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Review Article

Therapeutic challenges in renal cell carcinoma

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Abstract: Renal cell carcinoma (RCC) is a malignancy that in advanced disease, is highly resistant to systemic therapies. Elucidation of the angiogenesis pathways and their intrinsic signaling interactions with the genetic and metabolic disturbances within renal cell carcinoma variants has ushered in the era of "targeted therapies". Advanced surgical interventions and novel drugs targeting VEGF and mTOR, have improved patient survival and prolonged clinically stable-disease states. This review discusses the current understanding of diagnostic challenges and the mechanism-based clinical evidence on therapeutic management of advanced RCC.

Keywords: Angiogenesis, renal cancer, imaging, survival signaling, targeted therapies

Introduction

Renal cell cancer (most often adenocarcinoma arising from renal parenchymal cells) is the 16th most leading cause of death from malignancy globally, and the 9th most common malignancy among men and the 14th most common malignancy among women worldwide [1]. The mortality rates for renal cell carcinoma (RCC) in North America, Europe, and Australia have been on the decline since the mid 1990's, while incidence rates have continued to steadily rise in most countries [1]. This is due to advancements in imaging techniques (ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI)), leading to earlier detection of renal masses [1, 2]. The clinical disease of renal cancer is gender dependent, with preponderance for older males [3]. The incidence ratio of males to females in 'developed countries' is 12.4/6.2 (per 100,000 population) [4]. Worldwide, the male to female incidence ratio is approximately 6.0/3.0, making males roughly twice as likely to develop renal cancer [4]. In 2012 there were 338,000 estimated new cases of renal cancer globally, 2.4% of all malignancies [4], with a high incidence of RCC in Europe and North America compared to Africa, Asia, and South America [1]. Incidence varies widely among regions and among ethnic groups within countries. African Americans have higher incidence rates than Caucasian Americans, and within eastern Europe, RCC incidence in Bulgaria is 6.7 (per 100,000 men) while it is 22.1 for Czech Republic [1]. These marked incidence disparities implicate an array of lifestyle and environmental risk factors, and genetic susceptibilities unique to ethnic and regional populations which may contribute to RCC development [1].

Subtypes of RCC include clear cell renal cell carcinoma, papillary renal cell carcinoma, chromophobe renal cell carcinoma, renal collecting duct (Bellini's) renal cell carcinoma, mucinous tubular and spindle cell carcinoma, Xp11 translocation carcinomas, renal medullary carcinomas, carcinoma associated with neuroblastoma, and renal cell carcinoma unclassified type. Clear cell RCC (ccRCC) is the most common histologic subtype, representing 75-85% of all RCC, its name derived from the disproportionately high cytoplasmic glycogen and lipid content giving cells their characteristic appearance [5]. Morphologically, tumors may present in the solid, tubular, and cystic forms [5, 6]. ccRCC occurs sporadically, is often unilateral, and due to deletion of chromosome 3p harboring the VHL gene, a potent tumor suppressor [6]. About 80% of sporadic ccRCC are attributed to somatic VHL gene inactivation [2] and 2-3% of ccRCC to hereditary disease linked to von-Hippel Lin-

dau syndrome (VHL disease) [3]. The syndrome is inherited in an autosomal dominant fashion, is highly penetrant and promotes multiple vascular tumors including pheochromocytoma, pancreatic tumors and ccRCC [7]. Sporadic ccRCC occurs secondary to the accumulation of two mutation events [7].

Approximately 10-15% of RCC is attributed to the papillary histologic subtype, which remains the second most common variant of RCC. Papillary RCC exhibits diverse histological profiles; most commonly it is delineated as either of two distinct subtypes; type 1 is composed of cuboidal epithelium with 'low grade' nuclei and the more aggressive type 2 often displays pseudostratified epithelium with typically 'high grade' nuclei [8]. Multiple genetic mutations are associated with this cancer subtype, including trisomy of chromosomes 3q, 7, 8, 12, 16, 17, 20, and loss of the Y chromosome [6]. Chromosome 7 mutations with subsequent c-Met proto-oncogene (encoding a receptor tyrosine kinase) expression changes are often found in papillary RCC and fumarate hydratase (FH) gene mutations on chromosome 1 in the autosomal dominant type papillary RCC [6]. The third most common subtype of RCC is the chromophobe type, carrying with it the best prognosis, with tumors of low grade [9]. This subtype represents roughly 4-5% of RCC diagnoses, and is morphologically characterized by sheets of cells containing pale or granular eosinophilic cytoplasm [5, 9]. Loss of chromosomes 1, 2, 6, 10, 13, 17, and 21 and subsequent expression of the proto-oncogene c-kit often characterizes chromophobe tumors [9]. Oncocytomas, rare benign renal tumors, also arise from renal intercalated cells of the kidney. Approximately 15% of patients with Burt-Hogg-Dube syndrome (a rare autosomal dominant disorder characterized by hair follicle hamartomas) present with mixed renal chromophobe-oncocytomas [10]. The disease is characterized by BHD gene mutations, (encoding folliculin, a tumor suppressor), linking oncocytomas to chromophobe tumors [10].

