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RESISTANCE TO SYSTEMIC THERAPIES IN CLEAR CELL RENAL CELL CARCINOMA: MECHANISMS AND MANAGEMENT STRATEGIES

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Abstract

Renal cell carcinoma (RCC) is the most common form of kidney cancer. It is categorized into various subtypes, with clear cell RCC (ccRCC) representing about 85% of all RCC tumors. The lack of sensitivity to chemotherapy and radiation therapy prompted research efforts into novel treatment options. The development of targeted therapeutics, including multi-targeted tyrosine kinase inhibitors (TKIs) and mTOR inhibitors, has been a major breakthrough in ccRCC therapy. More recently, other therapeutic strategies, including immune checkpoint inhibitors, have emerged as effective treatment options against advanced ccRCC. Furthermore, recent advances in disease biology, tumor microenvironment, and mechanisms of resistance formed the basis for attempts to combine targeted therapies with newer generation immunotherapies to take advantage of possible synergy. This review focuses on the current status of basic, translational, and clinical studies on mechanisms of resistance to systemic therapies in ccRCC.

Introduction

Renal cell carcinoma (RCC) accounts for more than 330,000 cases of cancer world-wide and more than 140,000 cancer-related deaths each year. The incidence of kidney cancer has risen steadily over several decades and continues to increase. Approximately 65,340 new kidney cancer diagnoses are projected in the United States in 2018 that are expected to result in 14,970 deaths (1). The incidence in men is 1.5–2.0 times greater than in women, and the peak age of incidence is 60–70 years (2). Clear cell renal cell carcinoma (ccRCC) is the most frequent (75–80%) and the best studied subtype of RCC. Papillary RCC and chromophobe RCC represent the most common remaining histologic subtypes with an incidence of 7% - 14% and 6% - 11%, respectively (3). Advanced RCC is a lethal disease portending a 5-year survival of only 11.7% (4). Two distinct groups of patients are at

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particular risk of death from RCC: Those who present with metastatic disease and those who recur following surgery. Approximately 30% of RCC patients have metastatic disease at initial presentation (5). Recurrence occurs in about 30% of patients after complete resection of the primary tumor (6). This includes 10-25% of patients with localized (pT1-2N0) disease who demonstrate recurrence despite incidental detection and clinically complete surgical resection (7–10). Traditional chemotherapy and radiation therapy are largely ineffective in the treatment of all RCC subtypes (11,12). The lack of sensitivity to chemotherapy and radiation therapy prompted early research efforts into novel treatment options.

Treatment of metastatic RCC: historical and current concepts

Occasional spontaneous tumor regressions and the presence of tumor infiltrating immune cells suggested that adaptive immunity might play an important role in renal malignancy. For more than 20 years, immunotherapy using high-dose IL-2 or Interferon alfa (IFN-α) remained the primary treatment for patients with metastatic RCC (mRCC). Unfortunately, response rates to immunotherapy were disappointing, ranging from 15% to 25% (13).

The field of renal cancer therapy has undergone radical changes in the last decade. The clinical knowledge that ccRCC is a highly vascular cancer and that the Von Hippel Lindau (VHL) protein has an important role in sporadic ccRCC has made anti-angiogenic strategies an attractive approach (14,15). The introduction of therapeutic agents targeting Vascular Endothelial Growth Factor (VEGF) signaling, especially multi-targeted tyrosine kinase inhibitors (TKIs), has been a major breakthrough in ccRCC therapy. Various VEGF receptor TKIs have demonstrated considerable efficacy in RCC. Sunitinib (Sutent) and pazopanib (Votrient) are approved for first-line treatment in mRCC (16), whereas axitinib (Inlyta) and sorafenib (Nexavar) have demonstrated progression-free survival (PFS) benefits as second-line agents (17). As opposed to earlier TKIs, axitinib blocks receptors known to be involved in escape pathways that lead to treatment resistance. After its effectiveness was established, a head-to-head trial of axitinib vs. sorafenib (AXIS) demonstrated the clinical superiority of axitinib over sorafenib (PFS 6.7 mo vs. 4.7mo) in second-line treatment (17). Also, a consistent PFS benefit was demonstrated with bevacizumab + IFN-α (Avastin), a recombinant humanized monoclonal antibody that binds VEGF preventing its interaction with VEGF Receptor (VEGFR), in treatment-naïve mRCC patients (18–20). Most recently, cabozantinib (COMETRIQ), inhibitor of VEGF-R, MET, and AXL, demonstrated PFS and OS advantages over sunitinib, and received FDA-approval for frontline mRCC treatment (21). Cabozantinib appears specifically effective at blocking the combination of angiogenic pathways that emerges following oncogenic VHL inactivation. Lenvatinib (Lenvima) is another TKI targeting VEGF-R1-R3, FGF-R1-4, PDGF-R, RET, and KIT. Phase II analysis demonstrated a PFS superiority of lenvatinib + everolimus vs. either agent alone (22). The successful combination of lenvatinib with everolimus is especially notable because of the historical failure of combining VEGF and mTOR inhibitors due to excessive toxicity.

