Combined Blockade of Vascular Endothelial Growth Factor and Programmed Death 1 Pathways in Advanced Kidney Cancer

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Abstract: Targeted and immune-based therapies have improved outcomes in advanced kidney cancer, yet novel strategies are needed to extend the duration of these benefits and expand them to more patients. Combined inhibition of vascular endothelial growth factor (VEGF) and the programmed death 1 (PD-1)/programmed death ligand 1 (PD-L1) pathways with therapeutic agents already in clinical use may offer such a strategy. Here, we describe the development and clinical evaluation of VEGF inhibitors and, separately, PD-1/PD-L1 inhibitors. We present preclinical evidence of interaction between these pathways and the rationale for combined blockade. Beyond well-known effects on pathologic angiogenesis, VEGF blockade also may decrease immune tolerance and enhance PD-1/PD-L1 blockade. We conclude with the results of several early trials of combined VEGF and PD-1/PD-L1 blockade, which demonstrate encouraging antitumor activity, and we pose questions for future study.

Keywords
Combination therapy, kidney cancer, programmed death 1, programmed death ligand 1, renal cell carcinoma, VEGF inhibitors

Introduction

The American Cancer Society estimates that cancers of the kidney or renal pelvis will be diagnosed in 63,990 Americans in 2017, and that 14,400 will die of their disease.[1] According to 2006-2012 data, the 5-year survival for those with distant spread of disease—who account for 16% of cases—was 11.7%.[2] Historically, kidney cancer therapy has been notable for the failure of cytotoxic chemotherapy but also for the promise of immune-based therapies such as interleukin 2 (IL-2) and interferon alfa (IFN-α). The recent development of targeted therapies—inhibitors of vascular endothelial growth factor (VEGF) and mammalian target of rapamycin (mTOR)—and immune-based therapies has transformed the treatment of kidney cancer, improving response rates and survival beyond what was previously possible. Still, many novel therapies provide benefit to only a portion of treated patients, and for a limited time. Therefore, there is an ongoing need for improved approaches to the treatment of advanced kidney cancer. Here, we describe the role of pathologic angiogenesis and immune tolerance in kidney cancer. We discuss agents that target angiogenic signaling mechanisms and immune

checkpoints, as well as mechanisms of tumor resistance. Finally, we focus on evaluating the rationale for—and the outcomes achieved with—combinations of agents for antiangiogenesis and agents for immune checkpoint blockade.

Angiogenesis, Antiangiogenesis, and Resistance

VEGF, which is controlled by hypoxia-inducible factor (HIF),[3] binds to endothelial receptors. This results in mitogenic, angiogenic, and pro-permeability signaling, as has been reviewed extensively elsewhere.[4] Also reviewed elsewhere[5] is the action of tyrosine kinase inhibitors (TKIs), which target VEGF receptors and other receptors. Lenvatinib (Lenvima, Eisai) inhibits fibroblast growth factor (FGF) receptor and cabozantinib (Cabometyx, Exelixis) inhibits c-MET, both of which are potential mechanisms of VEGF resistance, discussed below. Large randomized clinical trials have led to US Food and Drug Administration (FDA) approval of sunitinib (Sutent, Pfizer)[6] and pazopanib (Votrient, Novartis)[7] as first-line therapies. Sorafenib (Nexavar, Bayer),[8] axitinib (Inlyta, Pfizer),[9] cabozantinib,[10] and lenvatinib (Lenvima, Eisai) in combination with everolimus (Afinitor, Novartis)[11] have been evaluated and approved as later-line therapies. Another strategy for targeting VEGF signaling is the use of monoclonal antibodies, especially bevacizumab (Avastin, Genentech), which is active as a single agent[12] and is FDA-approved in combination with IFN.[13,14]

Most patients who receive first-line VEGF-targeting agents exhibit disease stability or a partial response (10% to 20% may have primary refractory disease).[15] In addition, resistance to treatment will develop in most patients after a median of 6 to 11 months.[6-8,13,14] Interestingly, patients may still respond to other VEGF-directed TKIs[16] and antibodies[17] after failure of first-line VEGF therapy. Nonetheless, responses and survival are limited after resistance develops.

Activation of alternative angiogenic pathways may promote resistance. The receptor tyrosine kinase c-MET has been implicated in pathogenic angiogenesis[18] and is upregulated in response to sunitinib.[19] Cabozantinib has been shown to overcome sunitinib resistance in mice.[20] In trials, cabozantinib improved overall survival (OS) as second-line therapy compared with everolimus,[10] and it improved progression-free survival (PFS) as first-line therapy compared with sunitinib.[21] Similarly, FGF is a proangiogenic factor under the control of HIF, and its expression increases after VEGF inhibition.[19] It is frequently and strongly expressed in kidney cancer.[22] In vitro, FGF blockade has demonstrated reversal of pathogenic angiogenesis[22] and has overcome VEGF resistance.[23] Lenvatinib improved PFS as second-line therapy.[11] In contrast, the experimental agent dovitinib (a combined VEGF/FGF inhibitor) was not superior to sorafenib as third-line therapy.[24] Thus, c-MET and FGF are potentially targetable mechanisms of resistance to anti-VEGF therapy; 2 approved therapies employ this strategy.