RCC risk factors

Analysis of causal relationships between lifestyle and environmental factors with RCC progression has revealed multiple risk factor associations. The strongest association has been found with cigarette smoking; relative risk (RR) of developing RCC in 'ever-smokers' in comparison to 'never smokers' is 1.38 [11], with relative risk increasing in a dose-response relationship [11]. Cigarette smoking increases risk for RCC via chronic tissue hypoxia, carbon monoxide exposure, and tobacco specific N-nitrosamine induced DNA damage within tissues [12]. Obesity is also an important risk factor for RCC development. Relative risk of RCC development for overweight (BMI 25.0-29.9 kg/m^2) men is 1.28 while RR for obese (BMI > 30.0 kg/m^2) men is 1.82 according to one study [13]. Biological evidence implicates links between adipose tissue with VHL gene changes in the development of ccRCC, via angiogenic factors and inflammatory cytokines, 'adipokines', released from adipocytes to promote a premalignant environment [13]. Hypertension as a risk factor for RCC development has been identified by a Swedish retrospective study [14]. The precise mechanisms linking hypertension to RCC remain elusive, yet it is postulated that hypertension induced chronic renal hypoxia, as well as lipid peroxidation resulting in the formation of reactive oxygen species in the proximal tubules, may contribute to RCC development [15]. Other risk factors include diabetes, occupational exposures, nutritional intake, chronic renal disease, and physical activity [12, 16].

Imaging and diagnosis

The age adjusted incidence rate for RCC increased from 7.6 (per 100,000 population) to 11.7 [17], an increase thought to be a consequence of implementation of noninvasive imaging in current medical practices, with the incidence for localized tumors rising by 4.5% [17]. This is in contrast to the decreased incidence rates observed with distant stage RCC (0.57%) [17]. The mortality rates during this period increased for localized disease (3.16%) and decreased for distant disease [17]. Earlier data demonstrated that incidence of surgical intervention rose in an identical manner to RCC incidence in localized tumors ≤ 4 cm [18]. A recent meta-analysis study examining small renal tumors demonstrates an average growth rate of only 0.28 cm/year when these patients received no medical or surgical intervention, with 1% rate of metastasis [3]. The most important clinical criterion for determining suspicion of malignancy is via the presence or absence of renal mass enhancement > 20 Hounsfield units on CT [3]. Cystic components on CT imaging

require utilization of the Bozniak classification system, with grades III and IV being classified as likely malignant requiring further workup [5]. Ultrasound with IV contrast can be utilized as a primary imaging technique in patients with chronic renal failure with contraindications to IV contrast, although MRI is preferred [5]. Percutaneous biopsy is recommended for histological diagnosis when cryoablative approaches are considered, before systemic therapy is undertaken for advanced or metastatic RCC, or when active surveillance is the primary management [5].

Therapeutic strategies for RCC

The UCLA Integrated Staging System (UISS) and the stage size grade and necrosis score (SSIGN) provide valuable information regarding survival percentages at the two and five year time points [19]. The 'Heng score', determines median survival for patients with advanced disease according to six risk factors: 1) Karnovsky performance status (PS) < 80%; 2) Hemoglobin less than lower limit; 3) Time from diagnosis to treatment < 1 year; 4) Corrected calcium above the upper limit; 5) Platelets greater than the upper limit of normal; 6) Neutrophils greater than the upper limit of normal.

Surgical intervention

Partial nephrectomy (or nephron sparing surgery) is the preferred management option for T1 tumors (<7 cm) which are confined to the kidney. This is especially true for patients with compromised renal function and/or bilateral tumors. The laparoscopic approach to partial nephrectomy in comparison to open partial nephrectomy is associated with lower morbidity, although comparisons among recurrence and overall disease free survival rates remain equivalent [5]. Cryoablative and radiofrequency ablation are feasible in elderly patients with contraindications to conventional surgery, as well as in patients with tumors confined to the renal cortex [3]. Active surveillance with serial abdominal imaging in patients with significant comorbidities is a feasible option for small renal masses and indolent disease with low aggressiveness and metastatic potential [3].

The increased risk of cancer recurrence in T2 tumors (>7 cm, confined to kidney), commands laparoscopic radical nephrectomy in the management of these patients [5, 19]. T3 and T4 tumors commonly involve local structures outside the confines of the renal capsule including the renal vein, vena cava, peripelvic and perirenal fat, adrenal gland, and local structures beyond Gerota's fascia. The gold standard treatment for these tumors remains open radical nephrectomy which includes removal of the kidney, perinephric fat, renal fascia, ipsilateral adrenal gland, and extensive para-aortic and para-caval lymphadenectomy [20], which results in cure in 40-60% of patients [3]. Imaging with CT can help guide surgical rationale in locally advanced disease. Currently, adrenalectomy and extensive lymphadenectomy in the absence of abdominal CT evidence of tumor presence within these structures is not recommended [19]. Recent clinical trials showing improvement in overall survival after adjuvant administration of autologous tumor cell vaccines is an exciting recent development [20]. Neoadjuvant administration of tyrosine kinase inhibitors (TKI) has shown a median tumor shrinkage of 9.6% [20] with a relatively good safety profile, but no improvement in overall survival [19, 20]. Metastatic renal cell carcinoma (mRCC) is notoriously recognized as one of the most chemotherapy-resistant malignancies. Radical nephrectomy in mRCC patients, also known as debulking or cytoreductive nephrectomy (CN), performed alongside systemic targeted therapies (VEGF inhibitors) is currently the focus of two ongoing phase III clinical trials (CARMENA and SURTIME trails). These studies examining the effects of 'targeted therapies' with CN, built on evidence during the 'cytokine era' examining CN with and without IFN-α treatment, which demonstrated a near doubling of median overall survival time for the CN + interferon group versus the interferon alone group [21]. Cytoreductive nephrectomy may reduce the source of tumor-promoting growth factors and immunosuppressive cytokines, or simply increase the time needed to produce a lethal tumor burden by removing a source of metastases [3]. Some mRCC patients may also benefit from metastasectomy. Prognostic factors that deem patients favorable for metastasectomy include limited metastases offering complete resectability and prolonged time between initial diagnosis and metastases development [3].