In addition to the VEGF pathway, the Akt/mTOR mammalian target of rapamycin (mTOR) pathway was identified as a promising therapeutic target for the treatment of mRCC. Single agent activities led to market approval of two mTOR inhibitors, everolimus and temsirolimus, for the treatment of advanced RCC (23).

Improvements in molecular understanding of resistance mechanisms has led to the discovery of many new targetable pathways. Several new agents are under early clinical investigation, and these may play an important future role in combination therapy (20). Trebananib is a Ang/Tie-2 pathway inhibitor. This pathway is responsible for basal angiogenesis and vascular stability following VEGF blockade, so it potentially has significant utility when used in combination with TKIs (24). Dalantercept inhibits ALK-1, which is thought to play a role in vascular bed formation. Other agents are being tested in preclinical models that exhibit dual mTORC1/2 inhibition. Most mTOR inhibitors primarily target mTORC1, and may fail to disrupt the activity of HIF-2α, which is more highly regulated by mTORC2. By more effectively blocking HIF translation, dual mTORC1/2 inhibitors may offer unique treatment possibilities in the future.

Recent advances have led to new treatment approaches in the post-TKI second- or third-line settings. Nivolumab (programmed death (PD-1) checkpoint inhibitor), cabozantinib, and lenvatinib plus everolimus demonstrated promising clinical efficacy and have gained FDA approval in the last two years (25–28).

Mechanisms of primary and acquired resistance to systemic therapeutic agents

Response to anticancer therapy is currently defined by the Response Evaluation Criteria In Solid Tumors (RECIST) criteria as evidence of disease progression despite therapeutic treatment. This progression is defined as an increase of 20% or more in the sum of measurable lesions, the appearance of new lesions, or an unequivocal progression of non-measurable disease such as small lung nodules or bone lesions (29). Resistance to targeted therapeutics can be classified into intrinsic (primary) and acquired (secondary) resistance. Intrinsic resistance can be classified as an immediate inefficacy of therapeutic agents. This type of resistance can be attributed to the presence of resistant tumor clones prior to therapy due to inherited resistance or evolutionary clonal selection. Acquired resistance is characterized by tumor growth after initial tumor regression while the patient is still receiving therapeutic treatment. While the precise mechanisms of resistance to targeted therapeutics are still being elucidated, laboratory and clinical studies have identified several underlying factors contributing to both intrinsic and acquired resistance mechanisms.

Lysosomal sequestration of TKIs

Lysosomes contain about 50 different acid hydrolases, which are optimally active at the acidic pH of 4.6–5.0. To maintain the low pH, lysosomes utilize proton-pumping vacuolar ATPases (30). Lysosomal sequestration is a process in which hydrophobic weak base compounds accumulate within acidic lysosomes (31). Such compounds travel freely across the lysosomal membrane due to their hydrophobic nature. Upon encountering the acidic lysosomal compartment, these compounds become protonated due to their weak base properties and can no longer exit across the lysosomal lipid membrane (31). Most TKIs are membrane-permeable weak bases, and are therefore trapped in their protonated forms in the acidic lysosomal compartment away from their target sites. Lysosomal sequestration as a

mechanism of drug resistance is reversible; drug-free cultures of resistant tumor cells result in recovery of drug sensitivity (32).

Several TKIs have been shown to undergo lysosomal sequestration including sunitinib, erlotinib, and pazopanib (32,33). Interestingly, although lysosomal sequestration of sorafenib was not found in renal cancer cells (33), it has been demonstrated in hepatocellular carcinoma (34). Sorafenib does not belong to the same class of hydrophobic, membrane-permeable weak bases as sunitinib; and therefore, a different mechanism could explain its lysosomal sequestration, possibly involving the activity of drug pumps (35). Indeed, the lysosomal sequestration of both sorafenib and sunitinib was reported to be mediated by the ABC transporter P-glycoprotein (P-gp) (34). Treatment with verapamil, a P-gp inhibitor, enhanced the antitumor activity of sorafenib and sunitinib, supporting the role of P-gp in TKIs resistance (34).