The interaction between the tumor and its microenvironment is another important source of resistance.[25] Stromal cells (especially endothelial cells) may activate alternate proangiogenic pathways such as angiopoietin 2 and ALK1, both of which have been targeted in kidney cancer clinical trials.[26,27] Inflamed, hypoxic tissues may generate excess extracellular adenosine—also generated by regulatory T-cell (Treg) CD39 and CD73 ectoenzymes—which binds the A2A adenosine receptor on T cells, inhibiting antitumor response.[28] Finally, infiltrating immunosuppressive cells promote resistance, as discussed below.

The Programmed Death 1 Pathway in Kidney Cancer

The programmed death 1 (PD-1)/programmed death ligand 1 (PD-L1) pathway has been a critical target of cancer treatments. The physiologic role of this pathway is to terminate immune response after T-cell activation.[29] Binding of PD-L1/PD-L2 to PD-1 inhibits the activation of T-cell receptor proximal kinases, changing the balance in activation and inhibition of downstream signaling pathways and altering cell cycle progression, gene transcription, metabolism, and epigenetic programs in T cells.[29] PD-L1 is normally expressed by macrophage lineage cells and is inducible on activated T cells; tumors may acquire the ability to express PD-L1 aberrantly.[29] PD-L1 is expressed in both primary and metastatic renal cell carcinoma (mRCC), induced by the infiltrating immune cell production of interferons.[30] Its expression by kidney cancers or tumor-infiltrating mononuclear cells correlates with aggressive pathologic features, increased risk for disease progression, cancer-specific death, and overall mortality.[31-34] Shed PD-L1 is detectable in serum before nephrectomy and correlates with aggressive pathologic features and mortality.[35]

These data provoked considerable interest in using PD-1 as a therapeutic target. The development of PD-1/PD-L1 inhibitors has been extensively reviewed.[36] Nivolumab (Opdivo, Bristol-Myers Squibb) showed efficacy and a favorable toxicity profile in previously treated patients with kidney cancer enrolled in phase 1 trials,[37,38] achieving durable responses that sometimes persisted after drug discontinuation.[39] Phase 2 data were equally encouraging,[40] culminating in the randomized phase 3