Systemic therapy-immunotherapy in mRCC

Effective treatment of mRCC remains a significant clinical challenge. During the last two decades, 51 phase II clinical trials were performed which examined 33 different chemotherapeutic agents against RCC, revealing that no combination of drugs was superior to the immunotherapeutic agents interferon alpha and/or interleukin 2, both of which individually demonstrated relatively low response rates (10-20%) but with occasional curative outcomes in 5-10% of patients [22, 23]. The rationale for immunotherapy regimens in the treatment of metastatic disease stems from identification of the tumor cell as a potential target of T-cells, B-cells, antibodies and NK cells which may recognize and respond to the unique antigenicity conferred by the malignant cell phenotype. IL-2 and IFN-α function as non-specific immune system stimulators [24]. In 1992, the FDA approved high-dose IL-2 for use in mRCC due to its effectiveness in producing complete remission for a small number of patients [24, 25]. Confidence in high-dose IL-2 as a standard therapy has recently waned, as there is no proven benefit of high dose vs low dose IL-2, as originally believed [25]. High dose IL-2 treatment often produces a capillary leak syndrome in patients and requires frequent blood pressure monitoring in units capable of infusing pressors, limiting its clinical applicability [3]. IFN-α has been utilized after IL-2 treatment failure due to its response rate and survival benefit; combination or sequencing of the cytokines however failed to result in survival benefit [26].

Molecular profiling of RCC

Significance of VHL

The majority of RCC is of the clear cell type (ccRCC). In approximately 80% of sporadic ccRCC cases, VHL genes are inactivated by deletion, mutation, or methylation [27]. In the early 1990s a study of multiple families with von Hippel Lindau syndrome led to the identification of the previously elusive VHL gene, residing within the short p arm of chromosome 3. The gene product of VHL (pVHL) is produced as two different isoforms, the 30 kDa form $pVHL_{30}$ and the 19 kDa form $pVHL_{19}$. Both isoforms exert identical tumor suppressor activity *in vivo* [28, 29]. Subsequent elegant studies by Iliopoulos et al. showed that tumor cell lines containing VHL mutations (VHL -/-) exhibited more aggressive tumor growth compared to wild type VHL (VHL +/+) cell lines. Moreover, reintroduction of the wild type VHL gene into mutant cell lines resulted in significant suppression of tumor growth in experimental mouse models [30]. The characteristic function of pVHL is that it serves as a substrate recognition subunit of the E3 ubiquitin ligase complex, which labels the alpha subunit of the transcription factor hypoxia inducible factor (HIF) for destruction [31]. HIF is a DNA binding transcription factor regulating numerous genes that promote cell survival under conditions of hypoxia, often found within solid tumors that commonly outgrow the local oxygen supply. Activation of HIF is dependent on the α-subunit levels accumulating under hypoxic conditions; there are three isoforms of the alpha subunit (HIF1α, HIF2α, HIF3α). HIF1α and HIF2α are heavily implicated in RCC, both subunits are activated by hypoxia and dimerize with HIF1β, with subsequent nuclear translocation resulting in increased expression of the angiogenesis mediator vascular endothelial growth factor (VEGF) [32]. HIF action within the nucleus also results in increased expression of platelet derived growth factor (PDGF), transforming growth factor alpha (TGF-α), glucose transporters (GLUT1) and fibroblast growth factor (FGF) genes [7]. A growing body of evidence implicates HIF2, rather than HIF1, as the primary contributor to RCC [7]. HIF1 can promote the expression of pro-apoptotic factors such as BCL2 and BNIP3 in ccRCC cells, which also confounds traditional understanding of HIF subunit interactions, and further studies elucidating clearer differences are needed [7]. Cells harboring overexpression and stabilization of HIF have greater likelihood of undergoing metastasis by induction of the chemokine receptor CXCR4 which promotes renal-cell specific dissemination [33]. Under normoxic conditions within cells containing two functional VHL alleles, the HIF-α protein is efficiently degraded by the ubiquitin-ligase complex. Cells lacking functional VHL alleles and therefore no pVHL, overexpress VEGF mRNA even under conditions of normoxia, therefore highlighting the important angiogenic regulatory function of normal VHL protein [32]. Although VHL mutations are present in the majority of sporadic ccRCC cases, it remains unclear whether VHL mutational status impacts outcomes [7].

