

# Overcoming VEGF resistance in renal cancer: biologic and therapeutic implications

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Over the last few years, significant progress in the management of metastatic renal cell carcinoma (RCC) has been made. Although currently US FDA-approved therapies have shown dramatic efficacy, none of these have led to complete and durable responses. In addition to their complex side-effect profile, all patients eventually developed progressive disease and ultimately succumbed to their disease. Multiple clinical trials have addressed the importance of sequential therapy in RCC; however, none of them have built molecular correlative studies that would allow us to define the process of resistant disease. Emerging preclinical data demonstrate that VEGF remains an important driver in the resistance setting. Newer pathways such as the Tie2/ANG-2 pathway also appear to contribute to the resistant process. Future clinical trials will incorporate some of these concepts with the goal of providing a more rational selection of sequential therapies in metastatic RCC.

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Modern understanding of the molecular biology of clear cell renal cell carcinoma (RCC) has established the role of the VEGF pathway and its inhibition as a primary therapeutic target. Consequently, the management of RCC has undergone a transformation in the past decade. Metastatic RCC (mRCC) has witnessed the greatest change, with the advent of VEGF and mTOR targeting agents. Unfortunately, as is the case with most other cancers and new agents, the inevitable progression of disease signaling the emergence of resistance to the treatment has become the next challenge in treatment of mRCC. To date, there is not a clear understanding of the true mechanism of resistance, yet several clinical trials evaluating sequential therapy have either been completed or are underway. Although clinically relevant, none of these trials have been designed to address the most important question in the disease: how resistance emerges and how can one overcome it [1–5]? Future clinical trials require a thoughtful design process that incorporates biologic end points. Such a design will allow a better understanding of the management of RCC patients after they have received and failed primary VEGF therapy. This review provides an update on some of the proposed mechanisms behind the development of resistant disease and discusses recent preclinical and experimental development of novel next-generation agents that might be able to overcome this process and translate into a clinical benefit to RCC patients.

## Existing biologic rationale for current agents

The pathogenesis of RCC was elucidated by the discovery of the *VHL* gene from studies of familial VHL syndrome [6]. Angiogenesis is a critical component of tumor growth and metastasis, and central to this is VEGF. Solid tumors are

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frequently hypoxic and this correlates with clinical course and outcome [7]. The main pathway regulating gene induction in response to hypoxia is controlled by transcription factors HIF-1 $\alpha$  and -2 $\alpha$  [8–10], which are, in turn, regulated by ubiquitin-mediated proteolysis and are targeted for destruction by the pVHL in normoxia and stabilized under hypoxia [11–14]. In sporadic clear cell RCC, *VHL* gene loss of heterozygosity has been shown in 84–98% of cases and mutation in the remaining allele has been observed in approximately 50% of the cases [15]. Mutations in the *VHL* gene, as in sporadic renal cancer and VHL syndrome, result in expression of HIF-1 $\alpha$  and -2 $\alpha$  in normoxia and induction of hypoxia-responsive genes that play a significant role in migration, proliferation, tumor angiogenesis and progression, most notably VEGF, PDGF [16] and TGF- $\alpha$  [17]. Existing agents capable of targeting VEGF exert their function through different mechanisms, including direct inhibition of VEGF as a ligand, as is the case of the recombinant human monoclonal antibody bevacizumab, which binds and neutralizes all biologically active isoforms of VEGF [18–21]. Oral tyrosine kinase inhibitors, such as sorafenib, sunitinib, pazopanib and the newly US FDA-approved agent axitinib, exert their function by inhibiting the phosphorylation of VEGF receptor [22–35]. These agents also inhibit other receptors involved in tumor growth and proliferation. The off-target effects observed with these are in fact responsible for some of the adverse events (AEs) observed with many of these agents.

#### Clinical definition of resistance to VEGF inhibitors

The current standard of care is continuous treatment with VEGF-targeted agents until progression of disease or unacceptable toxicity. Given that there are other agents available in the treatment of mRCC, it is reasonable to consider progression of disease on an adequate treatment regimen as evidence of resistance. In this case, there is a ‘fundamental’ shift to alternative tumor-promoting pathways that require a change in agents and maybe a change in target. Currently, the most commonly used method is the Response Evaluation Criteria In Solid Tumors (RECIST criteria), although given the necrosis caused by VEGF-targeting agents, solely relying on size may be inadequate and other functional imaging techniques may prove more useful [36]. As such, any increase in size >20% in the sum of measurable lesions, appearance of new lesions, or an unequivocal progression in non-measurable disease, would constitute progression of disease and therefore resistance to therapy. It is important to note that the development of resistance to VEGF-targeting agents is consistently preceded by the restoration of

blood flow on perfusion scans, as well as infiltration of the necrotic tumor remnant by endothelial cells [37]. However, until these tools are more widely available in clinical practice, RECIST criteria remains the most common approach to determine presence of resistant disease in clinical practice.