Lysosomal sequestration of hydrophobic weak base TKIs induces lysosomal biogenesis (36). Increased lysosomal biogenesis, in turn, results in augmented lysosomal drug sequestration and multi-drug cross-resistance. Lysosomal biogenesis is regulated by the transcription factor EB (TFEB), which is in turn controlled by mTORC1 (37). These findings provide rationale for combined treatment with TKIs and mTOR inhibitors, though excessive toxicity was reported for TKI/mTOR combination therapy (38,39).

Mutations and modifications of expression level

Mutation rates can vary by as much as three orders of magnitudes between various cancer types and even patients (40). RCC is a complex disease characterized by mutations in many genes. Deactivation of the VHL gene is the most common mutation in RCC. Some studies have reported VHL gene inactivation in >90% of patients with sporadic ccRCC (41). Epigenetic silencing of VHL was present in 7% of ccRCC tumors, which is consistent with the role of epigenetic changes in renal carcinogenesis (42,43). Loss of VHL gene function results in the stabilization and increased expression of hypoxia inducible factors (HIFs) and up-regulation of HIF-responsive genes that mediate angiogenesis and cell growth. Nearly universal loss of VHL function and subsequent HIF activation in the pathogenesis of ccRCC would suggest that TKIs are a highly efficient treatment modality of this type of kidney cancer (44). However, a study by Choueiri et al. demonstrated that VHL mutation status has little effect on patient responses to VEGF-targeted agents in metastatic ccRCC (45). VHL mutations may confer aberrant activation of AKT/mTOR signaling (46) given that VHL inactivation subsequently activates mTOR, which in turn up-regulates HIF and jumpstarts other angiogenic pathways (47). Several clinical trials have evaluated the efficacy and safety of everolimus and temsirolimus in the treatment of mRCC after progression on TKIs. Whereas treatment with mTOR inhibitors offers significantly improved clinical benefits, such treatment rarely yields a complete response and is not curative (48). A potential mechanism that accounts for resistance to everolimus and temsirolimus is mutation in FKBP-12 domain in mTOR, which reduces the binding affinity of mTOR inhibitors (49). The mutational status of the mTOR pathway genes TSC1, TSC2, and REDD1 could also predict response to everolimus or temsirolimus in RCC tumors (50). REDD1 is a HIF-1 target gene that inhibits mTORC1 by activating the TSC1/2 complex under normal

conditions. Alterations in TSC1, TSC2, or REDD1 therefore, prevent the inhibition of mTORC1 (48). Mutations in all these genes have been observed in RCC (48).

The aberrant expression of proteins involved in the mTOR signaling pathway may also modulate sensitivity to mTOR inhibitors. Phospholipase D2 (PLD2)-derived phosphatidic acid binds to and activates ribosomal S6 kinase (S6K), which, in turn, activates a feedback loop inactivating PLD2 and decreasing phosphatidic acid levels (51). Phosphatidic acid is required for mTORC1/mTORC2 complex assembly and competes with rapamycin for mTOR binding (52). Critically, PLD2 expression and activity are greatly elevated in RCC tissue specimens as compared with the adjacent normal tissues (53). Taken together, these findings provide a mechanistic link between increased PLD2 activity and resistance to mTOR inhibitors.

A recent study by Adelaiye et al. demonstrated that the overexpression of the histone methyltransferase enhancer of zeste homologue 2 (EZH2) promotes tumor angiogenesis by inactivating anti-angiogenic factors via methylation at their promoter regions, causing resistance to sunitinib (54). Tumors resistant to sunitinib had an increased level of EZH2 expression. However, this increase was reversible upon dose escalation, suggesting that tumor adaptation to sunitinib was dynamic and was likely driven by epigenetic alterations (54).

The p4E-BP1/eIF4E axis represents a critical convergence point for several upstream signaling pathways such as EGF-R/ERK and AKT/mTOR, all of which are targeted in some respect by molecular-targeted therapies used in the treatment of RCC. Levels of phospho-4e-BP1 and total eIF4E are elevated in ccRCC (55). Accordingly, phosphorylated 4E-BP1 has recently been shown to be the single most accurate biomarker for predicting treatment response to mTOR inhibitors (56).

