivity to PDGFRβ inherent with sorafenib, sunitinib, and pazopanib, but pro-angiogenesis growth factors, including bFGF, TGFβ, HGF, angiopoetin, and ephrins, may mediate tumor escape in the face of VEGF, VEGFR, and PDGFR inhibition. Additional mediators of resistance have been postulated, outside of well-described pro-angiogenic factors, but many initial observations require validation in additional preclinical models [63].

The potential mechanisms of resistance to mTOR inhibition may be quite distinct compared to that observed with VEGF targeted therapy. One observation that deserves closer attention is that rapamycin analogs, including temsirolimus, inhibit only one of two signaling complexes of which mTOR is a part [64,65]. The TORC1 complex is potently inhibited by temsirolimus and everolimus, while the TORC2 complex is not [66,67]. As a consequence, one downstream pathway of mTOR activation is unopposed. It has been demonstrated in preclinical studies that mTOR inhibition with rapamycin analogs results in feedback upregulation of Akt, upstream of mTOR in the PI3K pathway [68]. Since Akt can activate the TORC2 complex, which is not inhibited by rapamycin analogs, it is possible that the upregulation of this pathway eventually compensates for TORC1 inhibition in tumors that are refractory to temsirolimus.

Attempts to identify predictive markers for response to VEGF and mTOR targeted therapies have not taken into account the complexity of angiogenesis and the full scope of patient and tumor factors. While this is not a unique issue in the RCC field, it is in this setting that these agents are given as single-agents, providing the most robust clinical scenario to pursue these investigations. We

believe that host germ-line genetic variation (polymorphisms in angiogenesis mediators and targets of therapy) as well as markers of tumor cell, endothelial, pericyte and, perhaps, additional tumor stroma constituents must be considered in multivariate models. This would permit the multi-cellular and multi-molecular nature of tumor angiogenesis to be encompassed in predictive algorithms.

4. Combinations of targeted agents for clear cell RCC

The mechanistic overlap of bevacizumab, sorafenib, sunitinib, pazopanib, temsirolimus, and everolimus have raised concern that these do not represent unique therapeutic maneuvers and that, once resistance develops to one of these agents, patients would be refractory to all of the remaining agents. However, the direct molecular target is unique for bevacizumab versus the VEGF receptor inhibitors versus the mTOR inhibitors. Thus, each possible two-drug combination, taking one from each of these three categories, has been or is being explored in RCC in an attempt to further augment the anti-angiogenic activity of single-agent therapy.

Sorafenib, sunitinib and pazopanib treatment is associated with significant increases in VEGF above baseline levels [20,45,69]. And while bevacizumab therapy is also associated with increased production of VEGF, bevacizumab is uniquely capable of neutralizing secreted VEGF. Thus, it is hypothesized that co-administration of bevacizumab with one of the VEGFR inhibitors may further decrease VEGF signaling and mediate greater efficacy than VEGF inhibitor therapy alone. Sorafenib has been combined with bevacizumab in a phase I trial restricted to patients with metastatic RCC [70]. Forty-eight patients were enrolled, 85% of whom had clear cell RCC. The doses of bevacizumab ranged from 3 mg/kg to 10 mg/kg every two weeks, and the dose of sorafenib were varied between 200 mg once daily and 400 mg twice daily, the latter being the standard single-agent dose. It was not possible to safely administer full doses of either agent along with even a quarter or a third of the other due to toxicity. The toxicity observed were those typically seen with either agent alone, but more frequent and more severe. This could be interpreted as mechanistic overlap at the level of normal tissue effects. Ultimately, half the usual dose of bevacizumab was safely combined with one-quarter the usual dose of sorafenib. Despite reduced doses of each being administered, the response rate of nearly 50% was far superior to the 10–15% rate observed with either agent alone when administered at full doses, supporting a possible synergistic effect [17,71,72]. In a separate phase I trial among patients with advanced solid tumors, by administering sorafenib for five days out of every seven, a dose of 200 mg twice daily could be tolerably combined with 5 mg/kg of bevacizumab every two weeks [73]. This regimen is being evaluated in an ongoing randomized trial compared to single-agent bevacizumab at the standard dose.