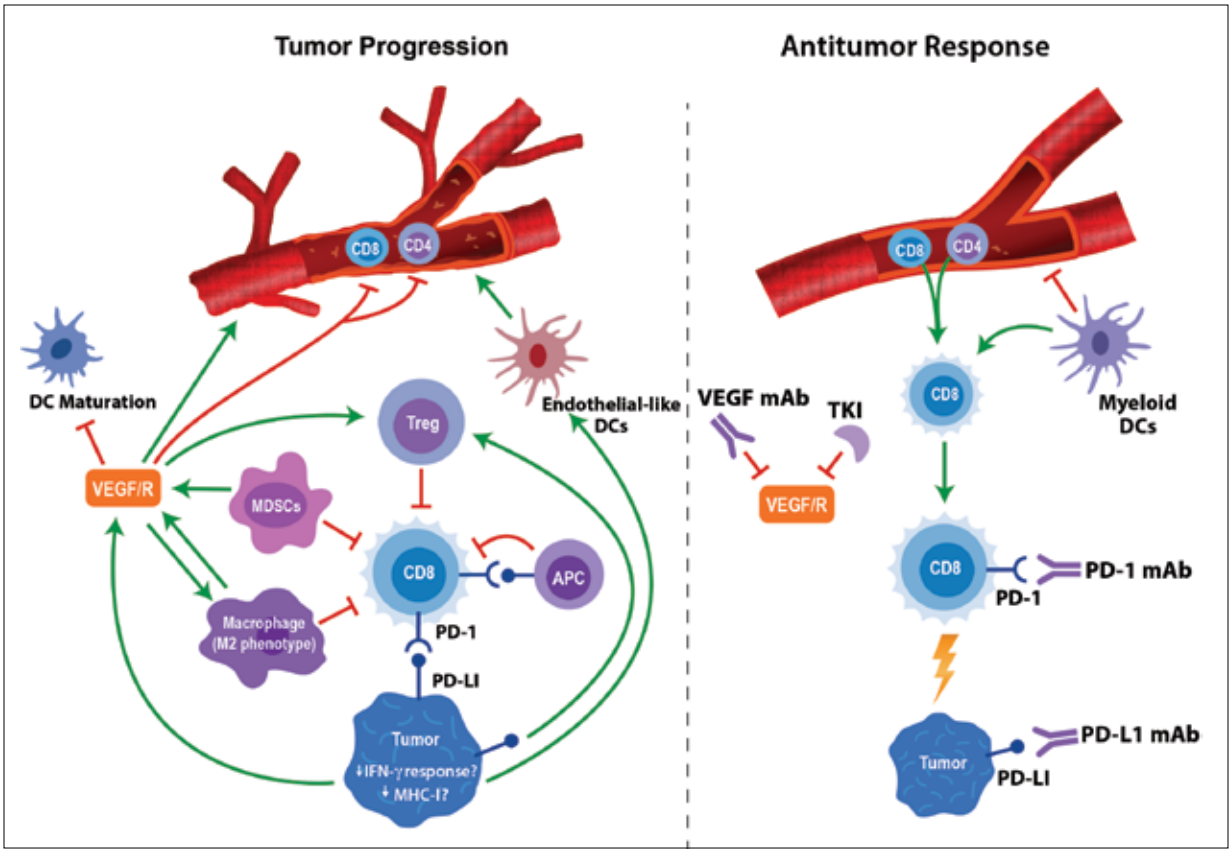

Figure. Shifting the balance toward antitumor response with combined VEGF/PD-1 blockade. APC, antigen-presenting cell; DC, dendritic cell; IFN-γ, interferon gamma; mAb, monoclonal antibody; MDSCs, myeloid-derived suppressor cells; MHC-I, major histocompatibility complex class I; PD-1, programmed death 1; PD-L1, programmed death ligand 1; Treg, regulatory T cell; TKI, tyrosine kinase inhibitor; VEGF/R, vascular endothelial growth factor receptor.

CheckMate 025 trial (Study of Nivolumab vs Everolimus in Pre-Treated Advanced or Metastatic Clear-Cell Renal Cell Carcinoma) comparing nivolumab with everolimus following failure of antiangiogenic therapy. Nivolumab was associated with improved OS and fewer serious adverse events (AEs),[41] leading to FDA approval in 2015. Finally, PD-L1–blocking antibodies are also under investigation in kidney cancer. Atezolizumab (Tecentriq, Genentech) has demonstrated activity in both clear cell renal cell carcinoma (ccRCC) and non-ccRCC,[42] discussed below.

The Rationale for Combined VEGF and PD-1 Inhibition

Aside from directly promoting tumor growth, dysregulated angiogenic signaling may also promote escape from immune surveillance. Multiple mechanisms may allow pathologic angiogenesis to mute the immune response to kidney cancer and to immune-directed therapies (Figure).

Mechanisms of Immune Tolerance

Failure of Immune Infiltration. Antitumor response depends on T-effector cells (Teffs; $CD8^+$ and $CD4^+Foxp3^-$) localizing to and infiltrating tumors. Tumor endothelium may prevent this process through VEGF- and endothelin-mediated regulation of vascular permeability. The endothelin axis contributes to pathologic angiogenesis and tumor progression,[43] stimulating the production of VEGF via HIF-1α[44] and inhibiting lymphocyte infiltration, which is dependent on intercellular adhesion molecule 1 (ICAM-1).[45] FGF decreases endothelial expression of ICAM-1 and prevents tumor necrosis factor α (TNF-α) from promoting endothelial adhesion molecule expression.[46] VEGF induces tumor endothelium to express the death mediator Fas ligand (FasL), with a resulting decrease in $CD8^+$ T-cell infiltration and predominance of immunosuppressive Tregs ($CD4^+Foxp3^+$). Importantly, inhibition of VEGF and prostaglandin E2 (PGE2) in mice markedly increased tumor-infiltrating $CD8^+$ T cells relative to Tregs and suppressed tumor growth.[47] Thus, multiple proangiogenic signaling

pathways may decrease tumor vascularity, permeability, and lymphocyte transmigration.

Immunosuppressive Immune Cells. The kidney cancer microenvironment often demonstrates a prominent immune cell infiltrate containing multiple cell types.[48] Many of these can induce immune tolerance and are influenced by angiogenic factors.[49]

Some lymphoid cells promote tolerance. Tregs inhibit the function of Teffs[50] and, in tumors, negatively correlate with survival.[51] Tumors recruit Tregs via the chemokine CCL22, induced by hypoxia.[51,52] PD-L1 expression regulates the development of induced Tregs and maintains their suppressive function via Foxp3 expression.[53] In addition, a subset of tumor-associated dendritic cells (DCs, discussed below) can promote the proliferation of Tregs.[54] Although promising in preclinical models, therapeutic Treg depletion with anti-CD25 toxin conjugates[55,56] failed to enhance immune therapies consistently, possibly owing to limited Teff infiltration.[57]

Other immunosuppressive cells are myeloid, such as DCs, myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs). DCs are central regulators of immune responses and can induce immunity or tolerance depending on their differentiation.[48] As the most potent antigen-presenting cells (APCs), DCs present tumor antigens to lymphocytes.[58] Normal myeloid DCs can produce IL-12 and induce the production of IFN-γ and IL-10, all strong antiangiogenic signals.[59] Thus, myeloid DCs can suppress tumor angiogenesis.[51] In contrast, some DCs are recruited by β-defensins (antimicrobial inflammatory peptides) to tumors, where, induced by VEGF, they undergo endothelial-like differentiation and can independently assemble neovasculature.[60] Thus, depending on the milieu, DCs can play both positive and negative roles in both angiogenesis and antitumor immune response.

