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Using Molecular Biology to Develop Drugs for Renal Cell Carcinoma

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Abstract

Background—Renal cell carcinoma is a disease marked by a unique biology which has governed it's long history of poor response to conventional cancer treatments. The discovery of the signaling pathway activated as a result of inappropriate constitutive activation of the hypoxia inducible factors (HIF), transcription factors physiologically and transiently stabilized in response to low oxygen, has provided a primary opportunity to devise treatment strategies to target this oncogenic pathway.

Objective—A review of the molecular pathogenesis of renal cell cancer as well as molecularly targeted therapies, both those currently available and those in development, will be provided. In addition, trials involving combination or sequential targeted therapy are discussed.

Methods—A detailed review of the literature describing the molecular biology of renal cell cancer and novel therapies was performed and summarized.

Results/Conclusion—Therapeutics targeting angiogenesis have provided the first class of agents which provide clinical benefit in a large majority of patients and heralded renal cell carcinoma as a solid tumor paradigm for the development of novel therapeutics. Multiple strategies targeting this pathway and now other identified pathways in renal cell carcinoma provide numerous potential opportunities to make major improvements in treating this historically devastating cancer.

Keywords

von Hippel-Lindau; Hypoxia Inducible Factor; angiogenesis; vascular endothelial growth factor; targeted therapy

1. Clinical significance

The treatment paradigm of renal cell cancer (RCC) has changed dramatically with the recent advent of several new targeted therapeutic agents. The discovery of these agents has been a direct response to increasing knowledge of RCC molecular biology. These new agents come at an opportune time as RCC has been increasing in incidence and has historically been resistant to therapy. In the United States, the incidence of RCC is predicted to reach 51,190 cases in 2007 and result in 12,890 deaths[1]. This increase reflects a 34% change in the last decade, and presents a major financial burden to society 2.

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The most common histological form of RCC is the clear cell type (ccRCC, which accounts for about 75% of cases), with other variants including papillary (15%), chromophobe (5%), and oncocytoma (5%). Currently, chance for cure is limited to patients in whom surgical resection of localized disease is possible. Historically, locally advanced and metastatic RCC (mRCC) has been treated with immunotherapeutic agents such as the cytokines, interferon and interleukin-2. These cytokine therapies have shown limited effectiveness with response rates in 10–20% of treated patients, and no effectiveness in nonclear cell histologies[3, 4]. In order to establish patients with the greatest likelihood to benefit from therapy, several prognostic scoring systems have been evaluated. One commonly used prognostic system to estimate survival in patients with metastatic disease is based on several clinical factors including functional status, levels of lactate dehydrogenase, hemoglobin, and calcium, and absence of prior nephrectomy. This prognostic system, known as the MSKCC system or Motzer criteria, is widely used to categorize patients as good, intermediate, or poor risk for achieving a lengthy survival5. Although designed in an era when physicians were determining to proceed with or forego cytokine therapy, this system provides the most familiar and useful algorithm currently available. A large portion of patients present with advanced disease and poor prognostic features using this prognostic system, such that only a small subset of patients will be appropriate candidates to even consider immunotherapy. Thus, there has been a great need for novel therapies to treat advanced RCC. Recent advances in our understanding of RCC biology have revolutionized the treatment of RCC, with the advent of several molecularly targeted agents, and many other promising strategies emerging on the horizon.

2. Molecular Biology of Renal Cell Cancer

Von Hippel-Lindau disease (VHL) is an inherited autosomal dominant syndrome that consists of a varied constellation of clinical disease including ccRCC, hemangioblastomas, pheochromocytomas, and visceral cysts[6]. Given the association of ccRCC in this patient population, this disease syndrome was a natural starting point for RCC research. The *VHL* gene is located on the short arm of chromosome 3 and encodes the tumor suppressor protein, pVHL [7]. Functional loss of pVHL occurs in the majority of sporadic and hereditary ccRCC and has been linked to somatic mutation events, loss of heterozygosity and silencing via hypermethylation [8–11]. pVHL plays an important role in the regulation of the cellular response to hypoxia. This was demonstrated by the correlation between the reintroduction of functional wild-type *VHL* into ccRCC cells and the inhibition of expression of hypoxia-induced genes such as vascular endothelial growth factor (VEGF), glucose transporter-1 (GLUT-1), transforming growth factor-α (TGF-α, also known as epidermal growth factor, EGF) and platelet –derived growth factor-β(PDGF-β) [12, 13].

pVHL binds to elongins C and B to form a functional E3 ubiqutin ligase complex which includes Cullin 2 (CUL2) and ring box protein 1 (Rbx1 or ROC1)14, 15. The pVHL-elongin B/C-Cul2 protein complex (VBC) was later discovered to be targeting the transcription factor farmily of hypoxia-inducible factors- α (HIF-1α and HIF-2α), key regulators of the cellular response to hypoxia, for ubiquitylation and subsequent degradation by the 26S proteasome16, 17. This process of pVHL and HIF-α interaction involves the oxygen-dependent hydroxylation of key prolyl residues on the HIF-α subunit by HIF-α prolyl-hydroxylase 18, 19. The functional loss of pVHL leads to an increase in the intracellular concentrations of HIF and subsequently to an increase in expression of HIF's transcriptional target genes[20](Figure 1). These target genes encode numerous factors, some of which are VEGF, PDGF-β, TGF-α, erythropoietin (EPO), carbonic anhydrase IX (CA-1X), and GLUT-1. It is the activation of these downstream target genes that leads to the proliferative, invasive, highly vascular, glycolytic and polycythemic phenotype seen in RCC.

Intracellular HIF levels are also affected by oxygen-independent mechanisms. Two well established pathways implicated in HIF protein synthesis are the phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways[21](See Figure 1). In the former pathway, growth factor ligands (e.g. EGF) engage the extracellular domain of their target receptor (e.g. EGFR) and initiate a cascade of phosphorylation events via PI3K and Akt (protein kinase B). Inhibition of the mammalian target of rapamycin (mTOR) suppresses HIF-1α expression and decreases activation of its target gene, VEGF[22, 23].

mTOR's role in HIF regulation has also been examined in the disease tuberous sclerosis, in which one of the tuberous sclerosis complex tumor suppressor genes (TSC1/TSC2) are inactivated via a germline mutation resulting in the development of hamartomas as well as an increased incidence of ccRCC. The TSC1/2 protein complex provides an important inhibitory signal to mTOR. Cells with TSC2 loss have been shown to have increased amounts of HIF-1α and VEGF and re-introduction of wild-type TSC2 or treatment with an mTOR inhibitor blunts this effect[24].

The MAPK pathway has also been implicated in HIF synthesis. The MAPK p42/44 serine/threonine kinases have been linked to post-translational modification and promotion of HIF-1α-mediated reporter gene expression[25]. HIF transactivation of reporter genes involves the binding of the N-terminal transactivation domain with cofactors p300 and CBP (CREB binding protein) and this interaction has been linked to MAPK signaling[26]. Expression of v-Src has also been linked to increased HIF-1 and HIF-1 target gene expression, suggesting multiple mechanisms of adapting HIF levels27.

RCC cells with upregulated HIF-2α as a result of VHL gene inactivation have been examined with regard to genetic manipulations which will abrogate tumor growth in xenograft models. The introduction of a single short hairpin RNA targeting HIF-2α in these transformed cells is sufficient to inhibit the growth of xenograft tumors, implicating this pathway as a primary target for drug development[28]. The understanding of the complex oxygen-dependent and independent regulation of HIF-1α and HIF-2α and their subsequent transcriptional gene targets has led to the identification of numerous avenues for drug development. Several key targets have included modulation of individual HIF target gene products, inactivation of receptor tyrosine kinase activity implicated downstream of HIF transactivation and inhibition of the mTOR pathway. Several of these drugs have been FDA approved or listed for use in RCC and others are still in development.

