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Beyond Conventional Immune Checkpoint Inhibition: Renal Cell Carcinoma at the Forefront of Solid Tumor Immunology

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Abstract

The management of advanced renal cell carcinoma (RCC) has been transformed by the development of immune checkpoint inhibitors (ICIs), but still most RCC patients do not receive durable clinical benefit. Importantly, RCC differs substantially from other immunotherapy-responsive solid tumors – it has a modest mutation burden, and paradoxically, high CD8⁺ T cell infiltration has been associated with a worse prognosis. Building on the successes of inhibitory antibodies targeting the PD-1 and CTLA-4 axes, multiple innovative immunotherapies are now in clinical development for the treatment of RCC, including new ICIs, co-stimulatory pathway agonists, modified cytokines, metabolic pathway modulators, cellular therapies, and therapeutic vaccinations. However, the successful development of such novel immune-based treatments and of immunotherapy-based combinations will require a RCC-specific framework for understanding the necessary immunotherapeutic interventions that underlie an effective anti-tumor immune response. Here, using the structure provided by the well-described cancer-immunity cycle, we outline the key steps required for a successful anti-RCC immune response, and describe the development of promising new immunotherapies within the context of this framework. With this approach, we summarize and analyze encouraging targets within the RCC microenvironment, and review the landscape of antigen-directed therapies in this disease.

Introduction

The management of renal cell carcinoma (RCC) has undergone vast changes over the past two decades. From a disease with few effective therapeutic options beyond surgical resection, RCC systemic therapy now includes a wealth of options including, VEGF pathway inhibition through VEGFR-tyrosine kinase inhibitors (VEGF TKIs) or the anti-VEGF antibody bevacizumab, mTOR pathway inhibition, and immune checkpoint inhibitors

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(ICI)¹. More recently, ICI-based combinations (either ICI-ICI or ICI-VEGF TKI) have shown remarkable efficacy in patients with metastatic RCC and today form the standard-of-care first-line therapies for patients with this disease^{2–4}.

Well before the development of modern ICIs, RCC already stood out as a tumor type known to be responsive to immune-based therapies. Historically, RCC was treated with cytokine-based forms of immunotherapy, including high-dose interleukin-2 (IL-2), which was associated with durable complete responses (CR) in a small subset of patients⁵. In addition, metastatic RCC tumors were, in very rare cases, reported to regress either spontaneously or after resection of the primary renal tumor (i.e. cytoreductive nephrectomy), with the mechanisms of such regressions suggested to be immune-related^{6,7}. Further, RCC patients treated with allogeneic hematopoietic stem cell transplantation (HSCT) have had durable CR rates in up to 9.5% of patients in some series⁸.

While ICI-based combinations have dramatically improved outcomes for patients with metastatic RCC, most patients still either have primary resistance to these therapies or acquire resistance after an initial response^{2–4}. The development of novel therapeutic strategies designed to overcome these mechanisms of resistance is therefore of paramount importance for patients with this disease. Given the established immune-responsiveness of RCC, novel immunotherapy-based agents hold great promise in the treatment of this disease.

In this review, we highlight novel immune-based therapies for RCC beyond conventional ICI, with a focus on their underlying scientific rationale and the potential for new immunotherapy-based combinations. This review focuses on the most frequent histological subtype of RCC, clear cell RCC (ccRCC), which comprises the vast majority of all RCC tumors, is highly immune infiltrated, and whose biology is best understood^{9,10}. When possible, we also draw parallels or elaborate on the implications for variant histology RCC (i.e. “non-clear cell”) based on the availability of published data.

Section 1. A Model of Effective Anti-Tumor Immunity in RCC: The RCC Cancer-Immunity Cycle

In their 2013 paper, Chen and Mellman outlined the biological steps underlying an effective immune response to cancer, termed the “cancer-immunity cycle”¹¹. This cycle conceptualizes the steps necessary for an immune response to cancer cells and provides a framework for understanding the role of specific immune system components – and therefore therapies – along this cycle. As we detail below, while RCC is among the most immune-responsive of solid tumors, it lacks many typical characteristics of other solid tumors that are responsive to immunotherapy. Building on the foundational work of Chen and Mellman, we outline a RCC-specific cancer-immunity cycle, highlighting how this general framework can be applied specifically in the context of RCC, which is critical to understanding the roles of conventional and novel immune therapies in this disease (Figure 1).

