New Strategies in Kidney Cancer: Therapeutic Advances through Understanding the Molecular Basis of Response and Resistance

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CCR New Strategies

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Abstract

The emergence of viable therapeutic strategies in metastatic renal cell carcinoma has invigorated translational and clinical research in this disease. Building upon the clinical activity observed with inhibition of vascular endothelial growth factor (VEGF) and mammalian target of rapamycin (mTOR) pathways, novel strategies are being investigated to extend existing clinical benefits. Preclinical study has identified potential molecular mechanisms of response and resistance, providing a rational basis for biomarker development as well as sequential and combination therapy strategies. Several treatment strategies have emerged that are in the early phases of clinical testing. Further clinical and translational research is needed to validate initial hypotheses and translate observations into novel treatment strategies. *Clin Cancer Res; 16(5); 1348–54. ©2010 AACR.*

Background

Metastatic renal cell carcinoma (RCC) has recently become a model disease in which an enhanced understanding of fundamental disease biology has been translated into therapeutic advances with the targeting of relevant pathways. Molecules with inhibitory activity against elements of the vascular endothelial growth factor (VEGF) and mammalian target of rapamycin (mTOR) pathways have revolutionized the therapeutic approach to RCC as a result of their significant antitumor effect (1–6). Indeed the rapid and simultaneous emergence of several active compounds has far outpaced the ability to critically understand precise mechanisms of response and resistance, and thus hampered both the ability for optimal current application and rational future advances. As such, the current practice in management of metastatic RCC is the empiric delivery of sequential monotherapy, based on noncomparative clinical trials and, especially in treatment-refractory patients, based more on practical issues such as route of delivery, cost, and physician familiarity than scientific evidence (4, 7). Significant new therapeutic strategies in RCC will result from a deeper understanding of the biology of response and resistance to targeted therapy (Fig. 1). This review will briefly summarize promising insights into this area and the emerging therapeutic strategies.

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On the Horizon

Understanding the basis for treatment response. The elusive holy grail of oncology therapeutics would be application of agents only to those patients with the highest chance of clinical benefit and/or the lowest chance of toxicity. With the recent emergence of active agents in RCC, the search for predictive biomarkers has intensified. To date however, none of the obvious biologic markers, such as von Hippel Lindau (*VHL)* gene status or serum levels of VEGF-related proteins have been shown to predict response to targeted therapy in RCC (8–10). Initial reports of *VHL* status suggested a possible link between silenced *VHL* and objective response to VEGF-targeted therapy, especially when segregating *VHL* abnormalities that were hypothesized to lead to a dysfunctional VHL protein (8). However, no correlation of *VHL* status and progression-free survival (PFS) or overall survival was shown, and actual VHL protein status was not investigated. Given that the vast majority of clear-cell RCC tumors have VHL silencing, this characteristic may not be discriminating enough by itself to segregate into distinct subgroups. Additional prospective investigation in larger sample sets is ongoing.

Biomarkers measured in the peripheral blood have also been investigated. VEGF-R inhibition has consistently lead to increases in circulating plasma VEGF and placental growth factor (PlGF), as well as decreases in soluble VEGF-R2 and VEGF-R3 (9, 10). These changes return to baseline levels off drug, and a greater magnitude of changes has been observed in patients showing an objective response to sunitinib. However, baseline levels of these soluble factors have not been shown to correlate with outcome, limiting the predictive ability of these markers to affect clinical practice at present. Further, prospective work on a larger number of patients is needed to delineate