3. Tyrosine Kinases Kinase Inhibitors

Tyrosine kinases cause phosphorylation of tyrosine residues that initiate intracellular signaling pathways to promote cell survival, proliferation and angiogenesis. There are several small molecule inhibitors of tyrosine kinases that are being studied in RCC. Two of these, sunitinib and sorafenib have been FDA approved for use in mRCC based on clinical evidence of efficacy in several recent trials. Currently, many others are being evaluated alone and in combination with other drugs for safety and efficacy in the treatment of mRCC and other advanced solid tumors. The clinical evidence and molecular evidence supporting the use of each of these drugs for RCC is discussed below (Table 1).

3.1 Sunitinib

Sunitinib (SU11248, ®Sutent, Pfizer Inc.) is an orally bioavailable multitargeted tyrosine kinase inhibitor that targets VEGFR-1–3, PDGFR-α, -β, FLT3, and c-kit. It has been evaluated for safety in a Phase I trial, with a recommended dose of 50mg per day for 4 weeks followed by 2 weeks off[29]. The main adverse side effects seen at this dose were sore

mouth, edema and thrombocytopenia. At higher doses (>75mg/day) side effects included fatigue, hypertension and bullous skin toxicity.

Based on this dosing regimen, two Phase II trials in metastatic RCC have been completed. In the first, 63 patients with good performance status who had failed at least one cytokine-based therapy were enrolled30. The primary end-point was overall response rate (ORR) with secondary end-points being time to progression (TTP) and safety. In this study, 40% of the patients (24 patients with clear cell RCC and one with papillary –cell type) were noted to have partial responses and 17 patients (27%) had stable disease. The median TTP was 8.7 months. The most common adverse events were fatigue, diarrhea, and lymphopenia. In a second Phase II study of sunitinib in mRCC, 106 patients with clear cell mRCC, good performance status, prior nephrectomy and previous cytokine-therapy failure were enrolled[31]. Results from this trial have recently been updated as data has matured, revealing an objective response rate of 33% (0 CR and 35 patients with a PR). Median duration of resonse was found to be 14 months and median survival was 23.9 months32.

Based on the activity and good tolerability of sunitinib, a multicentered, randomized, Phase III trial was performed evaluating sunitinib versus interferon alfa in previously untreated mRCC patients[33]. In this study, 750 patients with clear-cell histology and good performance status were randomized to receive either interferon alfa with a target dose of 9 million units (MU) subcutaneously (sc) three times weekly or sunitinib 50mg/day 4 weeks on, 2weeks off. The primary endpoint was defined as progression free survival (PFS), with secondary endpoints of objective response rate, overall survival (OS), and safety. The median PFS was found to be 11 months in the sunitinib arm compared to 5 months in the interferon arm (hazard ratio 0.42, $P<0.001$). In addition, 103 patients (31%) receiving sunitinib had objective responses compared to 20 patients (6%) with objective partial responses in the interferon arm ($P<0.001$). Diarrhea, vomiting, hypertension, and hand-foot syndrome were more common in the sunitinib group while grade 3/4 fatigue was more common in the interferon group. Despite these expected side effects, the patients reported a better quality of life in the sunitinib arm as opposed to the interferon arm. Based on clinical evidence of tolerability and efficacy, sunitinib has been approved for the treatment of patients with advanced RCC in both the United States and Europe.

3.2 Sorafenib

Sorafenib (BAY43–9006, ®Nexavar, Bayer Pharmaceuticals Corporation and Onyx Pharmaceuticals Inc.) is an orally bioavailible tyrosine kinase inhibitor of VEGF-R 1–3, PDGFR-β, Flt-3, c-kit, and Raf that has also been approved in the United States and in Europe for use in renal cell cancer. The basis of this approval is supported by the efficacy of the drug seen in several clinical trials. Phase I results from several trials of BAY43–9006 resulted in a recommended dose of 400mg orally twice daily with dose limiting toxicities including hypertension, rash, fatigue and diarrhea34–37. A multicenter Phase II randomized discontinuation trial of sorafenib was subsequently performed based on preclinical data predictions that treatment would result in tumor stabilization rather than tumor shrinkage38. In this study 502 patients with varied solid tumor types were enrolled; however the study was later refocused on RCC with 202 patients having renal cancer (75% clear cell, 7% papillary, 5% other, and 12% missing pathology). The primary endpoint of this study was progression free survival at 24 weeks of treatment. At the end of this 24 week period 50% of the patients receiving sorafenib were progression free while only 18% of those receiving placebo were progression free ($P=0.0077$).

With promising results in early clinical trials using sorafenib in ccRCC, a multicenter randomized, double-blind, placebo-controlled Phase III trial was performed[39]. In this study, 903 patients with metastatic ccRCC, good performance status who had progressed on one

systemic treatment within 8 months of enrollment were randomized for treatment with sorafenib or placebo. These patients were also required to have MSKCC prognostic scores of low or intermediate risk. The primary endpoint was OS, with secondary endpoints being PFS and objective response rate. Early cutoff results revealed a median PFS of 5.5 months for the sorafenib treated group compared to 2.8 months in the placebo group (P<0.01). Interim analysis of OS showed a trend towards survival benefit in the sorafenib group, although this was not significant given the limited number of events at this early timepoint (HR, 0.72; P=0.02). There was one complete response and 43 partial responses in the sorafenib arm (10%) compared to 8 partial responses in the placebo arm. The authors of this study concluded that treatment with sorafenib did result in a significant improvement in PFS, but was not surprisingly more toxic than placebo alone. In a separate study of this cohort, symptoms and quality of life were evaluated to determine the impact of treatment with sorafenib, no significant adverse impact on quality of life was evident in the sorafenib treatment group[40].

Recently, results from a Phase II intrapatient sorafenib dose escalation study have emerged[41]. In this study 46 patients with metastatic ccRCC, good performance status, and limited prior cytokine therapy (≤1) were started on sorafenib 400mg twice daily and doses were increased by 200mg twice daily every 28 days as tolerated to a goal of 800mg twice daily. Of 45 evaluable patients, 91% were able to tolerated titration to 1200 to 1600mg daily. An overall response rate of 52% was noted with a median time to progression of 8.4+months. This raises the intriguing possiblity that dosage and pharmacokinetics may be very tightly associated with tumor response, but at this time requires further investigation and validation before being adopted into clinical practice.

3.3 Axitinib

Simliar to sorafenib and sunitinib, other tyrosine kinase inhibitors are being evaluated for clinical efficacy. One such agent is axitinib (AG-013736, Pfizer) which is an orally bioavailable receptor tyrosine kinase inhibitor that targets VEGF-R type 1–3, PDGF, and c-kit. It has been evaluated in the Phase I setting in multiple advanced solid tumor types and demonstrated managable toxicities and efficacy42. In this trial of 36 patients, 6 patients had RCC and there were 2 partial responses within this group. Dose limiting toxicities were hypertension, hemoptysis, and stomatitis. The MTD was found to be 5mg twice daily. In two follow-up Phase II trials, axitinib continued to demonstrate tolerability and efficacy. In the first Phase II trial, 62 patients that were all sorafenib refractory were placed on axitinib 5mg twice daily and the dose was escalated to 7 or 10 mg or decreased to 2 or 3 mg based on tolerability43. In this study 21% of patients had a partial response and 34% had stable disease. At 8 months of follow-up, the median PFS was 7.4 months and median overall survival had not been reached. In a separate Phase II study of axitinib in the treatment of mRCC patients that had previously failed at least one cytokine-based therapy, 52 patients were enrolled to receive axitinib and twenty-one patients (40%) had a partial response44.

3.4 Vatalanib

Vatalanib (PTK787/ZK222584, Novartis Pharmaceuticals) is another receptor tyrosine kinase inhibitor with much more specific activity targeting VEGFR types 1–3. It is being evaluated in multiple tumor types, including RCC. A Phase I dose escalation trial of this drug in mRCC was reported in 2003[45]. Forty-five patients were enrolled and of 37 evaluable patients, 7 had responses with 1 partial response and 6 minor responses. The median TTP was 5.5 months. Although there are many factors in drug development that impact on an agents activity, it is tantalizing to speculate that the attenuated activity of this drug compared to the clinical experience with other drugs in this class occurs as a result of the highly specific nature of this drugs interaction with it’s target kinases, the VEGF receptors. In a

survey of kinase inhibitors binding to a panel of 116 known kinases, the pattern of interactions demonstrated by this drug confirmed it's very specific activity 46. While this may limit this drugs single agent activity in renal cell carcinoma, it may be a more well tolerated agent for other diseases which would benefit from specific inhibition of VEGF receptor signaling. Additionally, this observation promotes the speculation that the broad activity of other tyrosine kinase inhibitors is a direct result of activity at multiple target molecules simultaneously. This concept will be revisited below in the discussion of rationally combining agents to expand the potential clinical benefit.