Sunitinib has been combined with bevacizumab in two phase I trials, one exclusively among patients with RCC and the other among patients with advanced solid tumors [74,75]. In both cases, a standard 50 mg daily dose of sunitinib for 28 days out of every 42 days was tolerable in combination with bevacizumab 10 mg/kg every two weeks during the early course of therapy. While the severe toxicity rate was sufficiently low to allow these doses to be declared to recommended phase II doses, there were was one case of fatal myocardial infarction in the second cycle and a case of severe subcutaneous hemorrhage in the first month of treatment. Therefore, questions remain whether lower doses of one or both drugs should have been evaluated for longer period of time to carefully assess long term toxicities of the chronic use of this regimen. As this regimen was taken into a randomized phase II trial comparing sunitinib alone to sunitinib with bevacizumab in patients with metastatic clear cell RCC and patients continued to receive treatment in the RCC-specific phase I trials several cases of thrombocytopenia and renal failure were observed. Serologic markers of inflammation and fibrinogen consumption were identified, supporting a microangiopathic process reminiscent of thombotic thrombocytopenic purpura. Such a toxicity, very rarely observed with sunitinib alone suggests that this combination induced endothelial cell damage in a widespread fashion in normal vasculature. This could represent the limit of tolerable VEGF deprivation for even quiescent endothelial cells. While this effect is not completely understood, this observation provides evidence that complete inhibition of VEGF signaling will be limited by toxicity to normal vessels and that other innovative directions must be pursued to achieve greater degrees of angiogenesis inhibition than is possible with single-agent therapy with available agents.

Combining temsirolimus with either sorafenib or sunitinib provides another strategy for countering the presumed additional HIF activation the results from VEGFR targeted therapy. The combination of sunitinib and temsirolimus proved to be intolerable at doses that were 50% lower than the standard single-agent doses for each agent [76]. Sorafenib and temsirolimus could be safely combined, but a reduction in the dose of sorafenib to half the single-agent dose was required in order to administer temsirolimus at its standard single-agent dose [77]. This regimen is also being evaluated in a randomized trial compared to single-agent bevacizumab.

The combination of bevacizumab and temsirolimus has been evaluated in a phase I/II trial among patients with metastatic RCC [78,79]. The standard single-agent doses of both agents could be combined with an acceptable toxicity profile. Thus, this combination appears to be perhaps the least problematic with regard to enhancement of known toxicities. This could relate to the high degree of specificity of these agents for VEGF and mTOR, whereas the broad spectrum kinase inhibitors have been more problematic to combine with these agents. This last combination is the third one to be included in the randomized trial which single-agent bevacizumab serves as the benchmark.

5. Novel therapies in development

Sorafenib, sunitinib, and pazopanib are members of one of the largest classes of cancer therapeutics in development, with at least 15 other VEGF receptor inhibitors in clinical trials. As difficult as it is to discern what mechanistic differences exist between sorafenib, sunitinib, and pazopanib in this patient population, it is unclear what advantages may come from VEGF receptor inhibitors that have greater or less potency and specificity against members of the VEGF receptor family. Sorafenib, sunitinib, and pazopanib all exert relatively greater effect on VEGFR-2, compared to VEGFR-1 or -3 [30,32,45]. Given that VEGFR-1 is a receptor for VEGF-A and preclinical evidence supports it unique contribution to tumor angiogenesis [80], it may be that inhibiting VEGFR-1 is beneficial for complete VEGF-A signaling inhibition. Axitinib and AMG-706 are examples of agents that offer potent inhibition of VEGFR-1, in addition to VEGFR-2 [81,82]. Unlike the three FDA approved VEGFR inhibitors, these agents are associated with proteinuria in a minority of patients enrolled on clinical trials [83,84]. Bevacizumab frequently induces proteinuria, and had previously been the only VEGF signaling inhibitor to cause this toxicity [19]. Since bevacizumab only impacts VEGF-A signaling, it may be that the observation of proteinuria reflects more complete suppression of VEGF-A signaling, via VEGFR-1, with these novel VEGFR inhibitors.

