Emerging Role for Novel Immunotherapy Agents in Metastatic Renal Cell Carcinoma: From Bench to Bedside

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OVERVIEW

Therapies that augment the antitumor immune response have been an established treatment modality for metastatic renal cell carcinoma (mRCC) since the 1980s. An improved understanding of the factors that limit the immune response to cancer have led to the development of novel therapeutic agents. Most notably, monoclonal antibodies that block the programmed death (PD)-1 immune checkpoint pathway have demonstrated encouraging antitumor activity against mRCC in phase I and II clinical trials. However, as monotherapy these agents are unlikely to offer substantial clinical benefit for the majority of patients with mRCC. Combination approaches and improvements in patient selection will be essential to enhance their efficacy and ensure the rational application of immunotherapy. This review summarizes the clinical and preclinical data that support the use of novel immunotherapies for mRCC and looks forward to future directions for this promising therapeutic strategy.

Cytokine-based immunotherapies, such as interferon-alfa and interleukin (IL)-2, have been used for the treatment of mRCC since the 1980s.1-9 Although some patients experience a dramatic benefit with this approach, a majority fail to achieve long-term disease-free intervals. Substantial improvements in the outcomes of patients with mRCC have occurred over the past decade with the development of therapies targeting the vascular endothelial growth factor (VEGF) and mammalian target of rapamycin (mTOR) pathways,10-22 but mRCC continues to account for an estimated 13,860 deaths per year in the United States.23 Identifying therapeutic strategies that promote durable remission of metastatic disease remains critically important.

Conventional immunotherapies often fail to provide long-term control of cancers because neoplasms are able to evade immune-mediated attack through several mechanisms. These include: (1) preferential proliferation of T regulatory cells (Tregs), which leads to decreased acute inflammation, (2) increased intratumoral levels of immunosuppressive cytokines, such as interleukin (IL)-6, TGF-beta, and IL-10, (3) increased expression of immune checkpoint modulators that serve to limit the inflammatory response, and (4) poor trafficking of immune effector cells to the tumor itself.24-26 Gajewski et al have described two distinct tumor phenotypes as a model to conceptualize these different mechanisms of resistance to immune-mediated destruction: a “noninflamed phenotype” in which tumors have low levels of chemokine production and lymphocyte infiltration (and therefore might benefit from therapies designed to increase lymphocyte trafficking to tumors, such as tumor vaccination); and an “inflamed phenotype” in which tumors have rich chemokine levels and variable T-cell infiltration, but also contain increased levels of immunosuppressive Tregs and immune checkpoint modulators (and therefore might benefit from therapies designed to inhibit Tregs or immune checkpoint molecules).26

Although there is evidence that immunotherapy can be an effective therapeutic modality for mRCC, further efforts are needed to take full advantage of this approach for both tumors that display the inflamed phenotype and tumors that have the noninflamed phenotype. Novel immunotherapy strategies, including immune checkpoint inhibitors, suppressors of Tregs, T-cell agonists, and tumor vaccines, and combinations of these various modalities with FDA-approved therapies, have produced interesting preliminary data in ongoing clinical trials.

NOVEL IMMUNOTHERAPY FOR MRCC: A BRIEF SUMMARY OF THE ROLE OF IMMUNE CHECKPOINT INHIBITION

Interactions between molecules on the surfaces of T cells and antigen-presenting cells at the immune checkpoint can lead to the induction of immune tolerance. The most clinically relevant of these interactions are those between cytotoxic T lymphocyte associated protein-4 (CTLA-4) on T cells and its ligands B7-1 and B7-2 on antigen-presenting cells, and those between PD-1 on T cells and its main ligand PD-L1 on
antigen-presenting cells or tumor cells. It has been noted that mRCC tumor cells themselves can also express PD-L1 and thereby activate the immunoregulatory function of the PD-1/PD-L1 interaction to create an immunosuppressive tumor microenvironment. 