However, it is important to recognize that drug intolerance does not make a patient resistant to a particular agent. This is in fact one of the major challenges when interpreting data in the second-/third-line setting as these individuals are often enrolled in trials that are evaluating subsequent systemic therapy. Other cases where resistant disease might not represent a true change in the biology of the disease includes cases of reduced absorbance or increased clearance.

#### Mechanisms of VEGF inhibitor resistance

Mechanisms of resistance to VEGF inhibitors can be divided into two conceptual models: adaptive (evasive) or intrinsic (adaptive) [38]. The categories and mechanisms under each category are discussed in more detail in the following paragraphs (Figure 1).

##### ■ Adaptive mechanisms of resistance

This category includes mechanisms that involve a tumor response to the presence of antiangiogenic agents, namely, VEGF inhibitors. It is hypothesized that tumors acquire means to functionally evade the VEGF-inhibitor effects and go through ‘angiogenic escape’. Potential mechanisms are discussed below.

Gene mutation: a critical component of the pathway Although this is a common mechanism of drug resistance in some cancers [39–42], mutation is thought to be an unlikely explanation for resistance to VEGF inhibitors. The argument against this hypothesis is that the receptor that needs to undergo a mutational change resides in the endothelium of the tumor vasculature and multiple mutations with similar end results are needed to confer resistance to VEGF inhibitors in the primary tumor and metastatic sites; an extremely unlikely occurrence.

Experimental models lend support by showing that resistance to sorafenib is reversed if tumor cells are implanted. Mutations are not expected to reverse to normally functioning genes [43].

##### Emergence of alternative proangiogenic mechanisms

Xenograft models of tumor perfusion and VEGF inhibitors show that while on VEGF inhibitors, certain areas of the tumor microvasculature undergo necrosis that is detectable by perfusion scanning. As