MDSCs (Gr^+CD11b^+) are a heterogeneous population of immunosuppressive cells identified in tumors[61] that are correlated with refractoriness to antiangiogenic therapy.[62] They increase tumor angiogenesis via the production of matrix metallopeptidase 9 (MMP9), which releases VEGF from extracellular matrix[63]; this is abrogated by VEGF or MMP inhibition. In mice with tumors refractory to VEGF-directed therapy, the effectiveness of this therapy is increased when it is combined with a monoclonal antibody against myeloid cells.[62]

Tumors attract monocytes and, under hypoxic conditions, promote differentiation into the TAM phenotype, specifically the M2 phenotype that overexpresses VEGF and inhibits the T-helper cell type 1 (Th1) response (responsible for clearance of intracellular pathogens).[51,64,65] These studies suggest an integral role for lymphoid and myeloid cells in the promotion of tolerance, as well as a dependence on angiogenic signaling for this effect.

Functional Impairment of Immune Cells. The kidney cancer immune infiltrate is often polarized toward a Th1-type response.[66] In addition, the hypoxic tumor microenvironment may result in HIF-1α–mediated increased expression of major histocompatibility complex class I (MHC-I), resulting in an increased ability for antigens to be presented to effector lymphocytes.[67] So why should immune tolerance emerge? Teffs in the tumor microenvironment appear anergic owing to multiple alterations, including deficits in T-cell receptor signaling molecules.[48] Secondly, hypoxia, inactivation of the von Hippel-Lindau gene (*VHL*), and overexpression of carbonic anhydrases create an acidic tumor microenvironment rich in lactate,[68] which impairs lytic granule exocytosis.[69] Reversal of acidosis relieves inhibition. Thus, even Teffs that have infiltrated tumors may be functionally impaired.

Similar mechanisms could promote tolerance after PD-1/PD-L1 blockade, although little is known about mechanisms of resistance in kidney cancer. In a small study in patients with melanoma resistant to PD-1 blockade, tumor whole-exome sequencing revealed defects in pathways involved in IFN receptor signaling and antigen presentation.[70] Further research into the mechanisms of acquired resistance to PD-1/PD-L1 blockade in kidney cancer will be needed.

Immunologic Effects of VEGF and VEGF Blockade

VEGF overexpression results in abnormal hematopoiesis and the blockade of myeloid cell differentiation into DCs in mice[71-74] and in patients with cancer.[75,76] Bevacizumab was associated with both reduction in peripheral immature myeloid cells and enhancement of the antigen-presenting function of DCs.[76] Similarly, axitinib treatment in a mouse melanoma model induced differentiation of monocytic MDSCs toward an APC phenotype. In addition, sunitinib therapy depleted peripheral MDSCs in patients with kidney cancer, also linked to reversal of peripheral Treg elevation and Teff suppression.[77] Of note, the effects of VEGF on DC development and function may be independent of tyrosine kinase signaling,[58] suggesting an alternative pathway uninhibited by TKIs.

In mice, VEGF directly induces Treg proliferation. Conversely, in mice and humans, anti-VEGF TKIs and antibodies decreased Tregs in spleens, tumor-draining lymph nodes, and peripheral blood but did not affect their ability to suppress T-cell proliferation and IFN-γ secretion.[78] After recruitment to a hypoxic region, Tregs can also promote angiogenesis and VEGF expression.[52] Thus, there is a reciprocal relationship between VEGF

and Tregs. Sunitinib decreased Tregs in tumor-bearing mice[79] and decreased peripheral and tumor Tregs in patients with kidney cancer.[80] Reduction in Tregs after 2 to 3 cycles of treatment correlated with improved OS, although the decrease did not correlate with radiographic tumor volume, suggesting that benefits may be independent of radiographic response. Similar results were seen with sorafenib and a reduction in tumor-infiltrating Tregs.[81] Sunitinib increased tumor infiltration by Teffs in mice.[79] Anti-VEGF TKIs have been associated with reductions in Foxp3 expression, a maintainer of Tregs, and decreased tumor PD-L1 expression in mice[79] and in patients with kidney cancer undergoing nephrectomy.[19] Similarly, incubation of monocytic DCs with ovarian tumor cells increased DC PD-1 expression, inhibited by VEGF blockade.[82]

In one study, patients with kidney cancer received bevacizumab followed by the combination of bevacizumab plus atezolizumab, with serial evaluations to assess the effect of both phases.[83] After bevacizumab, the tumor immune microenvironment demonstrated increased chemokine signatures related to Th1-type response, increased tumor MHC-I expression, and infiltration of tumor-specific T-cell clones. After the combination of atezolizumab and bevacizumab, further increased trafficking and tumor infiltration by $CD8^+$ T cells and increased unique T-cell clones in tumors were observed. Chemokines increased, most notably fractalkine, expressed on activated endothelium in response to inflammation or hypoxia. Thus, antiangiogenic therapy alone had immunologic effects that were increased when it was combined with PD-L1 inhibition.