1) Tumor antigens: quantity, quality, or “going viral” (steps 1–3 of the cancer-immunity cycle)—Neoantigens are a class of tumor-specific antigens derived from somatic alterations (including single nucleotide variations [SNVs], insertions and

deletions [indels], and structural variations)¹² and represent an ideal target of effective anti-tumor immunity. Tumor mutational burden (TMB) is often used as a surrogate for neoantigen load and has been found to correlate with response to ICIs in many tumor types¹³, leading to a histology-agnostic approval for the ICI pembrolizumab for high TMB tumors¹⁴. However, RCC has only a moderate mutation burden (lower than most other immune-responsive tumor types)¹⁵, and TMB does not predict clinical outcomes on ICI in patients with RCC^{16,17}. Further, while the proportion of frameshift indels is highest in RCC compared to other tumor types and can be associated with increased immunogenicity¹⁸, the indel load does not associate with clinical outcomes on ICI in this disease^{16,17}.

Cancer-testis antigens are a class of antigens that are only physiologically expressed in male germ cells. These antigens are also expressed in multiple cancers because of demethylation of their promoter sequences and can induce anti-tumor immune responses¹². Expression of these antigens is limited in RCC, and they may therefore play a less prominent role in the anti-tumor immune responses in this disease^{19,20}.

Human endogenous retroviruses (ERVs) are yet another class of potential antigens in RCC. ERVs are remnants of retroviral infections that constitute up to 8% of the human genome and are transcriptionally silent in normal tissue²¹. ERVs can be aberrantly expressed in cancer due to epigenetic dysregulation and are able to stimulate anti-tumor immune responses through activation of non-specific innate immunity or a more specific adaptive anti-tumor response directed against ERV-derived peptides²¹. ERVs were demonstrated to be an antigenic target after a RCC patient who had a prolonged response to allogeneic HSCT was found to have CD8⁺ T cells that were reactive to a 10-mer protein derived from an ERV locus (ERVE-4)⁸. Other studies further supported a role for ERVs in RCC anti-tumor immune responses; in pan-cancer analyses, ERV expression was found to strongly correlate with immune cytolytic activity¹⁹ and was found to be associated with prognosis in ccRCC²². Specifically, elevated ERV3–2¹⁸ and ERV4700²² expression were also found to correlate with ICI response in RCC. However, in a recent study of ccRCC patients treated with ICI monotherapy, no correlation was found between the expression of these 3 ERVs (ERVE-4, ERV3–2, or hERV4700) and outcomes on ICI¹⁷. Of note, the latter study inferred ERV expression from RNA-seq on formalin-fixed paraffin-embedded (FFPE) tissue, and ERV3–2 expression did not appear to reliably correlate with gold standard qPCR-based quantification¹⁷.

While the antigens driving anti-tumor immune response in RCC remain largely undefined, the role of dendritic cells (DCs) and other antigen-presenting cells (APCs) in effective T cell priming also requires further study in this disease. DCs have been reported to form tertiary lymphoid structures (TLS) in the RCC microenvironment and these structures have been suggested to be crucial for DC maturation²³. Absence of these TLS was associated with immature DCs and ineffective antigen presentation²³. Moreover, presence of APC-dense regions that support “stem-like” TCF1⁺ progenitor CD8⁺ T cells within RCC tumors has been recently found to play an important role for anti-tumor immune response, with loss of these intra-tumoral niches associated with impaired CD8⁺ T cell response and tumor progression²⁴.

2) Trafficking, infiltration, recognition, and killing: the paradoxes of RCC

(steps 4–7 of the cancer-immunity cycle)—Clear cell RCC tumors are highly immune-inflamed tumors compared to non-clear cell RCC tumors^{19,25} and to other tumor types^{19,26}. Clear cell RCC is also a highly angiogenic tumor characterized by frequent two-copy losses of the *VHL* gene²⁷, inducing a pseudo-hypoxic state marked by HIF and VEGF-A over-expression²⁸. In fact, ccRCC has been shown to express VEGF-A at higher levels than all other cancer types²⁹. The co-occurrence of increased immune infiltration (including T cells) and increased angiogenesis in RCC (compared to other tumor types) contrasts with the pre-clinical and translational evidence that suggests that increased VEGF-A expression typically associates with decreased immune infiltration^{11,30}. This discrepancy may, in part, be explained by heterogeneity of the immune microenvironment of ccRCC. Multiple studies have converged on delineating distinct subtypes of ccRCC defined by different transcriptional and protein-based signatures: one subgroup is characterized by the highest level of immune infiltration (including CD8⁺ T cell infiltration), *BAP1* mutations, increased expression of antigen presentation machinery (APM) genes, and adverse prognosis^{31–33}. A second subgroup is characterized by increased VEGF expression, decreased immune infiltration, and a more favorable prognosis^{31,33}. A third subgroup has the lowest infiltration by immune cells, increased mitochondrial and metabolic signaling, and increased MYC and mTOR activity^{31,33}.