VEGFR-3 mediates lymphangiogenesis [85,86], which is clearly a component of tumor pathophysiology, but the therapeutic value of blocking lymphangiogenesis, as opposed to angiogenesis, in RCC is unknown. One intriguing observation is that increases in VEGF-C, the ligand of VEGFR-3, and decreases in soluble VEGFR-3 correlate with longest duration of response to sunitinib [69]. This supports the hypothesis that, after inhibition of VEGF-A signaling,

the next most critical determinant of benefit with this class of therapies is inhibition of VEGF-C signaling. Several of the VEGF receptor inhibitors currently in development have nearly equal potency against VEGFR-1, -2, and -3, with far less potency against other kinases. Axitinib, which potently inhibits all VEGF receptors, has demonstrated the highest objective response rate and longest median progression-free survival of any of the agents evaluated in RCC to date [87]. However, comparative trials against sorafenib or sunitinib will be required to determine if efficacy of axitinib is truly greater. Further preclinical studies to show relationship between changes in VEGF-C, soluble VEGFR-3 and clinical outcome with axitinib will need to be conducted. These should help us understand better the unique aspect of lymphangiogenesis inhibition with available and investigational drugs with respect to maximum efficacy.

At this point, it remains unclear how resistance develops to any of the available agents. Thus, rational strategies for designing therapies that would intercept such mechanisms are lacking. In the absence of that information, novel anti-angiogenic therapies largely represent attempts to broaden the coverage of previously described mediators of tumor angiogenesis.

Novel VEGF ligand inhibitors are in clinical development. VEGF trap has been the most extensively evaluated, but phase II data in RCC are not yet available [88]. This agent is a fusion protein made of up of VEGFR immunoglobulin domains which bind with high affinity all of the VEGF isoforms. The theoretical advantages of VEGF trap over bevacizumab are that the affinity for VEGF is higher and other isoforms of VEGF, beyond VEGF-A, are bound [89]. However, the affinity of bevacizumab for VEGF-A is quite high, with no detectable free VEGF remaining in patients treated at the doses used currently in clinical practice [19]. So, it is not clear that there is benefit in developing agents on the basis of higher affinity for VEGF.

VEGF and VEGF receptor activation are downstream consequences of HIF activity in RCCs. However, there are other consequences of HIF activity that contribute to carcinogenesis. For example, HIF2α controls the expression of c-myc which is critical cell cycle regulator [90,91]. Selective inhibition of VEGF signaling clearly alters the natural history of metastatic RCC, but concomitant inhibition of other HIF regulated signaling is a clear direction for novel therapy development. Amongst other potentials advantages, antagonism of HIF would potentially downregulate angiogenesis promoting growth factors other than VEGF. There are unique challenges to developing HIF targeted therapies, in that HIF is a transcription factor which lacks an enzymatic domain which can be blocked with a competitive inhibitor [92]. It is possible that non-competitive inhibitors interfering with protein-protein interactions that are essential for HIF function can be developed, but large peptides and proteins have very limited bioavailability and cellular penetration, and small molecules that could serve this function might simultaneously block signaling of transcription factors that are unrelated to cancer pathophysiology.

A promising new direction for RCC therapeutics are agents that modulate HIF stability and expression. As discussed previously, mTOR appears to serve this function and the mechanism of action of temsirolimus may, in part, derive from this effect. Other constituents of the PI3 kinase pathway have been independently demonstrated to regulate HIF protein stability, including Akt and GSK3β [93–95]. Inhibitors of Akt are in early clinical development and GSK3β inhibitors are in clinical trials for neurodegenerative diseases and, more recently, cancer. Given the large number of factors that have been associated with regulation of HIF expression and stability, it is not surprising that many different classes of novel cancer therapies have been observed to alter HIF levels in preclinical models. These include class II HDAC inhibitors, KIT inhibitors, STAT3 inhibitors, and HSP90 inhibitors [95–98]. While the ability of these inhibitors to downregulate HIF in vitro has been demonstrated, the clinical effects of these agents in RCC have not been thoroughly evaluated. Any one of these HIF regulators may offer incomplete inhibition of HIF activity, and thus minimal single-agent activity in RCC patients. However, these agents may be ideally suited to use in combination with VEGF targeted therapies which upregulate HIF activity.