Sequencing and Combining Therapies

VEGF-Directed Therapy After Checkpoint Inhibition

Are VEGF-targeted therapies less effective after prior failure of checkpoint inhibition? In patients with kidney cancer who had progressive disease after PD-1/PD-L1 blockade, second-line VEGF-directed therapy was as effective as expected after failure of first-line VEGF-directed therapy.[84] In addition, prior PD-1 blockade did not affect the safety of subsequent anti-VEGF TKIs.[85] However, decreased response rate and PFS were associated with a second-line anti-VEGF TKI after prior combination PD-1/VEGF-directed therapy vs PD-1 therapy alone. This analysis also demonstrated that a longer interval between the end of PD-1 therapy and the start of anti-VEGF TKI therapy decreased overall response to the TKI regardless of prior therapy, suggesting some possible overlapping activity between residual PD-1 and VEGF inhibition, although this conclusion is limited by retrospective analysis and possible confounders.

Clinical Trials of Combined VEGF/PD-1 Inhibition

Combined TKI and Checkpoint Inhibitor Trials. PD-1 inhibition has been combined with VEGF-directed TKIs in several ongoing trials; dosing and toxicity details appear in the table. Amin and colleagues described a phase 1 trial (NCT01472081), first presented at the 2014 American Society of Clinical Oncology (ASCO) annual meeting, of nivolumab plus either sunitinib or pazopanib in patients with previously-treated mRCC.[86] Following nivolumab dose escalation and on the basis of tolerability, the 5-mg/kg cohort was expanded to include treatment-naive patients; the pazopanib-containing arm was closed owing to 4 dose-limiting toxicities (primarily elevated transaminases), an effect also observed in a pembrolizumab combination trial discussed below. The overall response rate (ORR) was approximately 50%, with stable disease in another third of patients—substantially higher rates than those seen with any agent individually, especially given the prior treatment.

Pembrolizumab has also been evaluated, primarily in combinations. Preliminary results from a phase 1/2 study of pembrolizumab plus pazopanib in patients with treatment-naive, advanced, predominantly ccRCC were presented at the 2015 European Society for Medical Oncology (ESMO) annual meeting (NCT02014636).[87] The pembrolizumab dose was escalated in combination with pazopanib. After a safety review, the starting dose of pazopanib was reduced because of hepatotoxicity. The ORR was 60% in the pazopanib 800-mg group (with 1 complete response) and 20% in the 600-mg group. Given the significant hepatotoxicity associated with pazopanib, sequential dosing schemes were explored. This trial, like the nivolumab-plus-pazopanib trial discussed earlier, shows that not every combination strategy will prove safe and that PD-1 blockade following certain VEGF-targeting agents may also increase toxicity.

At the 2016 ESMO meeting, Atkins and colleagues presented preliminary results from a phase 1b study of pembrolizumab plus axitinib in treatment-naive patients with advanced RCC (NCT02133742).[88] The ORR was 71.2%, with another 19.2% exhibiting stable disease. Biomarker results were reported, and PD-L1 status did not seem to predict response.

Will the blockade of additional proangiogenic pathways improve results? At the same ESMO meeting, Apolo and colleagues presented preliminary results from a phase 1 study of nivolumab plus cabozantinib, the inhibitor of VEGF and c-MET (NCT02496208).[89] The ORR was 33%, including a partial response in the 1 patient with RCC, and the percentage of patients with stable disease was 38%. The lack of immune-related AEs was promising for future study of this combination, with attention to the unique occurrence of neutropenia.

Table. Clinical Trials of Combined VEGF-Directed Therapy and PD-1/PD-L1 Inhibition