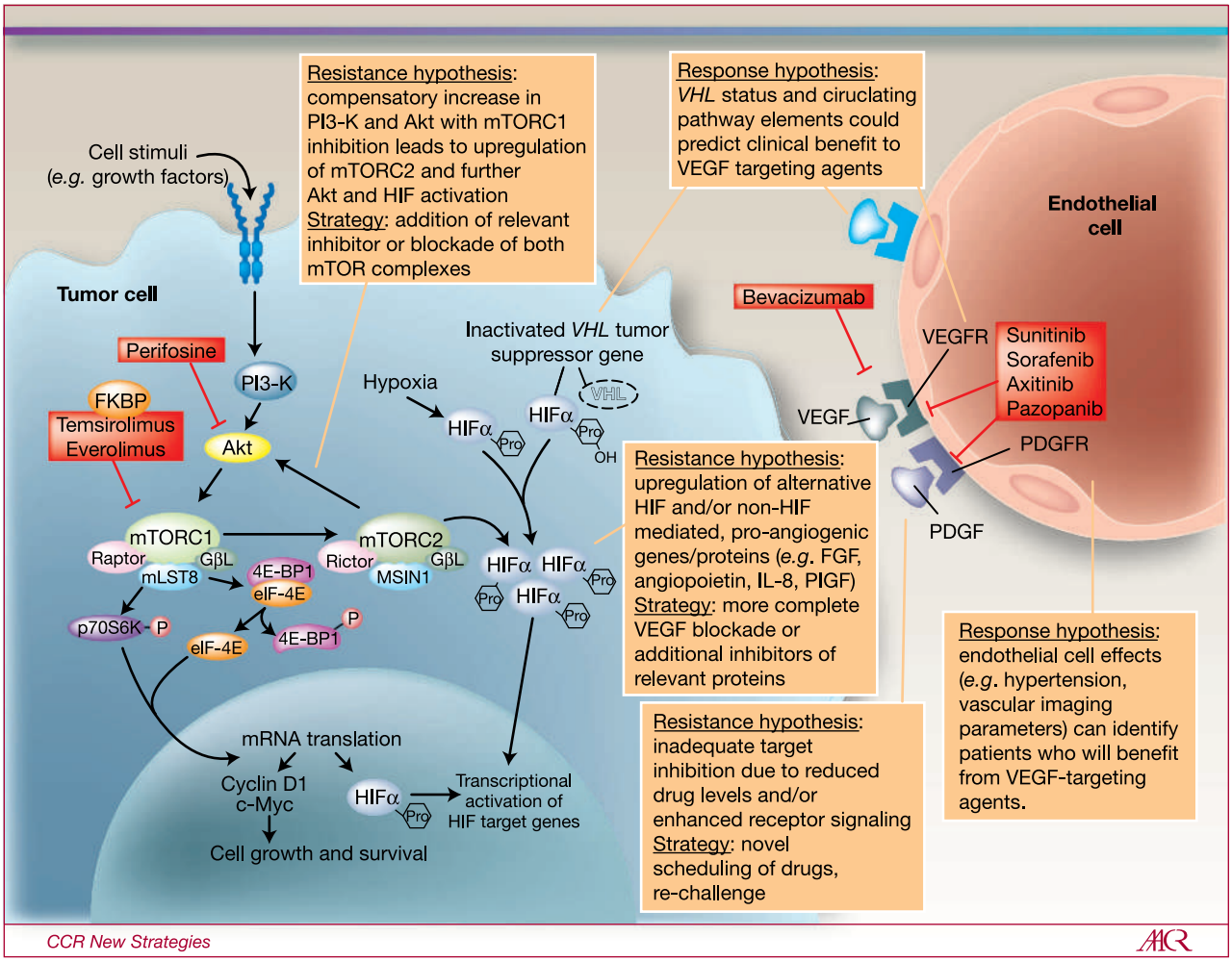

Fig. 1. Hypothesized mechanisms of response and resistance to targeted therapy in metastatic RCCC and resulting therapeutic strategies. Response to VEGF-targeted agents in RCC is postulated to be related to the status of VEGF pathway elements (e.g., *VHL* status) and/or concomitant endothelial cell effects (e.g., hypertension). Resistance to existing VEGF blocking agents may include upregulation of HIF- and/or non-HIF-mediated angiogenic proteins or inadequate target inhibition. mTOR therapy resistance may involve a compensatory increase in upstream elements leading to HIF production. Abbreviations: PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog; p70s6K, p70S6 kinase; 4E-BP1, 4E binding protein-1; eIF-4E, eukaryotic initiation factor-4 subunit E; HIFα, Hypoxia inducible factor alpha; Pro, praline; Ub, Ubiquitin; VEGFR, vascular endothelial growth factor receptor; PDGF, platelet derived growth factor; PDGFR, platelet derived growth factor receptor; IL-8, interleukin-8. Adapted with permission from Rini et al. (54).

whether these factors represent only a marker of pharmacodynamic drug effect or have predictive potential. In addition, given the putative anti-angiogenic mechanism of these agents, circulating endothelial progenitors (CEP), bone marrow-derived cells that contribute to pathological neovascularization, and mature circulating endothelial cells (CEC), which are shed from mature vasculature either as part of normal turnover or in response to vascular damage, have also been investigated. Limited data on patients treated with sunitinib or sorafenib have yielded conflicting results with regard to baseline CEP/CEC numbers or change in these parameters and clinical outcome (11, 12). This biomarker is thus far limited by several considerations including the fact that both CECs and CEPs are extremely rare events in normal blood, the lack of consensus on definitions of surface marker phenotype, and other technical considerations including heterogeneity in protocols for collection, analysis, and gating strategies. Additional investigation is needed to define the utility of measuring CEC/CEPs to provide insights into therapeutic advances.