3.5 Pazopanib

Pazopanib (GW 786034, GlaxoSmithKline) is a potent inhibitor of VEGF-types 1–3, PDGFR- α,-β and c-kit which has been studied in two Phase I trials and shown evidence of clinical activity and tolerability. Common adverse effects seen in these studies included hypertension, nausea, diarrhea, GI bleed, and fatigue47, 48. This drug has also been studied in the Phase II setting in RCC49. Patients with advanced or mRCC who were cytokine-naïve or refractory to cytokine or bevacizumab therapy were enrolled to receive pazopanib 800mg daily in a randomized discontinuation designed clinical trial. After the first 60 patients completed this 12 week period, an interim analysis was done which showed a PR in 24 (40%) and stable disease in 25 (42%). Based on the interim analysis results, it was decided that patients with stable disease would continue on active drug and not be randomized to placebo.

3.6 Semaxanib

SU5416 (semaxanib, Pfizer) is a small molecule inhibitor with highly selective activity at VEGFR-2 that has been studied in the Phase I setting and shown to have managable toxicity with the recommended dose of 145mg/m2 twice weekly. Common adverse side-effects include headache, nausea/vomiting, asthenia, and fever50, 51. This compound was examined in advanced RCC in a Phase II trial which combines SU5416 with interferon has been performed52. In this study, 30 patients were administered 145mg/m2 of SU5416 twice weekly and interferon 1 MU subcutaneously twice daily on a 6 week cycle. Fifteen patients (50%) were found to have stable disease. The median survival was 10 months and event-free survival at one year was 6%. Like valatanib, this drug's highly specific activity likely limits its activity in RCC, but may benefit it's utility in other disorders, and lends some credence to the notion that the activity of other compounds is largely contributed by the multiplicity of targets. Based on these results the authors concluded that this combination was not worth pursuing.

3.7 AZD-2171

AZD2171 (AstraZeneca International) is another highly potent orally bioavailable inhibitor of VEGFR types 1–3 which is being evaluated in RCC. In a Phase I clinical trial involving multiple tumor types it showed tolerability and disease responsiveness53. In this trial 83 patients were treated and there were 2 patients with a PR and 22 patients with stable disease. Common dose-related adverse effects included hypertension, diarrhea, and dysphonia. In a review of two Phase I trials which included patients with multiple tumor types, AZD2171 either as monotherapy or in combination with gefitinib showed early efficacy54. In the monotherapy study, 2 of 3 RCC patients showed tumor regression and in the combination study (AZD plus gefitinib 250mg or 500mg) there were 16 RCC patients evaluated with 6 PRs and 7 with stable disease. Also data from a recent Phase II study has been reported in patients with unresectable, advanced RCC without prior therapy55. In this study of 16 evaluable patients, there were 6 partial responses, 1 unconfirmed partial response and 5 with stable disease.

3.8 EGFR Inhibitors

Several inhibitors of the receptor tyrosine kinase, epidermal growth factor receptor (EGFR) including both monoclonal antibodies and tyrosine kinase inhibitors have been evaluated in RCC. TGF-α which is a ligand for the EGF receptor is also a gene target of HIF and is upregulated in RCC tumors, setting up a potential autocrine stimulation loop, as activated EGFR is frequently observed in these tumors. Therefore inhibition of the EGF receptor was a natural target for therapy. Unfortunately, as single agents receptor tyrosine kinase inhibitors of EGFR (erlotinib and gefitinib) as well as monoclonal antibodies against EGFR (cetuximab) have not performed well in the treatment of RCC[56–58]. Despite the failure of these agents as monotherapies, combination therapy with other agents has been theoretically appealing based on the known cell signaling pathways involved in RCC, but has unfortunately thus far failed to produce major responses or substantial disease improvement.

Early studies were provocative. Erlotinib (OSI-774, OSI Pharmaceuticals) is a receptor tyrosine kinase inhibitor of EGFR and has recently been evaluated in combination with bevacizumab. In a multicenter, Phase II trial 63 patients with metastatic clear cell RCC were enrolled to receive both bevacizumab 10mg/kg intravenously (IV) every 2 weeks and erlotinib 150mg po daily[59]. The patients were re-evaluated after 8 weeks of therapy. Fifteen patients had objective responses (25%, 14 PRs and 1 CR) and another 36 patients had stable disease (61%). The median PFS for all patients was 11 months. The most frequent adverse events were diarrhea and rash.

A follow-up Phase II trial comparing the combination of erlotinib plus bevacizumab compared to placebo plus bevacizumab has been performed[60]. In this trial previously untreated metastatic RCC patients (n=104) were treated with bevacizumab 10mg/kg IV every 2 weeks and with either erlotinib 150mg po daily or placebo. PFS for the bevacizumab arm with placebo was 8.5 months compared to the combined treatment arm which was 9.9 months (p=0.58). Despite the theoretical appeal of combining EGFR targeted therapy with VEGF targeted therapy, thus far clinical studies have not been supportive.

4. Monoclonal Antibodies

Monoclonal antibodies and peptide specific antibody based technologies have led the development of directed therapies owing to their high degree of specificity and in vivo stability. Below will describe the antibody based therapies in development directed against components of the hypoxia response pathway (summarized in Table 2).

4.1 Bevacizumab

Bevacizumab (Avastin, Genentech) is a recombinant humanized monoclonal antibody which binds and neutralizes all isoforms of VEGF-A, thereby acting as an anti-angiogenesis agent. It has been studied in many cancer types and is FDA approved for use in both metastatic colorectal cancer and nonsmall cell lung cancer. It has also been studied as single agent therapy and combination therapy in metastatic RCC. A randomized, double-blind Phase II trial has been performed comparing low dose and high dose bevacizumab to placebo[61]. In this study, 116 patients with clear cell metastatic RCC were randomized to receive either placebo or bevacizumab (3 or 10 mg/kg). Adverse events that were significantly greater in the high dose (10 mg/kg) treatment arm included epistaxis, hypertension, hematuria and proteinuria. The primary end-points of the study were TTP and OS. TTP was significantly longer in the high-dose treatment group compared to the placebo group. At 4 months after randomization the percentage of patients receiving high-dose, low-dose, and placebo therapy who were progression-free were 64%, 39%, and 20 %, respectively. There were 4 patients who had PRs (no complete responses) and all of these were treated with high-dose

bevacizumab, giving this group a 10% response rate. At the final analysis reported there was no significant difference in OS between the three groups.

Recently data on a Phase III multinational, double-blind placebo controlled trial, comparing interferon alfa-2a with or without bevacizumab, was presented[62]. This study enrolled 649 patients with untreated mRCC who were randomized 1:1 to receive either interferon alfa-2a (9MU) sc three times per week along with bevacizumab (10mg/kg) IV every 2 weeks versus interferon alfa-2a (9MU) sc three times per week plus placebo. ORR was significantly higher in the bevacizumab plus interferon group compared to placebo plus interferon (31% versus 13%, P <0.0001). Also there was a significant improvement in the median PFS in the bevacizumab group compared to the placebo group (10.2 months versus 5.4 months, HR 0.63 P <0.0001). Consistent with previous RCC trials, when stratified according to risk score, there was no difference in PFS in those with a poor MSKCC risk score. Median OS has not been reached in the bevacizumab plus interferon group, while the median OS in the placebo plus interferon group was 19.8 months. Most common grade 3/4 adverse events in the bevacizumab containing regimen included fatigue, proteinuria and hypertension. A similar Phase III trial (CALGB 90206) is being conducted and has met accrual, but has not yet undergone analysis[63].