Tumor vascularity in RCC

Renal cell carcinoma is characteristically a hypervascular tumor. A seminal study by Takahashi et al. more than 20 years ago demonstrated that VEGF mRNA was present at significantly higher levels in RCC tissues than in normal kidney tissue, which eventually led to the recognition of VEGF as a potent mediator of angiogenesis in renal cancer [34]. More recent evidence reveals that more than 90% of hypervascular renal cell tumors display elevated VEGF mRNA when compared to normal kidney tissues [33]. The biological effects of VEFG on target cells are mediated by the receptor tyrosine kinases (RTKs) VEGFR-1 and VEGFR-2. Interestingly, VEGFR-1 in particular is upregulated by HIF during hypoxia [35]. VEGF is an important regulator of angiogenesis throughout embryonic development. Inactivation by gene knockout of a single VEGF allele in mice results in embryonic lethality by 12 days [35]. VEGF functions to stimulate the growth of vascular endothelial cells (ECs) within arteries, veins, and lymphatics, and it also functions as a survival factor for ECs by preventing apoptosis via signaling through the phosphatidylinositol (PI)-3-kinase-Akt pathway [35]. VEGF also acts as a potent vascular permeability factor by increasing vascular leakage while also increasing fenestration in select vascular beds, making it a potential metastases-facilitator in many tumors including RCC [33, 35]. There have been significant associations between VEGF upregulation and nuclear grade, TNM stage, and poor prognosis [33].

PI3K/Akt/mTOR pathway in RCC

The PI3/Akt/mTOR pathway is considered one of the most important survival signaling pathways involved in diverse human malignancies. This signaling pathway is responsible for regulating cellular proliferation, differentiation, metabolism, and reorganization of cytoskeletal elements, all of which are intrinsically linked to both apoptosis and cell survival [36]. Somatic mutations of mTOR are associated with multiple cancers including RCC [36]. Multiple transmembrane tyrosine kinase receptors (ErbB receptor family, fibroblast growth factor receptors, etc.) along with G-protein coupled receptors (activated RAS) can initiate cell signaling through PI3K, an intracellular lipid kinase that cleaves phosphatidylinositol to generate PIP3, responsible for Akt activation, a serine threonine kinase that disinhibits mTOR activation resulting in increased protein synthesis [36]. Mechanistically, mTOR acts via its incorporation in two multi-protein complexes, TOR complex 1 (TORC1) and TOR complex 2 (TORC2). HIF-1 mRNA contains a 5' terminal oligopyrimidine (TOP) sequence which is obligatorily recognized by the ribosomal subunit P70 S6 kinase 1 (S6K) prior to translation [37]. TORC1 exerts translation regulation of mRNA containing 5' TOP sequences by phosphorylation of S6K, which is required for efficient translation of these mRNAs [37]. S6K can provide negative feedback to the upstream element PI3K by the repression of insulin responsive substrate 1 (IRS1), required for PI3K activity [38]. Significantly enough, mTOR activation is increased in the majority of ccRCC and most commonly occurring in high grade ccRCC tumors with poor prognostic features [39]. The therapeutic value of the mTOR inhibitor CCI-770 (temsirolimus) is enhanced by its ability to impair HIF-1 expression under both normoxic and hypoxic conditions [37].

Targeted therapeutics

VEGF inhibitors

Combination of bevacizumab with IFN-α: Bevacizumab is a recombinant humanized monoclonal antibody that directly inhibits VEGF. A randomized phase II clinical trial by Yang et al. indicated significant prolongation of time to disease progression with high-dose antibody treatments vs placebo [40]. Combination of bevacizumab with IFN-α in untreated mRCC patients led to a significant increase in progression-free survival (PFS) compared to the IFN-α only group (10.2 vs 5.4 months) in another study, with most significant improvement in low-risk and intermediate-risk patients [23].

Sunitinib: Sunitinib is an orally administered receptor tyrosine kinase inhibitor (TKI) with multiple targets including VEGFR and PDGFR and stem cell growth factor receptor (c-Kit) [41]. Clinically there was a significant increase in PFS in the sunitinib group compared to IFN-α (11 vs 5 months), with comparable adverse effects nevertheless (diarrhea, hypertension and left ventricular dysfunction) [27]. The post-baseline assessments of objectively reported quality of life were significantly higher in the

sunitinib group relative to the IFN-α control group [27]. A multi-center retrospective study in mRCC patients treated with TKIs identified 64 patients who had achieved CR, with the majority (59) of patients being exclusively treated with sunitinib [42]. Further studies will determine both patient factors promoting CR as well as necessity of treatment with TKIs following CR [27].

Pazopanib: Like sunitinib, pazopanib is an oral, highly selective tyrosine kinase inhibitor of VEGFR, PDGFR, and c-Kit. The pivotal study demonstrating its effectiveness was a phase III, international, multi-center clinical trial performed by Sternberg et al. which examined 435 mRCC patients, with 290 receiving pazopanib and 145 assigned to placebo. PFS was significantly increased in the pazopanib group compared to the placebo group (9.2 vs 4.2 months) as well as objective response rate for pazopanib (30% vs 3%) [43]. PFS with pazopanib treatment was comparable to that of sunitinib, however, the toxic profile of pazopanib was more favorable, with less incidence of hand-foot syndrome, cardiac dysfunction, and hematologic effects [44]. Further clinical evidence reveals comparable efficacy for pazopanib to sunitinib, with median PFS of 10.5 months vs 10.2 months respectively [44]. A phase III trial in 168 patients, indicated that 70% preferred pazopanib demonstrating patient preference in pazopanib's side effect profile [43]. Combination strategies of pazopanib with bevacizumab are currently ongoing and are listed in **Table 1**.