Combinations/ Trials	Phase	Population	N	Treatment Arms[a]	ORR	Notable AEs
PD-1 + TKI						
NCT01472081[86]	1	Treated mRCC	33	nivo (2-5 mg/kg) + sun (50 mg 4 wk on, 2 wk off) vs nivo + paz (800 mg)	52% (nivo + sun) and 45% (nivo + paz); SD in 33% (nivo + sun) and 35% (nivo + paz)	nivo 2-mg/kg + paz group closed owing to 4 DLTs (⇑LFTs, fatigue); grade 3-4 related AEs in 60%-73%
NCT02014636[87]	1/2	Treatment-naive aRCC (predominantly ccRCC)	20	pembro (2-10 mg/kg q2wk) + paz (800 or 600 mg)	60% (paz 800-mg group), 20% (paz 600-mg group)	Significant hepatotoxicity with paz 800 mg
NCT02133742[88]	1b	Treatment-naive aRCC	52	pembro (2 mg/kg q3wk) +axi (3-5 mg BID)	71.2%, SD in 19.2%	Grade 3+ AEs in 65.4%
NCT02496208[89]	1	Multiple tumor types	24 (1 RCC); expansion in RCC planned	Part 1: nivo (1-3 mg/kg) + cab (40-60 mg) Part 2: nivo + cab + ipi × 4	33%, 1 PR in RCC patient, SD in 38%	Grade 3 neutropenia seen, no irAEs
PD-L1 + TKI						
NCT02493751[90]	1b	Treatment-naive aRCC (with cc component)	Enrolling	avel (5-10 mg/kg q2wk) + axi (3-5 mg BID)	N/A	N/A
PD-L1 + mAb						
NCT01633970[91,92]	1b	Treatment-naive aRCC (with cc component)	12	atezo (20 mg/kg q3wk) + bev (15 mg/kg q3wk)	40%	No grade 3-4 AEs related to atezo, 3 cases of significant hypertension
NCT01984242[93]	2	Treatment-naive mRCC (cc or sarcomatoid)	305	atezo (1200 mg q3wk) + bev (15 mg/kg q3wk) vs atezo (1200 mg q3wk) vs sun (50 mg 4 wk on, 2 wk off)	32% overall in atezo + bev arm; in PD-L1+ pts, 46% (atezo + bev) vs 27% (sun) and 28% (atezo)	Grade 3-4 AEs in 40% of atezo + bev arm vs 57% (sun) and 17% (atezo)
NCT02420821[94]	3	Treatment-naive mRCC	Planned	atezo (1200 mg q3wk) + bev 15 mg/kg q3wk vs sun (50 mg 4 wk on, 2 wk off)	N/A	N/A

(Table continues on next page)

Another question is whether PD-L1 vs PD-1 blockade will alter results of this combination strategy. At the 2016 ASCO annual meeting, Larkin and colleagues presented the plan for a phase 1b study of avelumab plus axitinib in treatment-naive patients with advanced RCC (NCT02493751).[90] Enrollment had begun in 2015; preliminary results were not yet available. A phase 3 trial of avelumab plus axitinib vs sunitinib for the first-line therapy of patients with advanced RCC is planned.

Combined Monoclonal Antibody and Checkpoint Inhibitor Trials. Bevacizumab is also being studied in

Table. ***(Continued)*** Clinical Trials of Combined VEGF-Directed Therapy and PD-1/PD-L1 Inhibition

Combinations/ Trials	Phase	Population	N	Treatment Arms*	ORR	Notable AEs
PD-1 + mAb						
NCT02210117[95]	1	Treated mRCC pre-nephrectomy	Enrolling	nivo (3 mg/kg q2wk × 3) vs nivo (3 mg/kg q2wk × 3) + bev (10 mg/kg q2wk × 3) vs nivo (3 mg/kg q3wk × 2) + ipi (1 mg/kg q3wk × 2)	N/A	N/A
NCT02348008[96]	1b/2	Treated mRCC (ccRCC)	Enrolling	pembro (200 mg q3wk) + bev (10-15 mg q3wk)	N/A	N/A
NCT02298959[97]	1	Treated mRCC (post-TKI)	Enrolling	pembro + ziv-aflibercept	N/A	N/A

AE, adverse event; aRCC, advanced renal cell carcinoma; atezo, atezolizumab (intravenous); avel, avelumab (intravenous); axi, axitinib (oral); bev, bevacizumab (intravenous); BID, twice a day; cab, cabozantinib (oral); cc, clear cell; DLT, dose-limiting toxicity; ipi, ipilimumab (intravenous); irAE, immune-related adverse event; ⇑LFT, increased liver function test; mAb, monoclonal antibody; mRCC, metastatic renal cell carcinoma; N, number of patients; nivo, nivolumab; N/A, not available; ORR, overall response rate; paz, pazopanib (oral); PD-1, programmed death 1; PD-L1, programmed death ligand 1; pembro, pembrolizumab (intravenous); PR, partial response; pt, patient; q, every; RCC, renal cell carcinoma; SD, stable disease; sun, sunitinib; TKI, tyrosine kinase inhibitor; wk, week(s).