Monoclonal antibodies that inhibit the negative immune regulators present in inflamed tumors lead to upregulation of antitumor immune function and have demonstrated potent effects in both laboratory and clinical studies of various malignancies, including mRCC.

Nivolumab, a fully human monoclonal IgG4 antibody specific for human PD-1, demonstrated an objective response rate of 29% in a phase I trial and 21% in a phase II clinical trial of patients with mRCC who had failed standard therapy. Blockade of PD-L1, the primary ligand for PD-1, with MPDL3280A yielded an overall response rate of 15% in a phase I cohort of patients with mRCC. Additionally, a substantial number of patients in these early clinical trials experienced stable disease for longer than 24 weeks. Whether this level of antitumor activity will translate into prolonged overall survival remains the crucial question for phase III trials. A randomized trial of nivolumab versus everolimus demonstrated partial responses in 4 of 19 patients, with a tolerable toxicity profile. Although a subsequent phase I trial of intravenous recombinant IL-21 in metastatic RCC demonstrated partial responses in 4 of 19 patients, with tolerable toxicity profile. Although a subsequent phase I trial of recombinant IL-21 in combination with sunitinib demonstrated dose-limiting hematologic toxicities, a phase I/II trial of IL-21 and sorafenib revealed an acceptable toxicity profile with a response rate of 21%. A trial of IL-21 and PD-1 blockade might therefore be a rational combination to test in mRCC.

**FUTURE DIRECTIONS OF IMMUNOTHERAPY IN mRCC**

**Combinations of PD-1/PD-L1 Blockade and Other Immune Checkpoint Inhibitors or Angiogenesis Inhibitors**

Combination therapy in humans with advanced solid malignancies is currently ongoing. Therapies that increase T-cell activity, including IL-2, CD137 agonist antibodies, and interleukin-21 (IL-21), may improve the effectiveness of immune checkpoint inhibitors. CD137 (also known as 4–1BB), which serves as a costimulatory molecule for T cells and leads to increased cytokine production, effector cytolytic activity, and T-cell survival, has been demonstrated to eradicate mRCC in murine models. A phase I clinical trial of a CD137 agonist monoclonal antibody (PF-05082566) in combination with anti–PD-1 therapy in humans with advanced solid malignancies is currently ongoing.

The cytokine IL-21 is produced by activated CD4\(^+\) T helper cells and has been demonstrated to increase the density of CD8\(^+\) T cells in murine models of both melanoma and mRCC and lead to antitumor responses against these malignancies when administered to mice. A phase I clinical trial of intravenous recombinant IL-21 in metastatic RCC demonstrated partial responses in 4 of 19 patients, with a tolerable toxicity profile. Although a subsequent phase I trial of recombinant IL-21 in combination with sunitinib demonstrated dose-limiting hematologic toxicities, a phase I/II trial of IL-21 and sorafenib revealed an acceptable toxicity profile with a response rate of 21%. A trial of IL-21 and PD-1 blockade might therefore be a rational combination to test in mRCC.

**Elimination of Tregs**

Tregs are a subpopulation of CD4\(^+\)CD25\(^+\)FOXP3\(^+\) T cells that serve an immunosuppressive function. Therapeutic agents that deplete or eliminate Tregs, such as monoclonal antibodies against chemokine receptor 4 (CCR4) or CD25, or the cytokine–toxin conjugate denileukin diftitox, could therefore augment the immune response to malignancies.