4.2 VEGF Trap

VEGF Trap® (aflibercept, Regeneron Pharmaceuticals, Inc.) is a novel agent which is a fusion of the Fc portion of human IgG to the human VEGFR −1, and −2 extracellular domains. This results in binding and neutralization of all VEGF-A isoforms as well as placental growth factor. In a Phase I dose-finding trial 16 patients with advanced solid tumors were evaluated[64]. One patient with RCC had stable disease for greater than 6 months. Most frequent adverse events included fatigue, pain, and constipation. Further studies with this uniquely designed agent are anticipated.

4.3 AMG 386

AMG 386 is an interesting compound which represents a new class of agents designed for antiangiogenic therapy. AMG 386 (Amgen, Inc.) is a neutralizing peptibody designed to disrupt the engagement of the Tie-2 receptor by angiopoietin 1/2. This has immediate implications for the treatment of renal cell carcinoma, as blockade of the angiopoietin signaling pathway represents a novel strategy to inhibit angiogenesis, and angiopoietin 1 and 2 both represent transcriptional targets of HIF activation. A phase 1 study has demonstrated overall tolerability, and treatment causing declines in FDG-PET signal and DCE-MRI consistent with antiangiogenic activity[65]. The overall safety profile of this novel class of compounds, in particular the lack of effects observed with conventional blockade of VEGF signaling such as bleeding, hypertension, and proteinuria, make it a particularly attractive agent for combination approaches. Such approaches are ongoing to expand vertical blockade to maximize clinical efficacy.

4.4 Volociximab

α5β1 integrin is an extracellular protein on endothelial cells which binds and ligates fibronectin as a key step in new blood vessel formation. Volociximab (M200, PDL Biopharma/Biogen) is a chimeric monoclonal antibody which binds to α5β1 integrin, thus blocking its ability to interact with fibronectin. This results in apoptosis of proliferating endothelial cells. In a multicenter Phase II trial of 40 patients with metastatic clear cell RCC, volociximab (10mg/kg) was administered IV every 2 weeks. Endpoints for the study were safety and efficacy[66]. Frequent side effects included fatigue, nausea, dyspnea, and arthralgia. Thirty-two patients (80%) had stable disease and there was one PR. Median TTP was 4.8

months. The authors concluded that volociximab was tolerable and showed evidence of clinical benefit. Further trials of this drug are planned.

4.5 WX-G250

WX-G250 (Wilex) is a chimeric monoclonal antibody that recognizes CA-IX$^{MN/G250}$ which is an antigen that is commonly expressed (>95%) on clear cell RCC cells and is a target transcriptionally regulated by the HIF factors. WX-G250 has been evaluated alone and in combination with low dose IL-2. In a Phase II trial evaluating WX-G250 monotherapy, 36 clear cell mRCC patients received 50 mg IV once a week67. There was one complete response seen and 10 patients with stable disease. Median survival was 15 months after the initiation of treatment. None of the patients experienced drug-related grade 3 or 4 toxicity. This study led to a subsequent trial combining low dose IL-2 with WX-G25068. In this trial, 35 patients with clear cell mRCC received WX-G250 (20 mg) IV once weekly for 11 consecutive weeks, as well as IL-2 (1.8 MU) daily for 12 consecutive weeks (except for days 1–3 of weeks 3, 5, 7, 9,and 11 when 5.4 MU was given as “pulse doses”). Median OS was found to be 22 months. There were 3 PRs and 5 patients with stable disease. This agent is currently undergoing evaluation as an adjuvant to surgical resection for patients with high risk disease.

4.6 Infliximab

Tumor necrosis factor- α (TNF-α) is secreted by RCC cells and increased levels are associated with poor prognosis69. TNF-α, therefore, has been targeted for therapeutic intervention. Infliximab (Remicade®, Centocor) is a chimeric monoclonal antibody comprised of a human IgG Fc region and a murine antihuman TNF-α antibody. It is approved for use in rheumatoid arthritis and Crohn’s disease. Recently a review of two Phase II trials utilizing infliximab in RCC was reported70. In both studies, patients were included that had locally advanced or mRCC, had received prior immunotherapy, and had good performance status. In the first study (n= 19), infliximab 5mg/kg IV was given over 2 hours on weeks 0, 2, and 6 during an induction phase. This was followed by a maintenance phase of 5mg/kg IV over 2 hours starting on week 14 and then repeated every 8 weeks. In this study 3 patients (16%) had a PR and 3 patients (16%) had stable disease. Median duration of both PR and stable disease responses were 7.7 months. In the second study, there were 18 patients that were treated with infliximab 10mg/kg IV over 4 hours on weeks 0, 2, and 6 followed by 10mg/kg over 4 hours every 4 weeks, beginning at week 10. Interestingly in this group of patients, there were 11 patients (61%) with stable disease and no reported PRs. The median PFS was 4.1 months and median OS was 13.1 months. In both studies, the treatments were well tolerated and the potential for inhibiting this pathway will require further investigation.

5. Inhibitors of the Mammalian Target of Rapamycin (mTOR)

The mammalian target of rapamycin (mTOR) is a polypeptide serine/threonine nonreceptor kinase which is downstream from the kinase Akt and is an important regulator of the phosphoinositide3-kinase (PI3K) pathway. mTOR plays multiple cellular roles regulating cellular metabolism and global protein synthesis. mTOR is also important in the regulation of hypoxia-inducible factor production in a way which is separate from pVHL. This activity depends upon the phosphorylation dependent activation of the S6 kinase which promotes the translation of multiple factors such as HIF-1α. So named for it’s pharmacologic inhibition by the drug rapamycin, inhibition of this central cellular pathway has been an area of investigation of many years, with rapamycin playing a major role in post-transplant immunosuppression. Preclinical investigations have supported the rationale for drug design in this area, with suppression of HIF-1α and HIF-2α in response to hypoxia in cell cultures

treated with rapamycin[23, 71]. Recently, pharmacologic analogs have been developed with improved tissue penetration and safety profile. Inhibition of mTOR has therefore become a target of interest in the treatment of RCC (Table 1).

5.1 Temsirolimus

Temsirolimus (CCI-779, Torisel®, Wyeth) is a novel ester analog of rapamycin which acts by inhibiting mTOR kinase activity and has shown clinical efficacy in several clinical trials with mRCC patients. In a randomized, double-blind multicenter Phase II trial evaluating the pharmacokinetics, safety, and efficacy of temsirolimus, 111 patients with confirmed advanced RCC were given 25mg, 75mg, or 250 mg intravenously once weekly[72]. The primary end-point of the study was objective tumor response rate (CR + PR). The objective response rate was found to be 7% of all patients treated. There was one CR in the 250mg dose group. Median TTP was 6.3, 6.7, and 5.2 months for the 25mg, 75mg, and 250mg groups respectively with a median TTP of 5.8 months for the entire study group. Two year survival rates were 24%, 26%, and 36% for the 25mg, 75mg, and 250mg treated groups respectively. The most frequent adverse events were maculopapular rash, mucositis, asthenia, and nausea. The most common grade 3 or 4 related adverse events were hyperglycemia, hypophosphatemia, anemia, and hypertriglyceridemia. In a subset analysis, the cohort was divided into good, intermediate, or poor risk groups based on the MSKCC risk score and although the patients in the good and intermediate risk group did better than in the poor risk group (median survival 23.8 months, 22.5 months, and 8.2 months for the good, intermediate, and poor risk groups respectively), the benefit was perceived to be greatest for the poor risk group of patients.

In a follow-up multicenter Phase III trial, 626 patients with previously untreated mRCC inclusive of all histological subtypes and at least 3 poor prognostic risk factors were randomized to receive 25 mg of temsirolimus weekly, interferon-alpha 3 MU (with planned escalation to 18 MU) three times weekly, or combination therapy with temsirolimus 15mg weekly and interferon 6 MU three times weekly[73]. The primary endpoint was OS. Median OS was found to be 7.3 months, 10.9 months, and 8.4 months respectively for the IFN, temsirolimus alone, and IFN/temsirolimus treatment arms. Independently reviewed median PFS was 3.1 months, 5.5 months, and 4.7 months for the IFN, temsirolimus alone, and IFN/temsirolimus groups respectively. Additionally, the ORR was found to be 4.8%, 8.6%, and 8.1% for the IFN, temsirolimus alone, and IFN/temsirolimus groups respectively. Based on these findings, temsirolimus was found to be superior to interferon in the treatment of patients with poor risk mRCC with no additional benefit from the combination with interferon. This is the first therapeutic agent for mRCC to show an advantage in mRCC with poor risk factors. Further, the improvement in survival was observed regardless of histological sub-type. Temsirolimus has now been approved for the treatment of patients with advanced RCC.