[a] Oral medication doses are daily unless otherwise indicated.

combination with PD-1/PD-L1 inhibitors. A phase 1b study is evaluating atezolizumab plus bevacizumab in treatment-naive advanced RCC with a clear cell component (or with chemotherapy for other cancers; NCT01633970).[91] At the 2015 ASCO Genitourinary Cancers Symposium, Sznol and colleagues presented preliminary results from this trial.[92] Among 10 patients with at least 1 tumor assessment, the ORR was 40%. Increases in tumor-infiltrating $CD8^+$ T cells were observed.[83]

This result led to the phase 2 IMmotion150 trial (A Phase 2 Study of Atezolizumab as Monotherapy or in Combination With Bevacizumab Compared to Sunitinib in Participants With Untreated Advanced Renal Cell Carcinoma; NCT01984242), the first randomized study of combined PD-1/PD-L1 and VEGF inhibition in kidney cancer. Patients with treatment-naive RCC (either clear cell or with sarcomatoid components) were randomly assigned to atezolizumab with or without bevacizumab or to sunitinib. Patients were stratified by prior nephrectomy status, PD-L1 immunohistochemistry expression (<1% or ≥1% PD-L1 expression on the immune infiltrate, deemed "positive"), and Motzer criteria. Crossover from the atezolizumab monotherapy and sunitinib arms was allowed after disease progression (except in Europe, where crossover from atezolizumab monotherapy was prohibited). Data were recently presented at the 2017 ASCO Genitourinary Cancers Symposium.[93] Among all patients (intent-to-treat analysis), median PFS was longer with atezolizumab plus bevacizumab (11.7 months) than with atezolizumab monotherapy (6.1 months) or sunitinib (8.4 months). Subgroup analysis revealed that increased levels of PD-L1 expression correlated with more favorable PFS hazard ratios with combination therapy, indicating that patient selection might be possible according to PD-L1 expression. Of note, 56% of patients in the sunitinib arm and 43% of patients in the atezolizumab monotherapy arm crossed over to combined atezolizumab plus bevacizumab, causing bias toward the null hypothesis, and the study was not powered to detect differences at an alpha of 0.05 (only the comparison between investigator-assessed PFS in the combination arm and in the sunitinib arm reached significance). The ORR in the combination arm was 32% (intent-to-treat analysis), compared with 25% to 29% in the other arms. In the PD-L1–positive patients, the ORR was as high as 46% with combination therapy, and 12% to 15% of PD-L1–positive patients exposed to atezolizumab had complete responses. Analyses of clinical activity biomarkers and crossover treatment are ongoing.

The comparison between combination atezolizumab plus bevacizumab vs sunitinib will be continued in the

phase 3 IMmotion151 trial (A Study of Atezolizumab in Combination With Bevacizumab Versus Sunitinib in Participants With Untreated Advanced Renal Cell Carcinoma) for treatment-naive patients with kidney cancer (NCT02420821).[94] On the basis of the phase 2 findings, the phase 3 trial analysis will focus on the PD-L1–positive population (although PD-L1 positivity is not required for enrollment). Thus, IMmotion150 was the first randomized study of first-line PD-1/PD-L1 blockade in kidney cancer, the first randomized study of combined VEGF/PD-1 inhibition, and the first study to compare PD-1/PD-L1 blockers directly with anti-VEGF TKIs. In addition to the intriguing finding of longer PFS with combination therapy, this study demonstrated that toxicity was reduced with PD-1/PD-L1 blockade vs TKI, and it showed that a complete response was possible in more than 10% of PD-L1–positive patients treated with first-line atezolizumab.

Trials in which PD-1 inhibitors are added to antiangiogenic antibodies are also ongoing. One phase 1 study is investigating nivolumab vs nivolumab plus bevacizumab vs nivolumab plus ipilimumab—all treatments to be followed by nephrectomy—in patients with previously treated mRCC, excluding study drugs (NCT02210117).[95] A phase 1b/2 study is evaluating pembrolizumab plus bevacizumab in patients with previously-treated ccRCC (NCT02348008).[96] A phase 1 study is looking at pembrolizumab plus the fusion anti-VEGF/R antibody ziv-aflibercept (Zaltrap, Sanofi/Regeneron) in multiple cancers; in RCC, patients must have previously received a VEGF-directed TKI (NCT02298959).[97]

Combinations of Checkpoint Inhibitors. Meanwhile, additional combination strategies are being studied. For example, Hammers and colleagues presented preliminary data from CheckMate 016 (Nivolumab in Combination With Sunitinib, Pazopanib, or Ipilimumab in Subjects With Metastatic Renal Cell Carcinoma; NCT01472081), a phase 1 study of nivolumab combined with the cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) checkpoint inhibitor ipilimumab in patients with ccRCC, mostly previously treated.[98] Toxicity was prevalent but occurred mainly with the highest doses of ipilimumab and nivolumab; grade 3 to 4 occurrences were noted in only 34% of the patients in the dosing arm ultimately chosen for subsequent study. The ORR was approximately 40%, with another 40% of patients experiencing stable disease. CheckMate 214 (Nivolumab Combined With Ipilimumab Versus Sunitinib in Previously Untreated Advanced or Metastatic Renal Cell Carcinoma; NCT02231749) is a phase 3 trial that will compare the combination of nivolumab plus ipilimumab vs sunitinib for treatment-naive patients.[99]

Biomarkers for Predicting Response. Correlative studies have attempted to identify biomarkers to facilitate patient selection. Despite the link between *VHL* gene mutation and VEGF signaling, neither *VHL* gene status nor HIF-1α/HIF-2α expression correlates with response to or PFS with pazopanib.[100]