Angiogenic Switch

Inactivation of VHL in RCC cells lead to increased HIF-1 and HIF-2 activities. Interestingly, HIF-2 antagonist PT2399 was more active than sunitinib ($p=0.0126$) and inhibited tumor growth in several sunitinib-resistant RCC xenograft tumors (57). However, as discussed above, VHL mutation status by itself had little effect on patient responses to VEGF-targeted agents in metastatic ccRCC (45). Furthermore, studies by Bielecka et al. demonstrate that sorafenib and axitinib effectively inhibit the growth of primary and metastatic ccRCC cell lines in normoxia and hypoxia. Only the growth of papillary kidney cancer stem-like cells was inhibited in an oxygen-dependent manner in this study (58). Nevertheless, hypoxia and HIFs may contribute to resistance to targeted therapeutics by up-regulating expression of VEGF, platelet derived growth factor (PDGF), interleukin-6 (IL-6), interleukin-8 (IL-8), transforming growth factor α (TGF-α), erythropoietin (EPO), epidermal growth factor receptor (EGFR), hepatocyte growth factor receptor (HGFR/c-MET), placental growth factor (PlGF), and fibroblast growth factor 2 (FGF2) (59,60). Notably, hypoxia caused by the regression of tumor vasculature during the course of anti-angiogenic treatment may also lead to enhanced expression of various proangiogenic factors (61).

The findings relative to IL-6 and IL-8 are of particular interest given their well-established roles as prognostic and predictive factors associated with resistance to TKIs in ccRCC patients (62–65). In patients receiving pazopanib or sunitinib, high baseline serum IL-6 and IL-8 levels are associated with shorter PFS and/or overall survival (OS) (65–67). IL-8 promotes the expression of VEGF mRNA and protein in endothelial cells by binding to CXCR2, which subsequently leads to the autocrine activation of VEGFR-2, resulting in increased angiogenesis (68). Studies by Huang et al. showed that increased plasma levels of IL-8 were detected in the plasma of mice with sunitinib-resistant tumors compared to mice bearing sunitinib-responsive tumors (62). Treatment with IL-8 neutralizing antibodies reinstated sensitivity to sunitinib (62). Studies by Wu et al. evaluated the predictive value of polymorphisms in IL-8 in sunitinib and pazopanib resistance (69–71). These findings showed that variant alleles of IL-8 are associated with poorer survival outcomes in pazopanib- or sunitinib-treated patients with advanced RCC (69–71). Recent studies by Ishibashi et al. showed that treatment with sorafenib, sunitinib, and pazopanib stimulated the autocrine secretion of IL-6, which consequently lead to the activation of AKT/mTOR and STAT3 signaling, VEGF expression, and TKI resistance in RCC cells (63). Combination therapy with tocilizumab, a humanized antihuman IL-6 receptor (IL-6R) antibody reinstated TKI sensitivity in RCC cells (63). These findings are in agreement with previous studies by Zhu et al. showing that IL-6 signaling inhibition leads to the increased efficacy of sunitinib in cell and animal models of human RCC (64).

Studies by Mizumoto et al. suggested that the mechanism of acquired resistance to sunitinib in RCC cells may be also related to the activation of EGFR (72). Inhibition of EGFR signaling with erlotinib decreased the viability of RCC cells treated with sunitinib (72). The pro-angiogenic function of FGF2 may be also directly relevant for resistance to sunitinib. FGF2 suppresses the anti-angiogenic activity of sunitinib by directly stimulating pro-angiogenic signaling in endothelial cells (73). This is likely to be especially important for RCC, in which FGF2 expression is prominent (73).

VHL inactivation in ccRCC induces overexpression and activation of the receptor tyrosine kinases MET and AXL (74). Both, MET and AXL signaling have been implicated in clinical resistance to VEGF-targeted therapeutics. Therefore, targeting the MET and AXL represents a logical option after progression on initial VEGF therapy. Cabozantinib, VEGFR, c-MET and AXL inhibitor, was approved by the FDA in April 2016 for the treatment of advanced RCC, pretreated with at least one prior antiangiogenic therapy. Cabozantinib demonstrated clinical efficacy in advanced pretreated RCC, with statistically significant improvements in RR, PFS, and OS (27,75) (Fig. 1). Combination with another c-met inhibitor, crizotinib, not only increased axitinib-mediated inhibition of tumor microvessel density, but also suppressed growth of patient derived xenograft (PDX) RP-R-01 tumors. In this highly proliferative model, concurrent c-met inhibition and VEGFR blockade was required to inhibit tumor growth and improve survival (76). Lastly, the VEGF homologue PlGF binds to VEGFR-1 and displaces VEGF from VEGFR-1, resulting in an increased bioavailability of VEGF. The increase in VEGF bioavailability stimulates VEGFR-2 and thereby promotes angiogenesis (48,77). PlGF also stimulates angiogenesis through other mechanisms, such as up-regulation of VEGF-A, FGF2, and PDGFβ expression, and by recruiting bone marrow-

derived angiocompetent myeloid cells, which stimulate angiogenesis through the secretion of pro-angiogenic cytokines (48).