Dysregulated HIF activity results in the upregulation of numerous growth factors that stimulate angiogenesis. While VEGF may be the single most potent endothelial cell mitogen, angiopoetin, bFGF, HGF and TGFβ each have the ability to stimulate tumor angiogenesis [11,99–101]. Thus, any of these are plausible partners for VEGF targeted therapies, as a strategy for broadening the coverage of angiogenesis mediators. Inhibition of epidermal growth factor receptor (EGFR), the receptor for TGFβ, has been explored in phase II trials with single-agent EGFR inhibitors and in combination with bevacizumab or sunitinib [102,103]. In single-agent EGFR inhibitor trials objective response rates were observed among patients with metastatic clear cell RCC in 5–10% of cases, and a similarly small percentage of patients had disease stabilization for more than 6 months [104,105]. These data suggested that EGFR may be an important target for a subset of tumors. The most definitive test of EGFR inhibition was a randomized phase II trial in which bevacizumab was administered with or without erlotinib among 100 patients with clear cell RCC [17]. Although a trial of this size does not reliably rule out a small added clinical benefit, there was no difference detected in either PFS or objective response rate. Thus, among molecularly unselected RCC patients, this approach does not appear to warrant further testing. In light of the single-agent phase II data, it is still possible that a subset of RCC patients could benefit from this approach, but a potential marker of this subset has yet to be described. Phase I trials are being conducted with inhibitors of the receptors for angiopoetin, bFGF, and HGF: Tie-2, FGF receptor, and c-met. The latter two targets are genetically related to other receptor tyrosine kinases. Thus, it is not surprising that small molecule kinase inhibitors have been identified that have potency against either FGF receptor or c-met in addition to VEGF receptors and PDGF receptor β [106,107]. Such broad-spectrum inhibitors are, therefore, similar to sorafenib and sunitinib in some respects, but their spectrum is somewhat more tailored to some of the high priority angiogenesis targets.

6. Renal cell carcinoma histologies other than clear cell: papillary and chromophobe

Whereas papillary RCC has intact VHL, genetic amplification of c-met has been identified in some sporadic papillary RCC cases, and activating mutations in c-met in nearly all cases of familiar papillary RCC [108]. This receptor tyrosine kinase represents a unique target and one that is not inhibited by any of the six FDA approved RCC therapies. Broad-spectrum kinase inhibitors with potency against c-met are currently in clinical development and one, XL880, has entered phase II testing in papillary RCC [109]. Specific c-met inhibitors are also in development, and ARQ197 represents the most clinically advanced of these [110].

The molecular pathophysiology of chromophobe RCC is not well understood. The only commonly observed defect is mutation or deletion of the Burt-Hogg-Dube gene [111]. The function of this tumor suppressor is under investigation, and no upregulated pathway has been identified for which an inhibitor can be tested. Thus, this subtype remains without a rational therapeutic target.

7. Conclusion

While significant progress has been made in the treatment of RCC with the emergence of VEGF and mTOR signaling inhibitors,

two fundamental questions remain largely unanswered. First, to what extent do the six FDA approved therapies differ in their mechanism of action? Second, how can the next significant therapeutic advance be made given the lack of mechanistic understanding of resistance to those agents? In clear cell RCC, three strategies are being pursued in parallel: (1) more potent and specific inhibitors of HIF or VEGF signaling, (2) inhibitors of angiogenesis mediators other than VEGF and PDGF, and (3) combinations of individually active agents that act at distinct points in the HIF to endothelial cell axis. Certainly there are additional avenues that must be considered, particularly for clear RCCs for which VHL is intact. Arguably the greatest strides will come when a molecular classification can be defined that explains the heterogeneity of response to current therapy for RCC and identifies the best therapeutic targets for each subgroup.

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