PD-L1 has been investigated as a biomarker for response to PD-1/PD-L1 blockade, with inconsistent results. In patients with kidney cancer, tumor overexpression of PD-L1 may be less prevalent than in patients with other tumor types. In CheckMate 025, 76% of patients had less than 1% tumor PD-L1 expression. Survival benefit with nivolumab vs everolimus was seen regardless of PD-L1 status.[41] Evidence from other cancers has been mixed, with no prediction of response in squamous cell lung cancer[101] or melanoma[102] but some prediction of response in nonsquamous non–small cell lung cancer.[103] However, the level of PD-L1 expression on tumor-infiltrating immune cells may be higher and may better indicate response. In patients who had various cancers treated with atezolizumab, treatment response correlated better with immune cell PD-L1 expression than with tumor PD-L1 expression.[104] In the previously discussed trial of atezolizumab plus bevacizumab, half of the patients had immune cell PD-L1 expression, and a trend toward increased antitumor activity was observed with higher levels of expression.[93]

The power of PD-L1 expression to predict response to VEGF-directed therapy is limited. Tumor PD-L1 expression was a negative prognostic factor in the COMPARZ study (Pazopanib Versus Sunitinib in the Treatment of Locally Advanced and/or Metastatic Renal Cell Carcinoma; NCT00720941),[105] and this finding was corroborated in another series.[106] In a third series, pre-nephrectomy serum levels of PD-L1 correlated with a worse response to anti-VEGF therapy.[107] Some proposed that serum PD-L1 level might be useful as a predictive factor for VEGF-directed therapy. However, to establish predictive power, these results would have to be compared directly with outcomes in patients who had PD-L1 expression and received no VEGF-directed therapy.[108] Without such data, there is no role for PD-L1 as a predictive factor for VEGF-directed therapy. VEGF might predict response to PD-L1 blockade; plasma VEGF was decreased in patients who responded to atezolizumab but stable in patients with stable or progressive disease,[109] but there was no comparison treatment arm. Thus, identifying predictive biomarkers has been difficult, but PD-L1 expression by tumor-infiltrating immune cells may help investigators select the patients most likely to benefit from PD-L1 blockade, with or without concurrent VEGF-directed therapy.

Future Directions and Challenges

VEGF-targeted therapies and PD-1/PD-L1 inhibitors have profoundly altered the treatment of advanced kidney cancer, yet too few patients benefit, and for too brief a time. The many trials testing combined VEGF-directed and checkpoint inhibitor therapies bring excitement for the future, and the results may change the standard of care for treatment-naive kidney cancer. Several questions are being asked.

First, will any particular blockade of PD-1/PD-L1 or VEGF be superior? Anti-VEGF TKI activity may differ from that of antibodies, and the results of inhibition of different parts of the VEGF-VEGF/R axis may also differ. Simultaneous targeting of additional escape pathways may be useful.

Second, how does combined blockade compare with sequential blockade? Is there a survival benefit that justifies the risk for increased toxicity with concurrent therapy? Will it increase the "tail of the curve" of long-term responders who will need no further treatment? And although not currently standard of care, what if patients have previously been treated with neoadjuvant/adjuvant therapy? Adjuvant VEGF inhibition has been tested in the S-TRAC (Sunitinib Treatment of Renal Adjuvant Cancer)[110] and ASSURE (Adjuvant Sorafenib or Sunitinib for Unfavorable Renal Carcinoma)[111] trials, and neoadjuvant/adjuvant PD-1/PD-L1 inhibition is under investigation in PROSPER RCC (A Phase 3 Randomized Study Comparing Perioperative Nivolumab vs Observation in Patients with Localized Renal Cell Carcinoma Undergoing Nephrectomy; NCT03055013), MK-3475-031 (A Study Evaluating the Effect of Pembrolizumab in Participants With Renal Cell Cancer; NCT02212730), and IMmotion010 (A Study of Atezolizumab as Adjuvant Therapy in Participants With Renal Cell Carcinoma at High Risk of Developing Metastasis Following Nephrectomy; NCT03024996).

Third, how will this combination approach compare with other combinations, such as nivolumab plus ipilimumab, and in what sequence should different combination strategies be used?

Finally, can we identify the patients most likely to benefit (or not benefit) from the VEGF/PD-1 combination strategy? Some patients may require only checkpoint inhibitors; others may need the combination. It has been difficult to identify biomarkers of response to either VEGF-directed therapy or checkpoint inhibitors individually; it may be even more difficult to identify biomarkers of response to combined therapies. Further research may allow the use of biomarkers to direct an individualized immunotherapy strategy.[112]

In summary, the landscape of treatments for advanced kidney cancer is rapidly evolving, shaped by increasing understanding of underlying pathogenic mechanisms and interactions among them. Future studies may allow us to select patients for different strategies depending on the unique characteristics of their tumors, and so fulfill the goal of personalized medicine.

Disclosures

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