Axitinib/Sorafenib-second line options: In the search for second line agents to utilize in the treatment of mRCC patients who have failed bevacizumab or first line TKIs, both sorafenib and axitinib have shown efficacy in phase III clinical trials. In 2005, sorafenib was the first TKI to gain FDA approval for use in the treatment in mRCC. As multiple TKIs were developed and subsequently shown to outperform sorafenib, the investigative efforts focused on its value as a second line option for patients exhibiting resistance. The TARGET phase III trial supported its use as a second line agent (after IFN-α failure). In the phase III AXIS trial the second-generation TKI axitinib was more effective in increasing PFS compared to sorafenib, regardless of prior treatment [45]. The AGILE 1046 phase II trial examining axitinib treatment revealed a PFS of 14.6 months and OR rate of 54% (vs 34% placebo), while comparison of axitinib with sorafenib, failed to demonstrate significant differences thus terminating the development of axitinib [46]. Axitinib as a neo-adjuvant agent led to a considerable (28.3%) tumor reduction and favorable toxicity profile [47].

mTOR inhibitors

Temsirolimus: mTOR, or mammalian target of rapamycin, was discovered in the 1990s years after observing the potent *in-vitro* immunosuppressive and anti-fungal effects of the natural macrocyclic lactone rapamycin (also known as sirolimus) isolated from *Streptomyces hygroscopicus* bacterium from Easter Island. Sirolimus, upon cell entry, interacts with FKBP12, a peptidyl-prolyl isomerase, forming a toxic complex that binds only to TORC1 (not TORC2), preventing its kinase activity and resulting in cell-cycle arrest. Since its FDA-approval in 1999, multiple structural derivatives of sirolimus have been produced with reasonable efficacy in mRCC. A phase II clinical trial examining temsirolimus, a sirolimus derivative modified to increase solubility and bioavailability, demonstrated prolonged PFS of median 5.8 months as well as OS of 15.2 months [39]. Results from a phase III study on temsirolimus effect in a patient cohort with advanced, poor prognosis mRCC, demonstrated significantly increased OS, as well as PFS of 10.9 months for temsirolimus vs 7.3 months for IFN-α [39] (**Table 1**). Comparative analysis of patients with and without clear cell histology showed that OS advantage was similar in patients with non-clear cell mRCC [39].

Everolimus: Everolimus, another sirolimus derivative, emerges as an effective therapeutic agent for mRCC patients in the setting of failed first line treatments with the VEGF inhibitors bevacizumab and various TKIs. In a recent phase III trial, PFS in patients previously treated with one or more TKI or bevacizumab increased from 1.9 months to 4.9 months [39]. This second line therapeutic option is supported by level II-A evidence [19].

Overcoming resistance mechanisms in RCC

The anti-VEGF and anti-mTOR therapies have drastically altered the landscape of mRCC treatment, however prognosis for mRCC patients remains grim, with 5-year survival

Table 1. Ongoing Clinical Trials for Treatment of RCC (ClinicalTrials.gov)

Agent	Target	Design	Phase	Trial ID
AMG-386	Ang-1, Ang-2	As 1st line after combination with sunitinib after cytokine failure	II	NCT00853372
	Ang-1, Ang-2	With or without bevacizumab, pazopanib hydrochloride, sorafenib, or sunitinib	II	NCT01664182
Regorafenib	VEGFR, FGFR, PDGFR, others	BAY73-4506 in previously untreated patients with mRCC	II	NCT00664326
Cediranib	VEGFR, PDGFR, c-KIT	AZD2171 in metastatic or recurrent renal cell carcinoma	II	NCT00423332
Dovitinib	VEGFR, FGFR	1st-line activity of Dovitinib and correlation with genetic changes in RCC	II	NCT01791387
Tivozanib	VEGFR, PDGFR, c-KIT	Extension treatment protocol for patients in study of Tivozanib vs Sorafenib in RCC	III	NCT01076010
Aflibercept	VEGF-A, PlGF	As 1st line therapy for advanced mRCC	II	NCT00357760
Ramucirumab	VEGFR-2	As 2nd line therapy in combination with IMC-18F1 with/without Docetaxel	II	NCT01282463
INK-128	TORC1/TORC2	In combination with Bevacizumab for recurrent glioblastoma or advanced solid tumors	I	NCT02142803
BKM120	PI3K	In combination with Bevacizumab for mRCC patients	I	NCT01283048
GDC-0980	PI3K/TORC1/TORC2	GDC-0980 vs Everolimus in patients who have progressed on anti-VEGF therapies	II	NCT01442090
SF1126	PI3K/TORC1/TORC2	For patients with relapsed or refractory neuroblastoma	I	NCT02337309
BEZ235	PI3K/TORC1/TORC2	In combination with Everolimus in patients with advanced solid tumors	I	NCT01508104
MK2206	Akt	MK2206 vs Everolimus in patients with refractory renal cancer	II	NCT01239342
Nivolumab	PD1	In combination with Sunitinib, Pazopanib, or Ipilimumab in mRCC	I	NCT01472081
	PD1	Phase 1b study of MDX-1106 in advanced or recurrent malignancies	I	NCT00730639
	PD1	Alone or in combination with either bevacizumab or ipilimumab in mRCC	II	NCT02210117
Ipilimumab	CTLA-4	Ipilimumab and Lenalidomide in advanced cancers	I	NCT01750983
IMA901	Tumor antigens (vaccine)	In combination with Sunitinib in advanced mRCC	III	NCT01265901
AGS003	Tumor antigens (vaccine)	AGS003 vaccine vs standard treatment for advanced mRCC	III	NCT01582672
	Tumor antigens (vaccine)	Vaccine therapy before surgery in patients with localized kidney cancer	Pilot	NCT02170389
Other vaccines	Tumor antigens (vaccine)	Vaccine therapy in kidney cancer	I, II	NCT00014131
	Tumor antigens (vaccine)	Autologous vaccination of stage IV RCC combined with Sunitinib	I, II	NCT00890110
	Tumor antigens (vaccine)	Dendritic cell vaccine therapy combined with cytokine induced killer cells in RCC	I, II	NCT00862303
	Tumor antigens (vaccine)	Evaluating safety and efficacy of COMBIG-DC vaccine in mRCC patients	I	NCT01525017
	Tumor antigens (vaccine)	Vaccine therapy with or without Sirolimus in patients expressing NY-ESO-1 antigen	I	NCT01522820