5.2 Everolimus

Everolimus (RAD001, Certican ®, Novartis) is an orally bioavailable mTOR inhibitor which has shown some promise in the Phase I/II setting. In a review of two Phase I trials in advanced solid tumor patients, 12 patients with RCC were identified (10 clear cell, 1 papillary, and 1 sarcomatoid)[74]. Although doses and frequency of drug administration were variable, there were 6 patients with stable disease, one with a PR (papillary subtype) and no CRs. The most frequent adverse events included fatigue, diarrhea, rash, anorexia, mucositis, nausea, abdominal distension, vomiting, and headache. A subsequent Phase II study has been reported to evaluate mRCC patients with everolimus 10mg by mouth daily75. In this study, 37 patients were evaluated and results showed a PR in 12 patients and SD in 19 patients. Median OS was 11.5+ months at the time of report. Everolimus is also being

studied in a Phase III double-blind placebo controlled randomized trial which is evaluating it in the second line setting following the failure of one tyrosine kinase inhibitor.

6. Other Novel Agents

The development of novel drugs targeting the HIF pathway has exploded in recent years. The number of new agents reported currently tops 100 abstracts and peer reviewed articles annually which are designed or identified to target this disease. This review therefore will focus on those agents furthest in clinical development or whose mechanism of activity is specifically tied to the molecular biology of renal cell carcinoma. These compounds largely target HIF activation, as a central theme in the biology of RCC (Table 3).

6.1 Novel Topoisomerase Inhibitors

GL331 (Gene Labs Inc) is a novel synthetically modified compound derived from the plant toxin podophyllotoxin and is similar in structure to etoposide. Among its actions in tumor cells, it is able to prevent DNA damage repair via inhibition of topoisomerase II. Critical to this review, it has also been shown to decrease HIF-1 mRNA production in lung cancer cells via transcriptional repression[77]. In the Phase I setting, evaluating safety and tolerability in patients with various advanced cancers, the drug had dose-limiting toxicities of neutropenia and thrombocytopenia78. In the Phase II setting, it has been found to be ineffective for the treatment of refractory gastric cancer79, but has not been specifically evaluated in RCC patients to-date. Other drugs in this topoisomerase inhibitor category are in evaluation for their effects on HIF modulation, including topotecan and NSC 644221.

6.2 HSP inhibitors

By acting as chaperones to help stabilize various proteins, heat shock proteins (HSPs) serve to protect cells from stress which would otherwise result in apoptosis. Geldamycin, an inhibitor of HSP90 has been shown to promote HIF-1α ubiquitination and degradation in a *VHL* independent manner[80]. Later this *VHL*-independent process was shown to be related to HSP90 and RACK-1 (receptor of activated protein kinase C) competition for HIF-1α binding. Subsequently, RACK-1 is able to recruit the elongin-B/C ubiquitin complex for HIF-1α ubiquitination and degradation[81].

17-(Allylamino)-17-demethoxygeldanamycin(17-AAG), a derivative of geldamycin, has been evaluated in a Phase II clinical trial involving mRCC patients[82]. This trial enrolled 20 patients with metastatic papillary and clear cell RCC and treated them with 220mg/m2 twice weekly for two weeks on a 3 week cycle. The results were disappointing with no tumor responses observed. Median TTP was 1.6 months in the papillary subset and 3.3 months in the clear cell subset of patients. The potential causes of this apparent lack of activity can only be speculated, but may relate back to the biology of RCC with HIF-2α accumulation providing the primary tumor promoting activity of this family, and potential disparity in the activity of HSP-90 to act as a critical chaperone for both of the HIF factors. Currently 17-AAG and a related drug 17-DMAG (17-Dimethylaminoethylamino-17-Demethoxygeldanamycin) are currently being evaluated in several clinical trials for various tumor types.

6.3 Farnesyltransferase inhibitors with activity against HIF

Farnesyltransferase is required to post-translationally farnesylate many proteins, including ras, to make them biologically active. SCH66336 (lonafarnib) is an inhibitor of farnesyltransferase that has been shown to have anti-tumor effects in several tumor cell types *in vitro*[83] In addition to it's traditionally assumed role in preventing proper ras localization, SCH66336 has been observed to promote HIF1-α protein proteasomal

degradation in non-small cell lung cancer and head and neck cancer cell lines by blocking its interaction with HSP90[84]. This mechanism has clinical implications for HIF-1α overexpressing tumors including RCC and the drug is being evaluated in several early Phase I/II trials in patients with advanced solid tumors.

6.4 HDAC inhibition

Histone deacetylases (HDAC) are a group of enzymes that remove the acetyl group from specific lysine residues on histones and numerous other proteins. HDAC 1 and 3 have been shown to enhance HIF-1α stability[85]. The HDAC inhibitors Vorinostat (suberoylanilide hydroxamic acid, Zolinza™, Merck & Co., Inc.) and Trichostatin A have been shown to modulate HIF activity in vitro86. One mechanism of action for Vorinostat is the hyperacetylation of the p300 protein resulting in inability of HIF/p300 complex formation and subsequent downstream HIF transactivation. Vorinostat has been studied in numerous cancer cell types given its anti-tumor properties and is currently FDA approved for use in primary cutaneous T-cell lymphoma87. There are several early Phase trials in the midst of recruitment which are evaluating the use of Vorinostat in advanced renal cancer patients.

6.5 Inhibition of thioredoxin reductase

PX-478 (Biomira, Inc.) is a novel inhibitor of HIF-1α that is currently in early stages of clinical development. PX-478's mechanism of action is thought to be related to inhibition of thioredoxin reductase which is necessary for oxygen-dependent HIF-1α degradation. Interestingly, this agent inhibits HIF-1α but not HIF-2α. PX-478 significantly reduces tumor size in colon, prostate, breast, renal, and small cell lung cancer xenograft models[88]. There is currently a Phase I clinical trial that is recruiting patients with advanced solid tumors or lymphoma. Phase I results of a similar thioredoxin reductase inhibitor, PX-12 (Biomira, Inc.), has shown acceptable tolerability in patients with advanced solid tumors and currently is in Phase II evaluation in patients with pancreatic cancer 89.

6.6 Inhibitors of HIF transactivation

Transcription factors have been notoriously difficult drug targets due to the lack of an enzymatic pocket, and frequently retained function even in the face of substantial modification. Efforts to inhibit transcription factor oncogenes have therefore focused on disrupting the essential components of the complex required for transactivation. Such a mechanism is suggested for the potential activity of vorinostat, above. Chetomin, a metabolite of the fungus species Chaetomium, was identified in a high through-put screen (over 600,000 compounds evaluated) for compounds which disrupted HIF-1α and p300 binding[90]. Chetomin was found to disrupt the CH1 domain of p300, thereby interfering with HIF-1α interaction and subsequent transactivation of target genes. Chetomin treatment of mice with HCT116 colon cancer xenografts resulted in decreased erythropoietin and VEGF production, as well as tumor growth inhibition. Current limitations of this drug include substantial local toxicity with coagulative necrosis and leukocytosis at the injection site, although with chemical modification this avenue may provide a fruitful inhibitor.