Treatment-induced radiographic phenomena have also been studied. Several studies have established the feasibility of measuring various parameters (i.e., tumor size, attenuation, enhancement, and morphology) with a wide variety of radiographic techniques in metastatic RCC patients receiving targeted therapy and correlating to clinical outcome (Table 1; refs. 13–18). For instance, changes in tumor blood flow as measured by arterial spin labeling magnetic resonance imaging (MRI) have been observed at 1 month following treatment with PTK787/ZK222584, a small molecule VEGF receptor inhibitor, in metastatic RCC patients (14). These changes correlated with clinical

objective response observed at 4 months of therapy. An alternative MRI-based method, dynamic contrast enhanced (DCE) MRI, showed a high baseline Ktrans, the volume transfer constant of the contrast agent in which a high value indicates more vascular permeability, correlated with PFS on subsequent sorafenib therapy (15). However, no correlation between change in Ktrans with therapy and clinical outcome was shown. These two studies highlight some of the challenges facing the use of radiographic techniques as a biomarker, namely the variable and often labor-intense techniques for imaging and data analysis that can lead to variable results. What is critically missing from these early small studies, and will require much larger, prospective investigation, is whether baseline or treatment-induced radiographic changes, independent of alterations in tumor size, add meaningful clinical value to the care of metastatic RCC patients. The demonstration of baseline or early radiographic parameters that are independent of known baseline clinical prognostic factors and predict therapeutic success or failure (and thus allow an early switch to alternative therapy) are needed. Additionally, radiographic markers to predict long-term tumor burden control (in the absence of obvious size reduction) would represent a significant advance in clinical care of RCC patients. To date, it is not clear how to incorporate enhancement characteristics into clinical practice, and thus alternatives to standard computed tomography (CT)-based size assessment have not yet emerged as therapeutically relevant.

The development of treatment-induced hypertension has been shown to correlate with clinical outcome in RCC with sunitinib and axitinib, small molecule VEGF receptor inhibitors, and bevacizumab, a VEGF ligand-binding agent (19–22). These analysis have looked across several definitions of hypertension [i.e., according to grade of toxicity by Common Terminology Criteria for Adverse Events (CTCAE) criteria or specific systolic and/or diastolic thresholds], and with various clinical outcome measures (objective response, PFS, and overall survival). Consistently, patients who develop treatment-induced hypertension have a superior clinical outcome, correcting for duration of time of therapy and baseline prognostic variables. The precise mechanism underlying this phenomenon has yet to be elucidated, but strategies to optimize therapy on the basis of these observations are being prospectively tested (Table 1). A front-line trial of axitinib in metastatic RCC will initially treat all patients at standard dose (5 mg twice daily), with dose escalation for patients who do not develop hypertension or unacceptable treatment-related toxicity. This trial is based on analyses showing hypertension and axitinib drug levels are independently associated with clinical outcome (22). Further, this trial incorporates ambulatory blood pressure monitoring to more precisely

Table 1. Emerging therapeutic strategies in metastatic RCC

Biologic Hypothesis	Relevant Data	Therapeutic Strategy	Comments
Response to agents targeting vasculature will be greatest in tumors with enhanced vascularity	Baseline and/or post-treatment tumor vascularity correlates with clinical outcome to VEGF inhibitors in small, retrospective series (14–18)	Limit therapy to the most vascular tumors and/or use vascularity changes during treatment to guide therapy	Measuring tumor vascularity can be technically difficult, cumbersome and expensive; Reproducibility of techniques not validated
Hypertension reflects VEGF signaling disruption in the vasculature and is an on-target effect that parallels antitumor effect	Hypertension in RCC patients treated with VEGF-targeting agents is associated with good clinical outcome (19–22)	Therapy intensification (e.g., dose escalation) or early therapy discontinuation in patients not becoming hypertensive	Molecular basis of this phenomenon not understood; Requires initial treatment of all patients
Reemergence of VEGF-driven vasculature is a hallmark of resistance to anti-VEGF therapy	Preclinical models show restoration of blood flow with continued anti-VEGF therapy (14)	More complete VEGF blockade through combination therapy	Tolerability is uncertain; Combination therapy may compromise the ability to give efficacious single agent doses
Alternative proteins are upregulated in setting of persistent VEGF or mTOR blockade	Preclinical models have identified a number of candidate proteins (29–32, 42–45)	Combination therapy of an anti-VEGF or mTOR inhibitory agent with an agent blocking the relevant protein.	Multiple proteins and/or pathways likely involved in resistance and thus individualized treatment approaches may be needed
Non-anti-angiogenic mechanisms (e.g., immunostimulatory) contribute to the antitumor effect of existing agents	Immunomodulatory properties of sunitinib have been shown (49–51)	Combination therapy with immunostimulatory agents	Significant toxicity in early trials has precluded adequate dosing

characterize blood pressure changes and association with clinical outcome.