Bypass or alternative pathways activation

The tumor suppressor PTEN acts as a negative regulator of PI3K/Akt/mTOR pathway. The loss of PTEN function results in the constitutive activation of AKT/mTOR signaling downstream of TKIs cellular targets. PTEN mutations are rare in RCC (78). However, PTEN gene expression has been shown to be down-modulated in a large percentage of RCC (42,79). Our own studies demonstrate that lack of PTEN expression coincides with sunitinib resistance in renal cells, whereas restoration of PTEN expression or pharmacologic inhibition of AKT/mTOR signaling reinstates sensitivity of PTEN-deficient ccRCC cells to sunitinib-mediated apoptosis. (80). It has been reported that reduced expression of PTEN also correlates with a lower activity of bevacizumab (81).

ccRCC is a highly lipogenic tumor. Our recent studies demonstrate that the addition of LDL cholesterol increases activation of PI3K/AKT signaling, which coincides with reduced antitumor activity of clinically relevant TKIs such as sorafenib, pazopanib, lapatinib and sunitinib against ccRCC and endothelial cells. Furthermore, ccRCC xenograft tumors resisted treatment with sunitinib in mice fed a high fat/high cholesterol diet (82). Interestingly, results from the 3-arm phase III Global Advanced Renal Cell Carcinoma Trial (ARCC) demonstrated that increases in cholesterol potentially predicted temsirolimus efficacy. Longer survival in patients treated with temsirolimus was observed in those with larger increases in cholesterol (83). The exact mechanism underlying this phenomenon is not clear. The authors acknowledge that whether the increase in serum cholesterol merely reflects successful targeting of the mTOR pathway, or if it is mechanistically required for the antitumor response, cannot be determined from the results of this study (83).

ATP-binding cassette (ABC) efflux transporters

ABC transporters represent a large superfamily of active transporter proteins that mediate the efflux or uptake of specific substrates across different membranes including the plasma membrane, endoplasmic reticulum, Golgi compartment, peroxisomes, and mitochondria (84). Multiple TKIs have been reported to interact with ABC transporters (e.g. ABCB1, ABCC1, ABCG2, and ABCC10) (84). Investigation by Sato et al. demonstrated that sunitinib induced upregulation of ABC sub-family G member 2 (ABCG2) in 786-O RCC cells; and treatment with elacridar (GF120918) (85), a third generation P-glycoprotein inhibitor, enhanced the cytotoxic effect of sunitinib (86). Another study by Shibayama et al. revealed that sorafenib may serve as a substrate for multidrug resistance-associated protein 2 (MRP2) (87). However, TKIs may also serve as inhibitors of ABC transporters depending on drug concentration, the expression of specific pumps, and affinity for transporters. In general, at low concentrations TKIs display substrate-like properties; and at high concentration they can inhibit the function of pumps (84). A number of TKIs including sunitinib, sorafenib, and pazopanib have been established as inhibitors of various classes of ABC transporters (3,14).

Tumor heterogeneity

RCC tumor specimens from different patients with similar pathological grade and stage can be extremely heterogeneous, displaying different profiles at genomic and transcriptomic levels. This phenomenon drastically limits the utility of prognostic and predictive biomarkers. Furthermore, intratumoral heterogeneity (ITH) presents a considerable therapeutic challenge significantly limiting the efficacy of targeted therapies (88). Varying gene, microRNA, and protein expression signatures can be detected even within the same tumor specimen, and variations in the dysregulation of microRNA has been shown to impact ccRCC pathogenicity (89). Significant molecular heterogeneity was also detected between primary and metastatic lesions, with only a small subset of alterations present in both sites. In one study, post-sunitinib metastatic lesions carried mutations in FLT4, KMT2D, and BMP5, which were not detected in the primary tumor (90). Studies by Hatiboglu et al. demonstrated that although neoadjuvant treatment with sorafenib was clinically active in downsizing tumors in patients with locally confined, non-metastatic ccRCC, such treatment led to an enhanced functional ITH in the residual tumor tissue (91). These results are in agreement with findings by Stewart et al., demonstrating that primary ccRCC tissues from patients treated with sunitinib express greater morphologic heterogeneity compared with tumors from untreated patients (92). Unsupervised analysis did not reveal any significant effect of sunitinib therapy on ITH at a chromosomal or mRNA level. However, results from supervised analyses do show a consistent increase in ITH for both DNA and mRNA of several oncogenes. These findings were supported by protein expression results demonstrating an increase in ITH for selected tumor-specific proteins (92). According to Stewart et al., the results of this study do not support the hypothesis that a single resistant clone predominates from a number of clones after treatment commences; treatment was actually associated with more, rather than less, ITH. As such, the authors speculate that VEGF-targeted therapy may generate a polyclonal outgrowth of tumor cell subclones that can lead to acquired drug resistance (92).