6.7 Compounds with complementary lethality to HIF expression

Many new studies are underway to identify drugs which selectively kill cells which carry a particular genetic or molecular characteristic. This kind of unbiased screen provides the opportunity to identify drugs interfering in previously unrecognized pathways, but which when inhibited are incompatible with the survival of the abnormal cell. Chromomycin A3 is an aureolic acid compound which binds to the minor groove in DNA and thereby has the ability to change DNA conformation and interfere with transcription. In a screen of several NCI drug profiles, this compound was identified as an agent which targets *VHL*-deficient

clear cell RCC cells[91]. Treatment of RCC cell lines resulted in preferential cell death of *VHL* negative cells compared to *VHL* positive cells. Over-expression of HIF-2α also resulted in increased sensitivity to chromomycin A3 despite *VHL* positivity. Further, treatment of mice with RCC xenografts resulted in substantial tumor regression, supporting a mechanism of cell death that is HIF-dependent.

6.8 Echinomycin

In a high through-put screen of 140,000 small molecule agents, echinomycin was identified as an inhibitor of HIF-1α DNA binding activity[92]. This compound was found to inhibit HIF-1α/β complex binding to the HRE sequence. Treatment of U251 glioma cells resulted in decreased VEGF expression. Later work has demonstrated that echinomycin inhibits HIF-1α transactivation under hypoxic conditions but resulted in increased activity under normoxic conditions, presenting an interesting potential biological regulatory mechanism, but potentially limiting the clinical application of this drug for renal cell carcinoma[93].

6.9 2-Methoxyestradiol

2-methoxyestradiol (2ME2) is a naturally occurring derivative of estradiol that has been shown to have anti-tumor and anti-angiogenesis effects that are separate from estrogen receptor activation. 2ME2 has been shown to disrupt tumor microtubules and thereby down-regulate HIF-1α at the post-transcriptional level and decrease VEGF expression[94]. 2ME2 is currently being evaluated in multiple Phase I/II trials and is showing that it is well tolerated and has some evidence of efficacy. Although, the drug has not been evaluated specifically in RCC patients to-date, as a potential inhibitor of the HIF pathway is worthy of further attention.

6.10 YC-1

3-(5'-hydroxymethyl-2'-furyl)-1-benzylindazole or YC-1 is an agent that activates guanylyl cyclase (increases intracellular cGMP concentration) and was originally developed based on its circulatory properties of causing vascular contraction and blocking platelet aggregation. In the in vitro treatment of Hep3B cells it was shown to decrease HIF-1α protein levels but not mRNA, suggesting that it promoted HIF-1α protein degradation95. These results were confirmed with an in vivo mouse model experiment where hepatoma, gastric, renal, cervical, and neuroblastoma xenografts were implanted96. Treatment with YC-1 resulted in significant decreases in tumor size, vascularization, HIF-1α protein expression, and HIF-1α target gene expression (VEGF, aldolase, and enolase) *in vivo*.

7. Sequences and Combinations

Significant advancements in drug development for RCC have provided this bounty of active and potentially active agents. Determining the most appropriate patients to receive each drug remains a difficult daily decision for oncologists, and likely requires further discoveries about the tumor and patient biology that dictates the potential for response to a given agent. Therefore, studies investigating the sequences and combinations of drugs are essential to make the best use of these advancements for the benefit of patients. This section will overview the first investigations to address these important issues in the clinical development of these drugs.

7.1 Drug Sequence and Patient Selection

Anecdotal evidence initially supporting incomplete cross resistance to the antiangiogenic agents has received preliminary validation in sequence based studies. Sunitinib was evaluated in relation to bevacizumab therapy in a preliminary report of an ongoing Phase II study of sunitinib in bevacizumab-refractory patients[97]. Sixty-one patients who were found

to have progressive disease within 3 months of initiation of bevacizumab were enrolled to receive sunitinib 50mg/day 4 weeks on, 2 weeks off. These patients were required to have good performance status and prior nephrectomy. The primary endpoint was objective response rate, and recently presented results showed an objective partial response rate of 23% with an additional 57% of patients achieving stable disease. Median progression free survival was 30 weeks, and completed results are highly anticipated.

Sequential therapy of sunitinib and sorafenib has been evaluated retrospectively[98]. Sixty-eight patients who received sorafenib followed by sunitinib and 22 patients who received sunitinib followed by sorafenib were evaluated. The best responses for initial sorafenib treatment were 12 patients with a PR and 44 patients with SD. Further treatment with sunitinib in this group yielded 7 further PRs and 27 with SD. The best response in those initially treated with sunitinib was 5 PRs and 12 with SD. Further treatment of this group with sorafenib resulted in 3 further PRs and 11 further with SD. This led to the conclusion that there is a lack of cross resistance between these two agents and they can be used sequentially to yield a subsequent clinical benefit.

7.2 Rational Designs of Targeted Drug Combinations

Although blockade of vascular signaling molecules is often considered to be highly similar regardless of the level of inhibition, the concept of “vertical inhibition” promotes the idea that broader inhibition of a single pathway with drugs with overlapping activities may have more potent clinical activity. In a Phase I trial, the combination of bevacizumab and sunitinib in mRCC is being explored[99]. In this trial, 20 patients with clear cell mRCC were treated with bevacizumab 10mg/kg every 2 weeks and escalating doses of sunitinib (25, 37.5, and 50mg/day) for 4 weeks on, 2 weeks off. In combination with bevacizumab, the maximum tolerated dose of sunitinib was found to be 50mg/day. Of 19 evaluable patients, 7 had a partial response and 10 were found to have stable disease. Observed adverse events were common to the two agents including hypertension and elevated lipase, as well as the potential for serious adverse events such as grade 4 hemorrhage which was observed in two patients.

Sunitinib is also being evaluated in combination with gefitinib (an inhibitor of the epithelial growth factor receptor, EGFR). Preliminary data have been presented for a Phase I trial evaluating 42 patients with clear cell-type mRCC[100]. Patients were enrolled into either a Phase I arm evaluating the maximum tolerated dose (MTD) and safety or a Phase II arm evaluating ORR as a primary endpoint. Eleven patients were enrolled in the Phase I arm and 31 patients were enrolled in the Phase II arm. Of the 42 patients, 36 were evaluable and preliminary results revealed that 11 patients had partial responses (30%) and 15 patients had stable disease (42%), with no unexpected enhancement of toxicity.

In an effort to maximize disease response, combinations of sorafenib with other established agents have been evaluated in clinical trials. In a Phase II trial, the combination of sorafenib and interferon alpha-2b in first or second-line treatment of mRCC was evaluated[101]. In this study 40 patients with either metastatic or unresectable RCC (all subtypes included) were enrolled and received sorafenib (400mg bid) plus interferon alpha-2b (10 MU subcutaneously three times a week) for 8 week cycles of continuous. Of 36 evaluable patients there were 2 complete responses (5%), 11 partial responses (28%), and 18 patients with stable disease (45%). Median PFS was 10 months (95% CI, 2–24 months). Similar results were observed in a SWOG study combining the same two agents.[102]

Other complementary pathways which include a distant level of vertical inhibition include combinations of anti-angiogenic agents with mTOR inhibitors. Recently initial data from a Phase I/II trial evaluating the safety and tolerability of the combination of the tyrosine

kinase inhibitor, vatalanib, and the mTOR inhibitor, everolimus, has been reported[103]. Of 13 evaluable mRCC patients, there were 2 partial responses (15%) and 8 patients with stable disease (62%). These preliminary response rates suggest promising synergistic activity, with dose limiting toxicities including diarrhea, hypertriglyceridemia, asthenia, fatigue and mucositis.

In an extension of this type of combination, temsirolimus is being studied in combination with bevacizumab. In a Phase I trial of patients with advanced or metastatic clear cell RCC, twelve patients were enrolled to receive temsirolimus 25mg IV weekly and bevacizumab 5mg/kg IV every 2 weeks with plans to increase the bevacizumab to 10mg/kg if tolerated[104]. After a median of 6 treatment cycles, 8 patients had a PR and 3 had stable disease. Grade 3 adverse events reported were hypertriglyceridemia, mucositis, anorexia, nausea, hemorrhage, anemia, hypertension, proteinuria, weight loss and anorexia. The MTDs of temsirolimus and bevacizumab were 25mg and 10mg/kg, respectively. A Phase II trial in patients that are refractory to tyrosine-kinase inhibitor therapy has thus been started with this regimen.