rates at 10%. The majority of patients with metastases develop treatment resistance [48], via three mechanisms: (a) genetic modification results in structural changes of target proteins preventing drug binding; (b) cancer cells may engage alternative signaling pathways not targeted by drug therapies thus evading apoptosis and continue uncontrolled proliferation; (c) cancer cells may upregulate production of drug-targeted proteins to bypass drug-induced inhibition. The 'intrinsic' model of resistance is an alternate model characterized by pre-existing non-responsiveness to a drug resulting in no clinical benefit [49].

The angiogenic switch

Revascularization of tumors after initial inhibition of angiogenesis by VEGFR antagonists was postulated to occur via VEGFR mutations resulting in ineffective drug binding. RCC tumor xenografts, when removed from drug-resistant mice and reimplanted into untreated mice, lose their angiogenic properties quickly [48]. This physiologically compelling evidence negates the theory that permanent genetic alterations are driving therapeutic resistance to VEGF targeting in initially responsive tumors. One must consider the possibility of the tumor cells' reliance on alternative pro-angiogenic pathways supported by a dynamic surrounding stroma navigated by the tumor microenvironment [48]. Casanovas et al. demonstrated significant upregulation of pro-angiogenic factors FGF, ephrin, and angiopoietin in response to DC101 antibody blockade of VEGFR2 in murine models of pancreatic islet cell tumors, implicating alternative angiogenic pathway utilization. Angiopoietin-2 (Ang-2) expression follows a temporal decrease in sunitinib-responsive tumors, and dramatically increases after formation of sunitinib resistance in mRCC patients. Through its ligand binding interaction with Tie-1 and Tie-2 (tyrosine kinase receptors), Ang-2 directly stimulates endothelial cells to initiate new vessel assembly [49]. Contributing to therapeutic resistance, Ang-2 has also been shown to stimulate production of matrix metalloproteinases (MMPs) through integrin signaling in the tumor microenvironment [48]. MMPs are responsible not only for remodeling the basement membranes of endothelial cells to facilitate new capillary formation, but also for binding factor inhibiting HIF-1 (FIH-1), an HIF inhibitory protein, in the surrounding stroma [33, 50].

AMG-386 (trebananib) is a recombinant antibody composed of immunoglobulin linked to Tie-2 receptor, which binds Ang-2 preventing its interaction with native Tie-2 and has been shown to have anti-tumor effects in mRCC patients, leading to multiple clinical trials in combination with other anti-VEGF therapies [48] (**Table 1**). Furthermore, IL-8 (a pro-angiogenic cytokine), PIGF (placental growth factor), and MIFT (microphtalmia-associated transcription factor) are upregulated after bevacizumab or TKI induced hypoxia [49]. Functional studies identified the ability of these pro-angiogenic factors to promote re-sensitization to TKIs in murine models, further supporting the hypoxia-induced utilization of multiple angiogenic signaling pathways [49] in desensitizing advanced mRCC tumors to VEGF targeting. Mechanistically, down-regulation of angiostatic factors may be contributing to resistance, by preventing the actions of native anti-angiogenesis mediators. Pre-clinical studies in RCC xenografts indicate that resistance to TKIs is associated with the downregulation of IFN-γ and CXCL10, both chemokines heavily implicated in angiostasis [51]. Another angiostasis mediator, endothelial cell-specific molecule 1 (ESM-1), has also been assigned a role as a potential molecular marker of progression and therapeutic TKI resistance. Recently, HUVEC (human umbilical vein endothelial cells) clones, resistant to TKI and lacking VEGFR2 activation upon VEGF stimulation, exhibited downregulation of ESM1 [49]. This was clinically validated by the marked downregulation of ESM1 in mRCC patients undergoing TKI treatment and exhibiting therapeutic resistance [49]. Recent work by Wong et al. [52] highlights the potential benefits of the innovative approach of 'vascular normalization', which utilizes pro-angiogenic agents to actually improve rather than reduce tumor blood flow, thereby improving drug delivery. It is argued that by inhibiting vascularization of tumors, delivery of cytotoxic drugs is reduced while increasing tumor hypoxia. It was established that administration of cilengitide (an integrin receptor inhibitor developed as an anti-angiogenesis agent that has been shown to actually promote vascularization) with verapamil, resulted in improved gemcitabine delivery to tumors in preclinical models of pancreatic and small-

cell-lung carcinoma, via increased tumor blood vessel density, suppressing tumor growth and impairing metastasis [52]. The vascular promotion strategy in cancer therapy calls for further development in advanced tumors.