Tumor Microenvironment

The tumor microenvironment is critical for the initiation and maintenance of tumorigenesis (93). Growing evidence also indicates the direct involvement of the tumor microenvironment in the development of resistance to targeted therapeutics. The tumor microenvironment consists of tumor cells, extracellular matrix (ECM), signaling molecules, and stromal cells (e.g. fibroblasts, vascular endothelial cells, pericytes, and immune cells). Myeloid-derived suppressor cells (MDSC) are one of the major components of the tumor microenvironment. Accumulating evidence suggests that MDSC are recruited by tumors to mediate resistance to anti-angiogenic drugs by expressing various pro-angiogenic factors, which stimulate VEGF-independent angiogenesis (94). Studies by Ko et al. demonstrate that unlike the pronounced decline in peripheral blood MDSC observed in RCC patients treated with sunitinib, tumor tissues obtained from sunitinib-treated patients have not demonstrated declines in MDSC (95). MDSC resistance to sunitinib corresponded to compartmental availability of GM-CSF in renal tumors. Treatment with recombinant GM-CSF also conferred sunitinib resistance *in vitro* and *in vivo*. GM-CSF-induced sunitinib-resistance in MDSC was STAT5 mediated, as it was negated in STAT5ab(null/null) MDSC (95). Another population of stromal cells, which directly contribute to aberrant tumor angiogenesis and drug resistance are pericytes.

Increased pericyte coverage and increased production of VEGF by pericytes enhances survival of endothelial cells, rendering them less responsive to inhibition of VEGF signaling (59). Targeting pericytes and endothelial cells by inhibiting both VEGF and PDGF signaling has proven to be more effective in reducing angiogenesis and tumor growth than targeting either of these pathways alone (59).

Tumor endothelial cells express the Notch ligand Delta-like 4 (Dll4). The Dll4-Notch pathway functions as a key negative regulator of physiological and pathological angiogenesis downstream of VEGF (96). Dll4 is predominately found in the developing endothelium, with an almost 9-fold increased expression reported within the vasculature of ccRCC, as compared to normal kidneys (97). Miles et al. demonstrated the potent anti-tumor efficacy of combined treatment with anti-Dll4 and anti-VEGF therapy in a sunitinib-resistant metastatic ccRCC model (96). Notch signaling also plays a crucial role in the maintenance of “stemness” by cancer stem-like cells in RCC. Pharmacological blockade of Notch signaling reinstates the sensitivity of human RCC cells to sorafenib (98).

Tumor-associated fibroblasts can also contribute to resistance to targeted therapies via increased expression of PDGF-C, which could overcome the inhibition of VEGF-mediated angiogenesis (99).

Strategies to reverse or overcome TKI resistance

One of the most common approaches in cancer treatment is the use of combinations of agents with different mechanisms of action and molecular targets. Pharmacological inhibition of PI3K/Akt/mTOR signaling reinstates sensitivity to sunitinib and sorafenib in ccRCC cells with aberrant AKT activity (80,82). Sensitivity to sunitinib in PTEN-negative 786-O RCC cells was also successfully reinstated using temsirolimus (80). A meta-analysis of 22 randomized clinical trials supports earlier observations that combining targeted therapies is a promising strategy against advanced RCC. Yet, while efficacy may be potentiated, so can toxicity, as is seen in many combinations of TKIs and mTOR inhibitors. (38,39,100,101). Bevacizumab, for example, is an effective agent in combination with IFN; but combinations with sunitinib, sorafenib, and temsirolimus were all abandoned due to compounded toxicities (25).

Designing treatment sequences with different TKIs has also demonstrated positive outcomes in patients with advanced RCC. This approach relies on the fact that TKIs have varying target profiles and different affinities for common targets. For example, a randomized phase II study demonstrated clinical activity of axitinib in patients with sorafenib-refractory metastatic RCC and in patients who have been treated with additional prior therapies, including sunitinib, cytokines, temsirolimus, or bevacizumab plus IFN-α (102). The sequential use of TKIs followed by mTOR inhibitors, specifically everolimus, has also been established for the systemic treatment of metastatic RCC (103).

New options for immunotherapy

Immune checkpoint inhibitors have recently emerged as an effective treatment against advanced RCC. As opposed to therapies selectively targeting angiogenic pathways, immune checkpoint (PD1-PD-L1/CTLA4) inhibitors work to directly reverse the adaptive

camouflage tumor cells deploy to avoid host immunity. PD-1 is a molecule recognized by its ligand PD-L1 on antigen presenting cells (APCs) and tumor cells. This interaction acts as an immune checkpoint and promotes T-cell tolerance (20). Interestingly, preclinical studies have shown that targeted mRCC therapies have immunomodulatory effects, such as promoting T-cell infiltration into tumors and increasing tumor cell antigenicity (104). These findings formed the basis for attempts to combine targeted antiangiogenic therapies with newer generation immunotherapies to take advantage of possible synergy (25).