CCR4 is expressed selectively on Tregs among immune cells, and also on some tumor cells themselves. In preclinical studies, blockade of CCR4 with monoclonal antibodies decreased the level of Tregs and induced T-cell responses specific to human tumor antigens. Metastatic human RCC cells have increased proportions of both CD25\(^+\) Treg cells and CCR4\(^+\) T cells relative to primary neoplastic cells in the kidney, suggesting that selective depletion of CCR4 may lead to regression of metastatic lesions in mRCC. Denileukin diftitox and the anti-CD25 monoclonal antibody daclizumab have been shown to augment the effect of tumor an-
tigen vaccination in a variety of solid malignancies, including breast cancer and mRCC. Further efforts to eliminate or inhibit Tregs may enhance the efficacy of immunotherapeutic strategies for mRCC.

mRCC Tumor Vaccines

Interest in vaccine therapies for the treatment of various malignancies has increased following FDA approval of the dendritic cell vaccine sipuleucel-T for metastatic castration-resistant prostate cancer.90 In the specific context of mRCC, tumor vaccines may be of particular use in the noninflamed phenotype, as vaccination might be able to initiate a tumor-targeted immune response that would increase the initial trafficking of T cells to tumors.26 Several vaccines have been developed for clinical investigation of mRCC, including some targeted against peptide antigens (IMA901, TG-4010, and MVA-5T4)91-96 and some developed from autologous dendritic cells (AGS-003). Of these, IMA901 and AGS-003 are currently in late-stage clinical trials.

Phase I and II studies of the multipropeptide mRCC vaccine IMA901 have demonstrated encouraging overall survival in the subset of patients who also received a single dose of cyclophosphamide in addition to the vaccine.97-98 A randomized phase III study of IMA901, cyclophosphamide, and GM-CSF vs. sunitinib versus sunitinib alone as first-line therapy for patients with metastatic RCC is currently ongoing.99

AGS-003 is a personalized, autologous dendritic cell vaccine that has been examined in phase II clinical trials in combination with sunitinib. Preliminary data indicate improved progression-free survival and overall survival when compared to historic controls of patients with unfavorable-risk mRCC.100-102 A randomized phase III trial of AGS-003 plus standard therapy (sunitinib) for metastatic RCC is currently recruiting participants.103

Recently, multiregion whole-exome sequencing, chromosome aberration analysis, and ploidy profiling of primary and metastatic RCCs have demonstrated substantial levels of intratumoral genetic heterogeneity in this disease.104 Future research into mRCC vaccines will attempt to exploit this intratumoral heterogeneity by targeting the tumor neoantigens that arise from such genetic diversity. A study of such a personalized neoantigen cancer vaccine ("Neovax") is currently recruiting participants and should enter phase I clinical trials for RCC in 2015.105-106

Ongoing studies will also address the optimal combinations and sequence of mRCC vaccines, targeted immunotherapy, and small-molecular inhibitors in an effort to further improve immune responses.

**TABLE 1. Ongoing Trials of Combinations of PD-1/PD-L1 Inhibition and Angiogenesis or Other Immune Checkpoint Inhibitors in mRCC**

<table>
<thead>
<tr>
<th>PD-1/PD-L1 Inhibitor</th>
<th>Second Agent (Mechanism)</th>
<th>Phase</th>
<th>Estimated Enrollment</th>
<th>ClinicalTrials.gov Identifier</th>
</tr>
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<tbody>
<tr>
<td>Nivolumab</td>
<td>BMS-986016 (anti-LAG-3)</td>
<td>I</td>
<td>168</td>
<td>NCT01968109</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>Pazopanib (angiogenesis inhibitor)</td>
<td>I</td>
<td>228</td>
<td>NCT02004636</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>Axitinib (angiogenesis inhibitor)</td>
<td>I</td>
<td>60</td>
<td>NCT02133742</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>Ipilimumab (anti-CTLA4)</td>
<td>I/I</td>
<td>343*</td>
<td>NCT02089685</td>
</tr>
<tr>
<td>MEDI4736</td>
<td>Tremelimumab (anti-CTLA4)</td>
<td>I</td>
<td>102**</td>
<td>NCT01975831</td>
</tr>
<tr>
<td>MEDI4736</td>
<td>MEDI0680 (anti-PD-L1)</td>
<td>I</td>
<td>150**</td>
<td>NCT02118337</td>
</tr>
<tr>
<td>MPDL3280A</td>
<td>Bevacizumab (angiogenesis inhibitor), sunitinib (angiogenesis inhibitor)</td>
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<td>300</td>
<td>NCT01984242</td>
</tr>
<tr>
<td>Nivolumab</td>
<td>Ipilimumab (anti-CTLA4)</td>
<td>III</td>
<td>1,070</td>
<td>NCT02231749</td>
</tr>
</tbody>
</table>

Abbreviations: PD-L1, programmed death ligand-1; mRCC, metastatic renal cell carcinoma; CTLA4, cytotoxic T lymphocyte associated protein-4.