Novel VEGF, PDGF, FGF inhibitors (TKIs): Since the approval of axitinib as second line treatment for mRCC patients, the family of tyrosine kinase inhibitor has increased. Novel TKIs currently investigated in a spectrum of clinical trials in RCC patients, include regorafenib, tivozanib, cediranib, linifanib, dovitinib, and brivanib [53]. These ongoing clinical trials are listed in **Table 1**.

Novel mAB and other VEGF targeting agents: Aflibercept (VEGF-Trap) is a soluble fusion-protein comprised of both VEGFR-1 and VEGFR-2 and binds all forms of VEGF-A and placental growth factor [53, 54]. Ramucirumab is a recombinant human monoclonal antibody with high affinity for the extracellular domain of VEGFR2, blocking its interaction with VEGF [55]. Ramucirumab was unable to reach its primary endpoint of ≥15% overall response rate (ORR), although it exerted anti-tumor efficacy in TKI resistant patients [55].

TORC2 'open gate' resistance

TORC2 is not targetable by current clinically available mTOR inhibitors. TORC1 inhibition by rapamycin analogs results in loss of native TORC1/S6K induced inhibition of Akt resulting in compensatory increased activation of upstream PI3K and Akt [48]. TORC2 phosphorylates and activates upstream Akt by phosphorylation of Ser473 in a positive feedback loop [39]. At the molecular level HIF1α is dependent on both TORC1 and TORC2, while HIF2α expression is dependent only on TORC2. IRS (insulin receptor substrate) links the extracellular signal IGF-1 to intracellular PI3K, thus activating the PI3K/Akt/mTOR signaling pathway. Phosphorylation of IRS leads to uncoupling of the IGF/IRS signaling to PI3K [39]. With the evidence-based knowledge that the IRS-PI3K-Akt-TORC2 'gate' can be effectively targeted by current mTOR inhibitors, one may argue that loss of S6K negative feedback could confer therapeutic resistance. Significantly increased levels of PI3K and TORC2 correlate with high grade tumors and have potential value as prognostic indicators of survival [56].

Novel inhibitors of PI3K, Akt, TORC2: A number of pre-clinical studies examining novel inhibitors of the mTOR signaling pathway have demonstrated anti-tumor effects. The novel PI3K/TORC1/TORC2 inhibitor SF1126 leads to significant downregulation of Akt and HIF2α and suppression of RCC tumor growth [56]. Combination therapy with sirolimus revealed a marked tumor regression [56]. A phase I clinical trial examining SF1126 in multiple solid tumor types provided limited promise with one patient with ccRCC achieving stable disease for 84 weeks [57]. Other PI3K targeting agents currently under investigation include BKM120, BEZ235 and GDC-0980[56]. Moreover, perifosine, a novel Akt/MAP kinase inhibitor, has exhibited therapeutic effects in mRCC patients who failed treatment with sunitinib or sorafenib [53]; the therapeutic effect of perifosine and another novel Akt inhibitor MK2206 is also being interrogated in resistant mRCC [53]. Preclinical data from a first-in-class TORC1/TORC2 inhibitor AZD8055 demonstrated the drug's promising inhibitory effect on phosphorylation of TORC2 substrate Akt on Ser473 [58], translating into only partial clinical responses [59]. AZD2014 is another dual TORC1/TORC2 inhibitor exerting partial responses and antitumor activity, without affecting transaminase levels [59]. Ongoing clinical trials examining various inhibitors of PI3K, TORC, and Akt are listed in **Table 1**.

Novel therapeutic targets

Targeting c-Met

c-Met is a receptor tyrosine kinase, implicated as a proto-oncogene, that is normally involved in cell differentiation/growth, angiogenesis, and tissue repair. The ligand for c-Met is hepatocyte growth factor (HGF), which has been shown to be tumor promoting in a number of malignant conditions. As previously discussed, c-Met mutations often characterize sporadic and familial cases of papillary RCC, and c-Met/HGF levels have recently been shown to correlate with VHL mutation/loss of heterozygosity in clear cell RCC samples, thus, further development of Met targeting agents could perhaps be used to effectively treat both ccRCC and papillary RCC [60]. Prior to development of MET inhibitors, no specific treatments have been developed for papillary RCC, highlighted by the

fact that according to the National Comprehensive Cancer Network guidelines on kidney cancer, "clinical trials" remains the preferred treatment approach for advanced papillary RCC [61]. Foretinib is a recently developed oral inhibitor of MET and VEGFR among other kinases (AXL, RON, Tie-2) [61]. In a phase II clinical trial involving patients with papillary RCC, PFS was 9.3 months which is an improvement upon PFS obtained from recent trials examining sorafenib and sunitinib in papillary RCC (PFS 1.6 months to approximately 6 months for various trials) [61]. This same phase II trial demonstrated ORR of 13.5% which did not meet its efficacy goal of 25% [61]. Interestingly enough, patients harboring germline MET mutations were more likely to achieve partial responses with Foretinib, than papillary RCC patients without germline mutations or with somatic MET mutations [61].

Targeting PD-1

Since the replacement of IFN-α and IL-2 as standard monotherapies with 'targeted therapies', development of immunomodulation as a strategy to suppress tumor growth has been challenged. Programmed death receptor 1 (PD-1) is a major player in the immune checkpoint pathway, emerging as a therapeutic target towards induction of a more robust and viable anti-tumor immune response. PD-1 is expressed on T cells, B cells, and NK cells, and is a member of the CD-28 co-receptor family [62]. Upon ligand binding to PD-1, cell growth and cytokine secretion is inhibited in immune cells, thus playing a role in the vital process of peripheral immune tolerance within the body. Nivolumab is a recently developed human IgG4 anti-PD1 antibody. In a phase I clinical trial, Nivolumab has been shown to induce partial and objective responses (up to 16 months) in mRCC patients who failed previous therapies [63]. Currently ongoing clinical trials examining Nivolumab efficacy in mRCC are listed in **Table 1**.