It was previously demonstrated that ccRCC tumors have varying degrees of PD-L1 expression. An early study of ccRCC tumors after nephrectomy demonstrated that patients with tumors expressing PD-L1 had a significantly lower 5-year CSS (41.9%) vs. those not expressing PD-L1 (82.9%) (105). The degree of PD-L1 expression on mRCC cells directly correlates with aggressive pathologic features (25).

CTLA-4 is a receptor checkpoint inhibitor expressed on T cells, and ligand binding promotes immune tolerance. A Phase II study of ipilumumab (anti-CTLA-4 mAB) in mRCC demonstrated partial responses; and intriguingly, those patients who had autoimmune related side effects (Grade 3/4) tended to see the most tumor regression, indicating that characteristics of host immunity can play a significant role in tumor control (106). Despite the significant toxicities from this agent, there may still be a role for combination therapies with ipilimumab, including for treatment of non-ccRCC tumors.

The clinical success of checkpoint inhibition in other solid tumors led to several trials demonstrating the efficacy of agents such as nivolumab (anti PD-1 mAB).

Single agent IO

Checkmate 025 was a randomized phase III clinical trial comparing nivolumab to everolimus in patients who failed initial antiangiogenic therapy. The median OS for nivolumab vs. everolimus was 25mo vs. 19.6mo, with a ORR of 25% vs. 5%, respectively, favoring nivolumab over everolimus. Importantly, Grade 3/4 treatment side effects appeared to be lower in the nivolumab group (19% for nivolumab vs 37% for everolimus), which makes this an attractive treatment option (107) (Fig. 1).

Other agents are in the early phases of clinical study. Atezolizumab, an IgG mAB against PD-L1 (as opposed to nivolumab which targets PD-1), showed efficacy in phase I studies of patients with advanced RCC, with OS of 28.9 months and 15% ORR (108). Pembrolizumab, another anti-PD1 agent and anti-PDL1 therapies durvalumab and avelumab are being studied as monotherapy in trials NCT02212730, NCT02669914, NCT02493751, respectively.

IO+ VEGFR TKIs

A phase 1 trial was conducted to investigate the combination of escalating doses of nivolumab with sunitinib or pazopanib. While the pazopanib arm was closed due to hepatotoxicity, the sunitinib combination arm was dose escalated. Although toxicity was increased, high response rates were observed in this study (52% in sunitinib arm and 45% in pazopanib arm) (109). Similarly, the combination of nivolumab with cabozantinib is

currently being studied in NCT02496208. Pembrolizumab (anti-PD-1) is currently under phase I/II study as a combination drug with several VEGF-targeted therapies in both the first-line and post-TKI spaces (110). These include a phase III first-line trial of pembrolizumab with axitinib (NCT02853331), and other trials of pembrolizumab in combination with lenvatinib (NCT02501096), ziv-aflibercept (NCT02298959), and bevacizumab (NCT02348008). A 3-armed phase III study (IMmotion 151) is currently underway evaluating the efficacy of atezolizumab + bevacizumab, atezolizumab-alone, and sunitinib in first-line therapy. Early findings appear to indicate that patients with positive PD-L1 expression experience a higher PFS, with response rates of 25-46% (111) (Fig. 1). AVELIN Renal 101, a randomized multicenter, phase 3 study (NCT02684006) comparing the combination with sunitinib in treatment-naïve patients with mRCC, began enrollment in March 2016.

IO+ IO combinations

The results of the phase III, randomized, open-label CheckMate-214 study evaluating the combination of nivolumab and ipilimumab compared to sunitinib in patients with previously untreated advanced or metastatic RCC were recently reported at ESMO 2017. The study met its primary endpoint of OS compared to sunitinib in intermediate and poor risk patients in the front line setting. Pembrolizumab is also being evaluated in combination with ipilimumab as one of the three arms of a phase I/II study in patients with metastatic RCC or melanoma (NCT02089685). Anti PD-L1 therapy durvalumab is also evaluated in combination with anti CTLA4 antibody tremelimumab (NCT01975831). Checkmate 214 is a phase III randomized trial of nivolumab + ipilimumab vs. sunitinib in previously untreated mRCC (112) (Fig. 1). The recently-announced results are very promising: After 17.5 months follow-up, the ORR for nivolumab + ipilimumab vs. sunitinib was 41.6% vs. 26.5%, respectively, with 9.4% of combined immune-blockade patients achieving complete response (113).