8. Expert Opinion

Historically, advanced RCC has been resistant to conventional chemotherapies and cytokine therapy, resulting in responses in only a small subset of patients. With an increase in the understanding of RCC molecular biology, new targeted therapies are being developed that hold great promise in changing our ability to manage this disease. Particularly, *in vitro* evidence points to maximal inhibition of the HIF pathway, specifically the HIF-2α transcription factor, as key to effectively controlling the growth of RCC. Several drugs intercepting this pathway have recently been approved for the treatment of mRCC and new combinations of these drugs are being evaluated. Currently, numerous agents are in the pipe-line which will potentially expand our therapeutic arsenal. These drugs modulate the hypoxia pathway at multiple levels including interference with HIF synthesis, HIF protein-protein interaction, HIF transactivation, and downstream target protein function (See Figure 2). Based on clinical trials involving many of these agents, modest response rates and disease stabilization are the norm, with fewer complete responses and no cures. This leads to the idea that combination therapy with several drugs affecting this complex pathway may be necessary to improve clinical responses and change the natural history of renal cancer to one of a chronic disease.

Therefore, new clinical trials must continue to address the proper sequence and combination of targeted therapies. By targeting multiple pathways both upstream of HIF regulation and downstream of HIF activity, a more robust disease response would be expected. Trials exploring both simultaneous and staggered administration of multiple agents may be required to avoid drug-drug interaction and accumulation of toxicities. If these complications can be managed, current findings of drug resistance may be overcome with the addition of new agents. As an obvious limitation of combination therapy may be increased toxicity, sequential therapy algorithms should also be explored. With results of an early phase clinical trial demonstrating further benefit of sequential use of sorafenib and sunitinib, further investigation needs to be done with mTOR inhibitors and agents of other classes as they become available. The success seen with sequential use of sorafenib and sunitinib is likely explained by the differing tyrosine kinases they target and therefore, a rational experimental design of sequential therapy should include agents, either within the same class or of a different class, that offer different mechanisms of action. In addition, as experience with these novel agents increases, an effort to define a standard of care should be done with head-to-head clinical trials and abandonment of cytokine-only treatment arms.

Finally, further investigation of therapies in patients with poor risk MSKCC criteria and non-clear cell RCC tumor types needs to be performed.

Future directions will expand the pool of clinically valuable agents which target HIF activation, and specific analysis of drug activity to target HIF-2α is essential from this point forward. We have currently only begun to understand the essential molecular biology of RCC, and future combinations of drugs which maximally inhibit HIF signaling as well as take advantage of other tumor attributes will likely be the most effective therapeutic strategies. Finally, identifying tumor or patient specific factors which would support the selection of one agent or combination in the new era of customized therapy is an ideal platform for further development of treatments for RCC.

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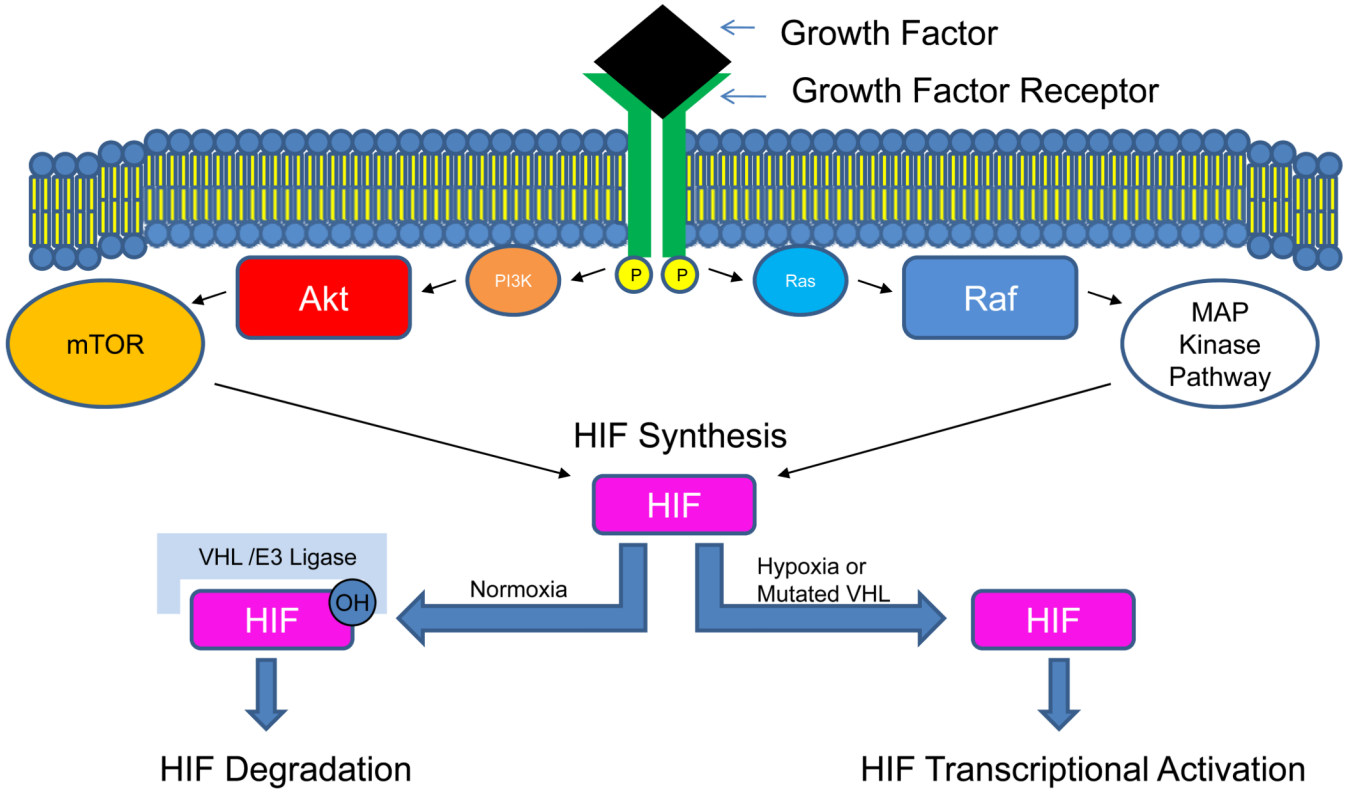

Figure 1.
Molecular Biology of Renal Cell Cancer and Its Association with the Hypoxia Pathway. Hypoxia inducible factor (HIF) synthesis is upregulated by growth factor signaling via the phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways. The mammalian target of rapamycin (mTOR) is an important regulator of the PI3K pathway. The Von Hippel-Lindau (VHL)/E3 ubiquitin ligase targets HIF for degradation in normoxic conditions. In hypoxic conditions or in absence of functional pVHL activity, HIF transcriptional activation results in target gene activation causing the angiogenic, proliferative, glycolytic phenotype of renal cell cancer.