Targeting CTLA-4

Another immunomodulatory strategy being explored is the targeting of CTLA-4, another player in the immune checkpoint pathway. It functions as a co-receptor on inhibitory T cells and serves to down-regulate CD28 expression on effector T-cells after binding to B7 ligand present on antigen presenting cells. Targeting CTLA-4 has been achieved by development of the novel human monoclonal CTLA-4 antibody ipilimumab. In a recent phase II trial examining 40 mRCC patients, five participants achieved partial responses with duration of 7-21 months [64].

Tumor vaccines

A more specific and 'targeted' approach in immune system modulation may be found within the developmental schema of the fairly new class of tumor vaccines. Rather than targeting immune regulatory pathways, tumor vaccines show more potential in evoking tumor antigen specific responses by forcing dendritic cells (the primary professional antigen presenting cell) with tumor antigens, which facilitates T and B cell maturation and priming against these antigens. Developing anti-tumor vaccines has been a challenging task clinically. Walter et al. recently developed the first tumor vaccine containing multiple RCC tumor specific peptides, IMA901. The vaccine is comprised of nine peptides shown to be naturally present on RCC cells, which were proven to be immunogenic in *in vitro* studies: PLIN2, APOL1, CCND1, GUCY1A3, PRUNE2, MET, MUC1, RGS5, MMP7, HBV; nucleocapsid protein (HBcAg) [65]. In a phase II trial utilizing co-adminstration of cyclophosphamide (which was shown to reduce inhibitory T_{Reg} cells), IMA901 demonstrated a disease control rate of 31% at 6 months in patients previously treated with cytokine therapy, and 14% for patients previously treated with TKIs [65]; a randomized phase III clinical trial with IMA901 is currently ongoing (**Table 1**). A newly developed vaccine, AGS003, is created by first removing tumor tissue as well as the patient's dendritic cells (DC) that are subsequently transfected with RCC specific amplified RNA as well as human CD-40 ligand that may improve immune responses induced by DCs. A recent phase II trial examining AGS in combination with sunitinib demonstrated an improvement in PFS compared to historical data regarding sunitinib treatment alone in newly diagnosed mRCC patients (11.9 months vs 8 months, respectively) [64]. A phase III trial examining AGS003 administration in combination with sunitinib, as well as several other clinical trials (**Table 1**) with developed vaccines (5T4, autologous tumor cell lysate, etc.) are ongoing [64].

Conclusions

In summary, the treatment of patients facing diagnosis of renal cell carcinoma has undergone extensive change within the past few decades, catalyzed by advancements in the understanding of the consequences of genetic alterations of VHL leading to hypoxia induced increases in tumor vascularity and survival, and highlighted by the exploitation of angiogenesis pathways via agents such as TKIs, anti-VEGF antibodies, and mTOR inhibitors. Understanding of resistance mechanisms utilized by tumor cells during treatment with these agents (including alternative angiogenic pathways, reliance on HIF2α, modification of the tumor microenvironment, etc.) has coincided with the appearance of more potent and specific TKIs, novel anti-VEGF antibodies, and multiple novel agents targeting previously untargeted TORC2, PI3K, and Akt. Novel therapeutic directions point to the return of immunomodulatory strategies targeting immune checkpoint pathways to bolster anti-tumor responses and developing patient individualized tumor vaccines specific to patient tumor antigens.

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Disclosure of conflict of interest

None.

Abbreviations

ccRCC, clear cell renal cell carcinoma; mRCC, metastatic renal cell carcinoma; PDGF, platelet derived growth factor; VEGF, vascular endothelial growth factor; FGF, fibroblast growth factor; mTOR, mammalian target of rapamycin; TORC, target of rapamycin complex; VHL, von-Hippel Lindau; HIF, hypoxia inducible factor; FIH-1, factor inhibiting HIF-1; IFN-α, interferon alpha; IL-2, interleukin-2; ROS, reactive oxygen species; GLUT, glucose transporter; ESM-1, endothelial cell specific molecule 1; c-MET, mesenchymal epithelial transition factor; PI3K, phosphatidylinositide 3-kinase; S6K, ribosomal protein S6 kinase; PlGF, placental growth factor; MIFT, microphtalmia-associated transcription factor; HGF, hepatocyte growth factor; AKT, protein kinase B; FH, fumarate hydratase; BNIP-3, Bcl-2/adenovirus E1B 19kD-interacting protein 3; IRS1, insulin responsive substrate 1;CXCR4, CXC chemokine receptor type 4; c-Kit, tyrosine protein kinase KIT; BHD, Birt-Hogg-Dubé; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PD-1, programmed death receptor 1; Ang2, angiopoietin 2; Tie-1, tyrosine kinase with immunoglobulin-like and EGF-like domains 1; TKI, tyrosine kinase inhibitor; BCL2, B-cell lymphoma 2; PFS, progression free survival; OS, overall survival; ORR, overall response rate; CN, cytoreductive nephrectomy; FDA, Food and Drug Administration; UISS, UCLA Integrated Staging System; SSIGN, stage size grade and necrosis score.

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