As with targeted therapies, there is evidence that primary resistance to PD-L1 inhibition may form in some tumor microenvironments, with adaptive resistance developing in others. The mechanisms behind these resistance patterns are still unclear; but as with TKIs and mTOR inhibitors, combination therapy will likely be helpful in preventing evasion of host immunity (114).

Vaccines have been used effectively in other solid tumor malignancies, and new options have recently emerged for RCC. AGS-003 is a dendritic cell vaccine prepared with amplified tumor RNA that can prime the immune response. A randomized phase III trial (ADAPT) of sunitinib + AGS-003 vs. sunitinib-alone for previously untreated mRCC was launched after promising phase II study results, which found that the magnitude of increased effector/memory T cell production correlated with improved OS (115) (Fig. 1). IMA901 is a multipeptide vaccine utilizing a combination of HLA class I & II-binding tumor-associated peptides that underwent a phase III randomized study for first line therapy in combination with sunitinib. Unfortunately, it appeared that the magnitude of immune response was not sufficient to demonstrate an OS difference when IMA901 was used in conjunction with

sunitinib, though this doesn't completely close the door on this strategy in the future (116) (Fig. 1).

Conclusions and perspectives

Targeted therapies are nowadays the standard treatment options for renal cancer that have changed the treatment landscape of advanced ccRCC compared with the cytokines era. However, nearly all patients treated with currently approved targeted drugs will eventually experience disease progression. Furthermore, a significant number of RCC patients are primarily refractory to targeted therapeutics, showing neither disease stabilization nor clinical benefits. Newer agents that augment tumor/immune-system interactions and that concurrently target multiple oncogenic pathways hold great promise. Further characterization of the RCC oncogenic pathways and the ongoing clinical trials should help to optimize the management of patients with advanced RCC.

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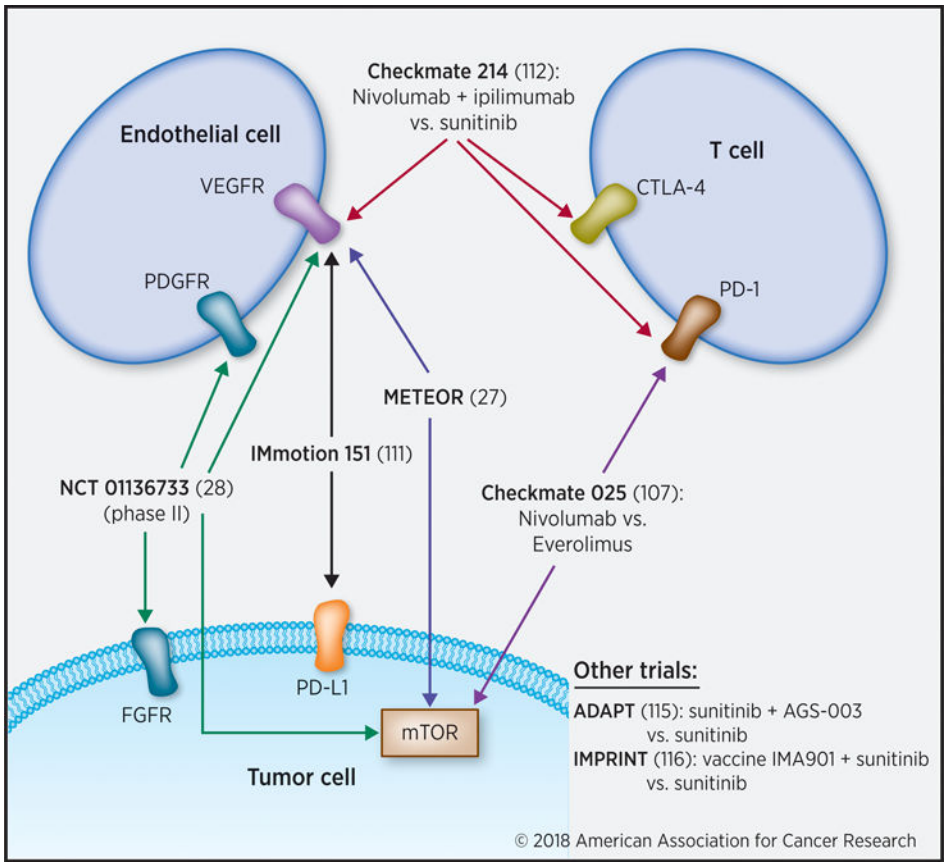

Figure 1. Recent clinical trials in RCC

The current treatment landscape for advanced RCC includes combination strategies targeting tumor, endothelial and immune cells.