Another area of investigation is analysis of molecular predictors of toxicity to targeted agents in RCC, with implications for drug selection, dose and/or schedule optimization, and early intervention for specific toxicity. An initial report in 219 metastatic RCC patients treated with sunitinib examined 31 single nucleotide polymorphisms in 12 candidate genes and identified several significant associations of certain haplotypes with specific toxicities (23). Separate analyses in a similar patient population identified the VEGF-634 C/C genotype associated with increasing frequency and duration of sunitinib-induced hypertension (diastolic > 90 mmHg and/or systolic > 150 mmHg), which remained significant adjusting for baseline blood pressure and use of antihypertensive medication (24). These preliminary retrospective reports generate hypotheses that specific and clinically useful genotypes can be identified but require much larger prospective patient samples to validate and ultimately incorporate into clinical care.

Understanding drug resistance and strategies to overcome resistance. The precise mechanisms of resistance to targeted therapy have yet to be fully elucidated, but initial evidence suggests that angiogenic escape, i.e., reemergence of tumor-associated vasculature, occurs with continued VEGF suppression (Table 1). Immunohistochemical analyses of tumors in RCC xenograft models resected shortly after beginning treatment with sorafenib, a VEGFR inhibitor, reveal a pruning of the microvasculature visualized by immunohistochemical and radiographic perfusion techniques (14). The development of resistance, however, is consistently preceded by the restoration of blood flow suggesting that resistance involves reestablishment of a vasculature that is less dependent, but not necessarily independent, of VEGF.

Therapeutically, the resulting strategy could involve several approaches. Combinations of VEGF-inhibiting agents (either at initiation of therapy or the addition of another agent at progression) could theoretically further suppress the VEGF pathway, enhancing the immediate therapeutic anti-angiogenic effect and/or forestall angiogenic escape. Additional data in a murine RCC model (Renca) using endostatin, neuropilin, and thrombospondin support superior reduction in microvessel density and reduction in lung metastases tumor volume with a combination approach (25). Whether these data apply to current clinical agents or whether greater initial effect would translate into delay of resistance is unknown at present. In RCC, however, initial attempt at combinations of VEGF agents have been met with limited success owing to toxicity (26–28). Most if not all patients do not tolerate full doses of both agents, and thus continued combination therapy for the duration of time required to surpass the expected duration of sequential single agent efficacy is not achievable with current agents. Additional clinical trials of combinations are ongoing, and this approach may require novel dose and/or scheduling of agents, or entirely new agents altogether to be of therapeutic utility.

Another potential resistance mechanism is the upregulation of alternative proteins and/or pathways that could drive tumor angiogenesis and/or growth independent of VEGF (Table 1). Preclinical studies, involving RCC and non-RCC models, have identified a variety of candidate proteins that may be involved in resistance to VEGF therapy. These proteins include fibroblast growth factor (FGF), ephrin and angiopoietin family proteins, interleukin-8, and PlGF, and stimulate angiogenesis directly or indirectly (29–32). Inhibition of these proteins in these models was shown to inhibit tumor growth in the setting of resistance to VEGF therapy. The therapeutic strategy that emerges from these data are combination therapy of an anti-VEGF agent with an agent blocking the relevant protein. Bevacizumab has shown benefit when combined with interferon alpha, an agent reported to have bFGF inhibiting activity, compared with interferon monotherapy in two phase III trials (2, 6, 33). However, the lack of a bevacizumab monotherapy arm in either trial precludes the ability to estimate the additive effect of interferon. Another relevant agent in clinical development is an angiopoietin-2 inhibitor, AMG386 (Amgen; refs. 34, 35). AMG 386 is a construct that involves the Tie2 receptor linked to an immunoglobulin. AMG386 combined with sorafenib produced tumor response in 29% of patients with RCC including some with resistance to prior VEGF-inhibiting therapy (36). These results have led to a randomized phase II trial of sorafenib ± AMG386 in previously untreated patients with RCC, which has completed accrual. In addition, a multi-institutional phase II trial of sunitinib and AMG386 has recently been initiated. Other such studies await further identification and validation of relevant proteins and/or appropriate inhibitory agents.