**This study will enroll patients with both mRCC and metastatic melanoma.

**This study will enroll patients with various advanced solid malignancies (not solely mRCC).**

**IMPROVING PATIENT SELECTION: DEVELOPING BETTER PREDICTIVE MODELS**

The determination of appropriate biomarkers to better predict which patients with mRCC will benefit most from immunotherapy is an active area of investigation. Multiple clinical trials have indicated that responses to immunotherapy with checkpoint inhibitors have been observed both in patients whose tumors express PD-L1 and in those without such expression.107-108 There are several potential explanations for this discrepancy, including: (1) heterogeneous expression of PD-L1 across the primary tumor and metastatic sites in individual patients, (2) inconsistent definitions of the threshold PD-L1 immunohistochemical positivity (e.g., 1% vs. 5% staining), (3) immunosuppressive effects of PD-L1 expression by other cells in the tumor microenvironment (e.g., macrophages or myeloid-derived suppressor cells), and (4) the potential impact of PD-L2 expression on responsiveness to anti-PD-1/PD-L1 therapy.

Preclinical and clinical trials of agents targeting PD-1/ PD-L1 have used cutoffs of PD-L1 immunohistochemical positivity ranging from 1 to 5% staining, resulting in differences in response rates depending on which definitions are employed.54 Standardization of the appropriate cutoff for a positive test will be essential for future development of meaningful predictive models.

Furthermore, a recent small series has demonstrated discordant PD-L1 expression between primary RCC sites and metastatic sites in individual patients, (2) inconsistent definitions of the threshold PD-L1 immunohistochemical positivity (e.g., 1% vs. 5% staining), (3) immunosuppressive effects of PD-L1 expression by other cells in the tumor microenvironment (e.g., macrophages or myeloid-derived suppressor cells), and (4) the potential impact of PD-L2 expression on responsiveness to anti-PD-1/PD-L1 therapy.

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metastatic RCC sites in 15% (5 of 33) of patients, suggesting that differential PD-L1 expression profiles between primary and metastatic lesions may contribute to the heterogeneity of responsiveness to anti-PD-1/PD-L1 targeted therapies. In the setting of such heterogeneous PD-L1 expression, it is likely that the most useful models for prediction of response to immunotherapy will integrate PD-L1 status with other clinical, pathologic, molecular, and cytogenetic variables. These may include PD-L2 status,110 gene expression profiling,111 and/or clinical characteristics such as the Memorial Sloan Kettering Cancer Center (MSKCC) risk score96 or International Metastatic RCC Database Consortium (IDMC or Heng) criteria.112 Development of comprehensive predictive models for immunotherapy in mRCC is currently an active area of investigation.

CONCLUSION
Novel immunotherapies have produced encouraging initial results in the treatment of metastatic RCC; however, these agents are unlikely to be effective as monotherapy in the majority of patients. At present, intensive clinical study is focused on devising combinations of therapies and developing more accurate predictive biomarker models in an effort to improve clinical outcomes.

Disclosures of Potential Conflicts of Interest

Relationships are considered self-held and compensated unless otherwise noted. Relationships marked “L” indicate leadership positions. Relationships marked “I” are those held by an immediate family member; those marked “B” are held by the author and an immediate family member. Institutional relationships are marked “Inst.” Relationships marked “U” are uncompensated.


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59. NCT01968109. Safety study of anti-LAG-3 with and without anti-