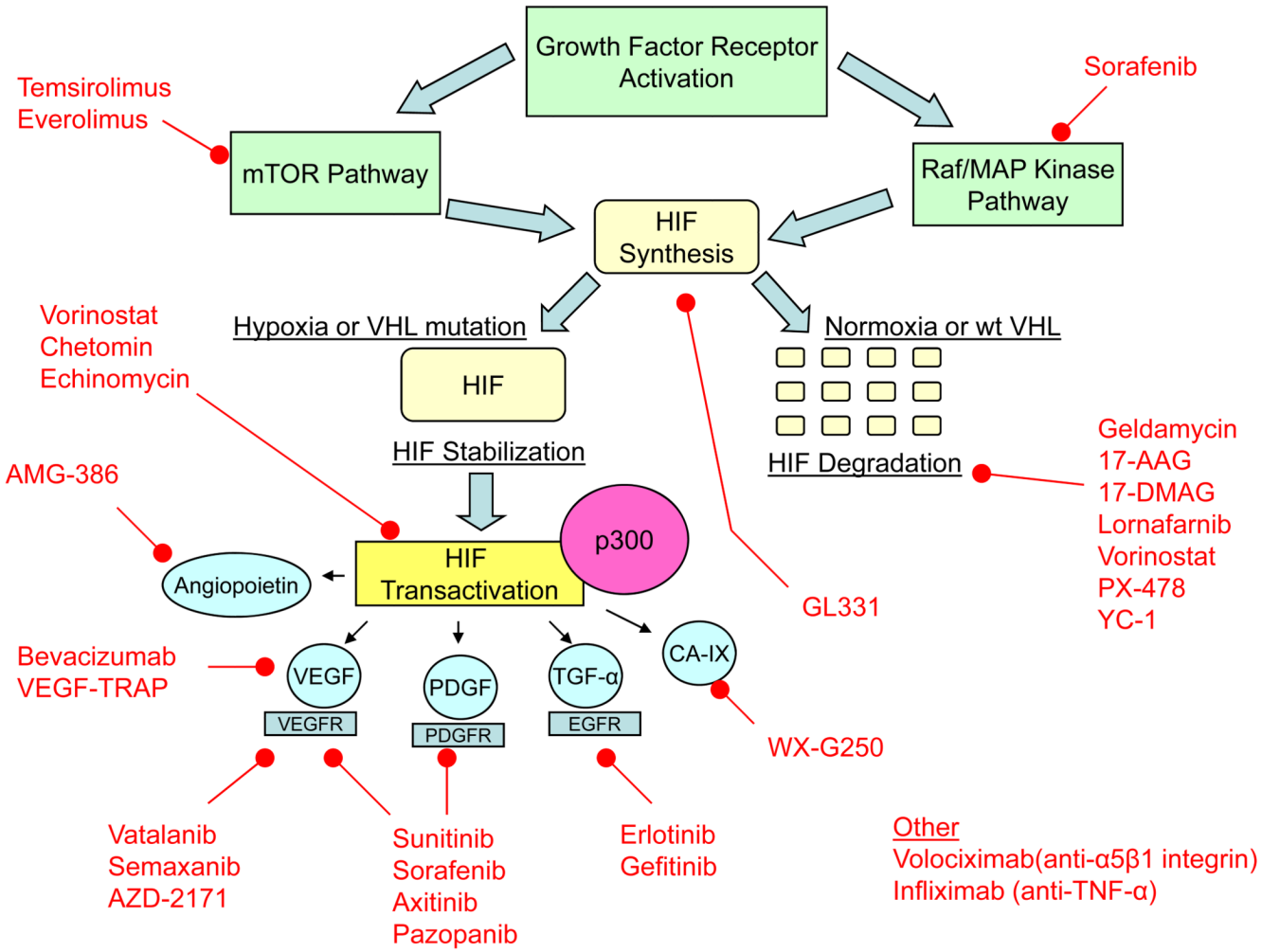

Figure 2.

Sites of Action of New Molecularly Targeted Therapies. Therapeutic compounds discussed in this review are depicted where they intersect with known RCC signaling pathways. **mTOR** mammalian target of rapamycin, **MAP** mitogen-activated protein, **HIF** hypoxia inducible transcription factor, **VHL** Von Hippel-Lindau gene, **wt VHL** wild type Von Hippel-Lindau gene, **VEGF** vascular endothelial growth factor, **VEGFR** vascular endothelial growth factor receptor, **PDGF** platelet derived growth factor, **PDGFR** platelet derived growth factor receptor, **TGF**-α transforming growth factor alpha, **EGFR** epidermal growth factor receptor, **CA-IX** carbonic anhydrase IX.

Table 1

Summary of Kinase Inhibitors.

Agent	Class	Target(s)	Clinical Activity	Common Toxicities	Reference(s)
Sunitinib	Receptor Tyrosine Kinase Inhibitor	VEGFR 1–3 PDGFR-α, -β FLT-3 c-kit	31–40% PR 37–39% SD	Hypertension Hand-foot Syndrome Nausea Diarrhea	30, 31, 33
Sorafenib	Receptor Tyrosine Kinase Inhibitor	VEGFR 1–3 PDGFR-β FLT-3 c-kit Raf	10% PR 74% SD	Fatigue Rash Hand-foot Syndrome Hypertension Diarrhea	39
Axitinib (AG-013736)	Receptor Tyrosine Kinase Inhibitor	VEGFR 1–3 PDGF c-kit	21–40% PR 34% SD	Fatigue Hypertension Hand-foot Syndrome Proteinuria Nausea Diarrhea	43, 76
Vatalanib (PTK787/ZK222584)	Receptor Tyrosine Kinase Inhibitor	VEGFR 1–3	19% ORR (PR and minor responses)	Diarrhea Hypertriglyceridemia Asthenia Fatigue Mucositis	45
Pazopanib (GW786034)	Receptor Tyrosine Kinase Inhibitor	VEGFR 1–3 PDGFR-α,-β c-kit	40% PR 42% SD	Hypertension Nausea Diarrhea GI Bleed Fatigue	49
Semaxanib (SU-5416)	Receptor Tyrosine Kinase Inhibitory	VEGFR 2	50% SD (combined with interferon)	Headache Nausea Vomiting Asthenia Fever	52
AZD-2171	Receptor Tyrosine Kinase Inhibitor	VEGFR 1–3	37% PR 31% SD	Hypertension Diarrhea Dysphonia	55

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Agent	Class	Target(s)	Clinical Activity	Common Toxicities	Reference(s)
Temsirolimus (CCI779)	Nonreceptor Serine/threonine Kinase Inhibitor	mTOR	7–8.6% ORR (CR+PR) 17–32% SD	Rash Mucositis Asthenia Nausea	72, 73
Everolimus (RAD001)	Nonreceptor Serine/threonine Kinase Inhibitor	mTOR	8–32% PR 50–51% SD	Mucositis Rash Pneumonitis	74, 75

Table 2

Summary of Monoclonal Antibody Therapies.

Agent	Class	Target(s)	Clinical Activity	Common Toxicities	Reference(s)
Volociximab	Monoclonal Antibody	A5β1 Integrin	80% SD	Fatigue Nausea Dyspnea Arthralgia	66
WX-G250	Monoclonal Antibody	CA-IX	27% SD	No Grade III or IV Toxicities Reported	67
Infliximab	Monoclonal Antibody	TNF-α	0–16% PR 16–61% SD	Infusion Reaction Headache Nausea	70
AMG-386	Neutralizing Peptibody	Tie-2 Receptor	50% SD (multiple tumor types)	Fatigue Nausea Peripheral Edema	65
Bevacizumab	Monoclonal Antibody	VEGF-A	10% PR	Epistaxis Hypertension Hematuria Proteinuria	61
VEGF-TRAP	Fc IgG-VEGFR 1–2 Fusion Molecule	VEGF-A	6% SD (multiple tumor types)	Fatigue Pain Constipation	64

Table 3

Summary of Select Agents That Modulate HIF.

Agent	Mechanism of Action	Effect on HIF	Stage of Development	Reference(s)
GL331	Topoisomerase- II Inhibitor	Decreases HIF mRNA production	Phase I/II Trials	77–79
Heat Shock Protein Inhibitors (Geldamycin, 17-AAG, 17-DMAG)	HSP-90 Inhibitor	Promotes HIF Degradation	Phase I/II Trials	80–82
Lonafarnib (SCH66336)	Farnesyltransferase Inhibitor	Promotes HIF Degradation	Phase I Trials	83, 84
Vorinostat	Histone Deacetylase Inhibitor	Promotes HIF Degradation and Inhibits HIF Transactivation	FDA Approved for Primary Cutaneous T-cell Lymphoma and Phase I RCC Trial Development	85–87
PX-478	Thioredoxin Reductase Inhibitor	Promotes HIF-1α Degradation	Phase I Trials	88
Chetomin	Disrupts the CH1 Domain of p300	Inhibits HIF Transactivation	Preclinical Development	90
Echinomycin	Inhibits HIF-1α/β binding to HRE Sequence	Inhibits HIF Transactivation	Preclinical Development	92, 93
2-Methoxyestradiol	Disrupts Tumor Microtubules	Down-regulates HIF-1α Levels	Phase I/II Trials	94
YC-1	Activates Guanylyl Cyclase	Promotes HIF-1α Degradation	Preclinical Development	95, 96