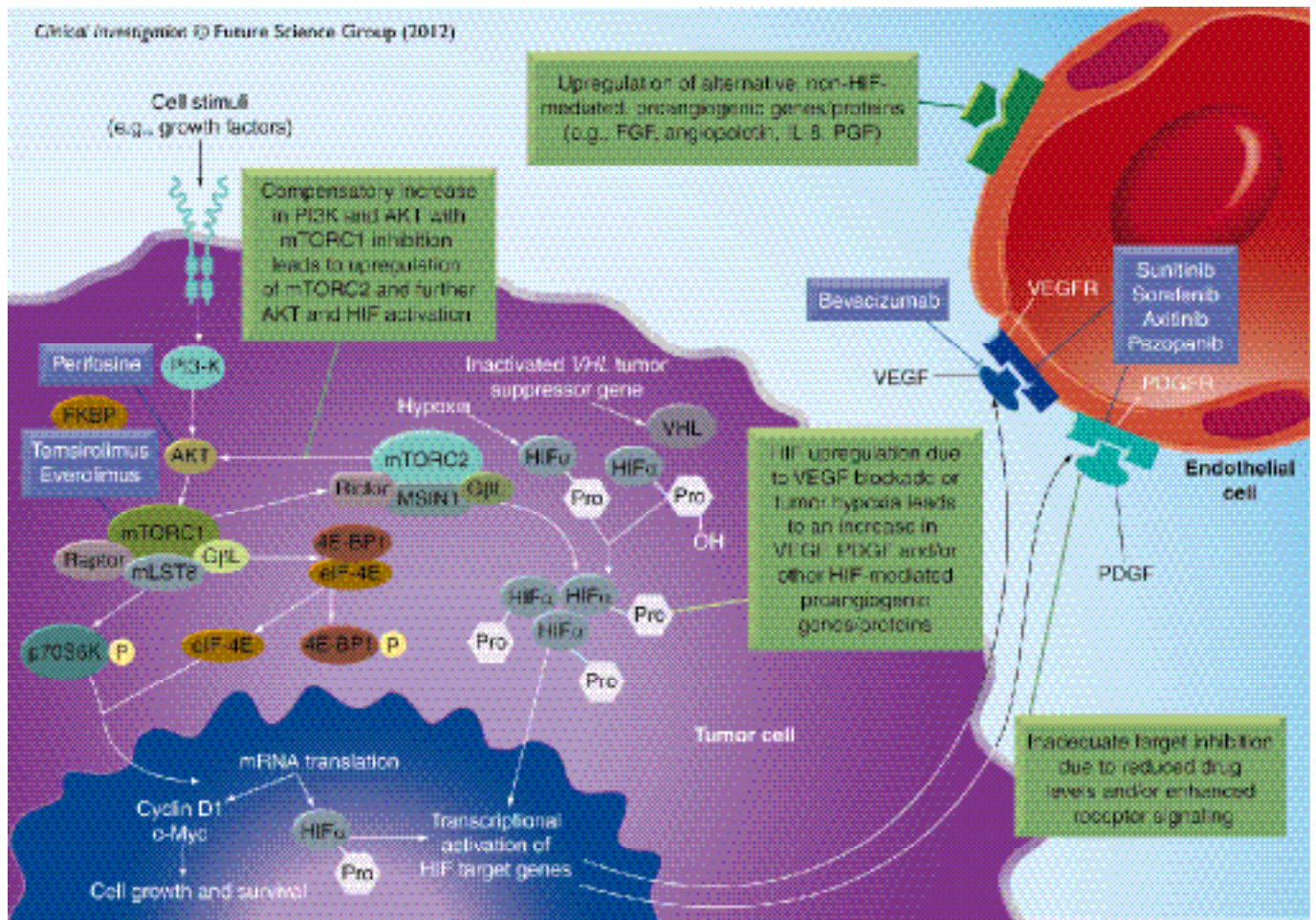


Figure 1. Mechanisms of VEGF inhibitor resistance.

Reproduced from [1].

discussed above, development of resistance is heralded by restoration of the perfusion. This supports the hypothesis that an alternative mechanism supports the growth of microvasculature [37].

In a mouse model of a pancreatic neuroendocrine tumor, investigators demonstrated that tumor growth is transiently inhibited by blocking the VEGF receptor-2 and the resistance coincides with increased levels of mRNA for FGF1 among others; FGF2, ephrin A1, A2 and ANG-1. This resistance was delayed by the introduction of an FGF trap suppressing the FGF signaling mechanism, providing evidence for alternative angiogenic regulators [44,45]. Of interest is the fact that IFN- $\alpha$  has been reported to have basic FGF-inhibiting activity [46].

#### Upregulation of HIF1- $\alpha$

A conceivable mechanism of resistance is upregulation of the HIF-1 $\alpha$  pathway leading to increased levels of circulating VEGF and PDGF overcoming the receptor

blockade. Although no molecular evidence directly supports a role for this mechanism, indirect evidence from clinical trials, which have used a subsequent VEGF inhibitor after tumor progression on first-line therapy with a VEGF inhibitor [32–34,47], lend some support to the fact that even after development of resistance, the tumors, at least in part, depend on VEGF and therefore may respond to a different VEGF inhibitor.

#### IL-8

A xenograft model that mimicked clinical resistance to sunitinib was shown to have a higher microvessel density in sunitinib-resistant tumors. This can be interpreted as an escape from antiangiogenic agents. This escape was found to coincide with increased secretion of IL-8 from tumors into the plasma. In this experimental model, administration of an IL-8-neutralizing antibody resensitized tumors to sunitinib [48].

In patients who were refractory to sunitinib



treatment, IL-8 expression was elevated in clear cell RCC tumors, supporting the concept that IL-8 levels might predict clinical response to sunitinib [48]. Similar findings were noted in models of other cancers, giving further support to the role of IL-8 in angiogenesis [49].

#### PGF

PGF is a VEGF homolog. It has been shown that levels of PGF increase after treatment with bevacizumab as well as VEGF tyrosine kinase inhibitors [26,50,51]. This is an indicator that antiangiogenic escape may also be linked to PGF, although, it is important to underline the questionable relevance of this particular mechanism as sunitinib is also an inhibitor of PGF.

#### Angiopoietin pathway

The Tie2/ANG-2 axis is a powerful pathway, perhaps as important as the VEGF pathway. Its inhibition has been shown to suppress tumor growth. It is also indirectly involved in secretion of VEGF, through matrix metalloproteinases (MMPs). Furthermore, similar to PGF, its levels increase in the plasma of patients treated with sunitinib and is correlated with tumor resistance. Theoretically, inhibition of ANG-2, either at the time of resistance or in the first-line, could be an important target.