A distinct approach to overcoming angiogenic escape would be novel scheduling of existing agents. Sunitinib is a VEGF receptor inhibitor approved as an intermittent schedule of 4 weeks on drug and 2 weeks off. Alternative, lower dose continuous therapy has also been investigated (37). Comparison of these approaches is limited at present due to nonrandomized trials with varying lengths of follow up, but there may be important clinical and toxicity differences that may allow for more individualized dosing. A randomized trial of continuous versus intermittent sunitinib dosing has completed accrual with results expected soon. These data will provide further insight into optimizing therapy through dose and schedule modification. In addition, given the universal resistance to targeted therapy that develops with continued therapy, several lines of evidence support an approach of initial treatment followed by several weeks or longer off therapy. Recent data from RCC xenograft models indicate that resistance to sorafenib, as well as most of the associated changes in gene expression, are reversed by reimplantation of the resistant xenografts into untreated mice (38). Clinically, antitumor effect of therapy reinitiation after holding drug for a period of time has been shown. In the randomized discontinuation trial of sorafenib, patients on placebo who crossed over at progression to reinitiate sorafenib had an identical PFS (24

weeks) as those patients who continued sorafenib (39). Further, a recent retrospective report of 23 metastatic RCC patients who were rechallenged with sunitinib (after previous progression on sunitinib and administration of other agents) showed a 22% partial response rate and a median PFS of 7.2 months (40). Prospective trials are planned, and such strategies may most appropriately balance the goal of tumor burden control with other issues such as toxicity and quality of life.

mTOR inhibitors have emerged as therapeutically relevant in RCC, most notably in patients with adverse risk features (temsirolimus) and patients refractory to one or more VEGFR inhibitors (everolimus). As such, they have broadened the therapeutic approach to RCC and are part of the strategic armamentarium moving forward. Existing mTOR inhibitors form a complex with the FK binding protein (FKBP) and prohibit activation of one subpopulation of a multiprotein complex termed mTOR complex 1 (mTORC1). An additional complex, mTOR complex 2 (mTORC2), holds mTOR in a form that may not be inhibited as readily by these agents (41). Thus, the use of small molecule TOR kinase inhibitors that target both mTOR complexes could enhance the therapeutic effect of blocking this pathway. Such agents have yet to be tested in RCC. Additional data suggest that inhibition of TORC1 with rapamycin and its analogs leads to a compensatory activation of PI3 kinase and protein kinase (AKT). These molecules can potentially drive resistance via upregulation of mTORC2, which activates AKT and HIF2 alpha with resultant downstream signaling implications (42–45). Therapeutically, administration of Akt inhibitors after mTOR therapy resistance could be effective. A small subset (n = 16) of a study with perifosine, an AKT inhibitor, in patients refractory to both prior VEGF- and mTOR-targeted therapy, however, showed minimal activity (46). Additional sequential and combination studies are needed to further define the utility of this approach. The compensatory hypoxia inducible factor (HIF) activation noted could also be addressed by combining mTOR inhibitors with VEGF blocking agents, as the tumor-promoting effect of HIF upregulation is presumably through enhanced VEGF secretion. Initial clinical trials of each mTOR inhibitor with bevacizumab have shown tolerability and promising signs of clinical efficacy (47, 48). Large scale randomized trials are underway and planned to further investigate the therapeutic benefit of this approach.

Another emerging therapeutic strategy stems from data identifying potential alternative mechanisms of antitumor effect for existing therapy (Table 1). Sunitinib has been shown in preclinical models and in patients to favorably alter the immune cell environment through promotion of a T-helper 1 phenotype, reduction in T-regulatory cells and reduction in myeloid derived suppressor cells (49–51). Although the contribution of these observations to the antitumor mechanism of sunitinib is unclear at present, they provide rationale for combination of sunitinib, and perhaps similar agents, with immunotherapeutic approaches. A clinical trial was undertaken with sunitinib and an inhibitory antibody against cytotoxic T lymphocyte antigen-4 with this preclinical rationale. This combination was found to produce unacceptable toxicity, mostly in the form of renal failure, underscoring the complex biology and potential off-target effects of such combinations (52). Similarly, sunitinib plus interferon produced unacceptable toxicity, even at low sunitinib doses (53). Further trials of VEGF suppression and immunotherapy are ongoing.

Conclusions

The abundance of active drugs in metastatic RCC has resulted in unprecedented tumor burden control and survival in this patient group. However, harnessing the activity of these agents and building upon the clinical activity to take the next step in further extending the lives of more metastatic RCC patients and achieving definitive cure will be challenging. Translational initiatives have resulted in preliminary observations about the biology of response and resistance to targeted therapy in RCC. Further investigation, both preclinically and clinically, is needed to validate the hypotheses generated and translate them into significant new treatment strategies in RCC.

Disclosure of Potential Conflicts of Interest

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