A Phase II clinical trial of AMG 386, an inhibitor of angiogenesis through sequestration of ANG-1 and -2, in combination with sorafenib in previously untreated mRCC patients (clear cell), did not improve PFS compared with sorafenib plus placebo. Increased objective-response rate and the observed reduction in tumor burden are suggestive of an antitumor effect of AMG 386 in mRCC [52]. The role of this pathway is still not clear.

#### Pericytes & the PDGF receptor

Pericytes are recruited by newly formed microvasculature and decrease the sensitivity of the newly formed endothelial cells to VEGF inhibitors. Animal models have demonstrated that dual inhibition of endothelial cells by VEGF inhibitors and pericytes by PDGF receptor inhibitors may increase efficacy, therefore implicating PDGF receptor as a potential mechanism of resistance to VEGF inhibitors [53–55].

#### ■ Intrinsic resistance

This group includes tumors that do not respond to VEGF inhibitors whatsoever. Clinically, these are patients who progressed either clinically or radiographically, soon after initiating first-line VEGF therapy. It is likely that in these patients, their

tumors are driven by completely different pathways to VEGF or mTOR. It is unclear whether this category has any clinical relevance, but it has been shown to exist in at least one mouse model [56]. Adequacy of treatment and aggressiveness of the tumor are among the factors that make determination of a primary resistance to VEGF inhibitors difficult to discern.

#### Strategies to overcome resistance

Although current clinical trials are primarily focussed on clinical end points, multiple trial designs can help elucidate possible pathways of resistance. Adding a second agent at the time of clinical/radiographic failure or initiating combination therapy to delay the process of resistant disease are some of the simple strategies currently undergoing exploration. To date, we have learned that although attractive, combination strategies such as bevacizumab plus temsirolimus increased side effects in a significant manner and did not appear to improve clinical efficacy when compared with single agent therapy [57]. Perhaps the most elementary of all would be to clearly define who is truly resistant to front-line therapy, that is, only those patients with a fundamental shift in the biology of their tumor, rather than those with physiological changes related to pharmacodynamics and kinetics of drug delivery and metabolism. Further exploration of existing and newly proposed mechanisms will continue to be the source of major research efforts in years to come. To that end, using frameworks such as personalized RNA interference to enhance the delivery of individualized cytotoxic and targeted therapeutics to investigate the biomarkers of response and resistance to targeted therapies will allow effective collaboration among the investigators in the field [58].

#### Future perspective

With the continued surge of novel agents in mRCC, a better understanding of the biology responsible for the development of resistant disease is of utmost importance. In the era of personalized medicine, understanding this process would allow for a rational selection of therapy for patients with progressive disease after primary VEGF-targeted therapy. Emerging hypotheses should be further explored and incorporated in clinical trials design. To date, efforts to delay resistance by using combination therapies have resulted in significant AEs. Therefore, a monotherapy given in a sequential manner using biology of resistant disease might become an optimal strategy to maintain control of a ‘chronic disease’ while minimizing AEs and maintaining the quality of life of patients.

Additional trials aimed at defining which agent

is best have become less attractive as most of the existing agents shared similar efficacy and side-effect profiles and none of them has led to durable complete responses. Perhaps understanding the appropriate sequence that can provide patients the best quality of life during therapy would be a more relevant question. Several trials are in fact already addressing such questions. Biologically, we will be pushed to define biomarkers that can be used, not only for treatment selection, but also to define treatment outcome. Existing trials have failed to demonstrate the 'sort-of-expected' association between tumor biology and treatment efficacy. To this end, primary and metastatic tissue will be essential to correlate with serum/plasma markers in future clinical studies. Such trials should consider

### Executive summary

#### Existing agents in renal cell carcinoma

- A dramatic change in the treatment paradigm of advanced renal cell carcinoma has occurred with the availability of at least seven agents that target either VEGF or mTOR signaling.

#### Disease resistance

- All renal cell carcinoma patients eventually develop progressive disease and require subsequent therapy.
- Sequential therapy with either a VEGF or mTOR inhibitor is an appropriate strategy in the second-line setting.
- Clinical resistance appears to be different to biological resistance. Thus, a true biologic definition of VEGF-resistant disease is needed.
- Multiple pathways outside VEGF and mTOR have been identified as potential contributors in the VEGF-resistance process.

#### Strategies to overcome resistant disease

- Various clinical trials evaluating sequential and combination therapy are currently underway.
- A consensus definition for resistant disease is desperately needed.

utilizing biologic rather than traditional clinical end points for clinical trial design.

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