Beyond VEGF-A, other cytokines and growth factors play key roles in shaping the RCC microenvironment and regulating immune infiltration. Interleukin-8 (IL-8) is a chemokine produced by both intra-tumoral and circulating myeloid cells and plays key roles in regulating the microenvironment of RCC³⁴. Increased circulating and intra-tumoral IL-8 was found to correlate with worse outcomes on systemic therapy, including ICI^{34,35}. In addition, increased IL-8 correlated with an immunosuppressive myeloid-enriched microenvironment characterized by increased neutrophil and monocyte infiltration and decreased T cell and interferon-gamma (IFN γ) signatures^{34–36}. Some of this phenotype appeared to be driven by the downregulation of APM genes within monocytes that overproduce IL-8³⁴.

APM gene expression may also play a role in RCC immunotherapy response. RCC tumors have higher APM gene expression compared to normal kidney tissue³¹ and the tumors with the highest expression appear to be the most T cell infiltrated^{31,33}. In addition, the subset of RCC tumors with sarcomatoid or rhabdoid dedifferentiation features, which have exceptional responses to ICI^{37–42}, also have increased APM gene expression³⁷.

In contrast to almost all other solid tumors, it has long been noted that increased infiltration of RCC tumors by CD8⁺ T cells associates with poor prognosis⁴³. Multiple hypotheses have been proposed to explain this paradoxical relationship. First, the relative low density of TLS may lead to a larger proportion of immature DCs. This in turn can result in infiltration by polyclonal CD8⁺ T cells that do not recognize tumor-associated antigens^{23,43,44}. Second, the CD8⁺ T cells infiltrating RCC appear to be highly heterogeneous in terms of their states of activation and cytotoxic potential^{24,44–46}. As such, regardless of the overall abundance of CD8⁺ T cells, the presence of specific subpopulations such as stem-like TCF1⁺ or PD-1⁺ TIM-3[–] LAG-3[–] CD8⁺ T cell subsets^{24,46} may be key for an effective anti-tumor response. Third, CD8⁺ T cells in RCC appear to have a specific metabolic dysregulation whereby their

glucose uptake, glycolysis, and mitochondrial function are impaired. These metabolic impairments can limit CD8⁺ T cell activation and cannot be restored by PD-1 axis inhibition^{47,48}. Fourth, the relationship between CD8⁺ T cells and outcomes may be confounded by their associations with specific intra-tumoral genomic alterations that themselves alter RCC behavior. RCC tumors that are highly infiltrated by CD8⁺ T cells also appear to be relatively depleted of *PBRM1* mutations, which are typically associated with a better prognosis, and enriched in deletions of chromosome 9p21.3 (containing *CDKN2A* and *CDKN2B*), which are associated with unfavorable clinical outcomes in RCC^{17,49}. Exclusion of CD8⁺ T cells, which has been described in other tumor types^{50,51} and associated with ICI resistance³², does not commonly occur in RCC¹⁷.

Section 2. The Tumor Immune Microenvironment: Novel Potential Targets for Immunotherapy

Despite the relatively high rates of response to combinations of PD-1 and/or CTLA-4 axis inhibition in RCC that form the new standard-of-care, most patients with RCC do not receive durable clinical benefit from these therapies²⁻⁴. A deeper understanding of the RCC tumor immune microenvironment of RCC and early clinical signals hold promise that targeting novel microenvironmental cues could further improve clinical outcomes in this disease (Figure 2, Table 1 and S1).

1) Targeting novel immune checkpoints—Immune checkpoints physiologically regulate immune responses, maintaining homeostasis by sending appropriate co-stimulatory and co-inhibitory signals to T cells. These signals help protect normal tissue from an overactive immune system and direct the immune response to exogenous pathogens. However, these immune checkpoints can be co-opted by cancer cells to evade the immune system⁵². The effectiveness of PD-1 and CTLA-4 axis blockade has led to increased interest in targeting other immune checkpoints that could further reinvigorate anti-tumor responses.

i. Inhibitory immune checkpoints: T cell immunoglobulin and mucin-domain containing-3 (TIM-3, encoded by the *HAVCR2* gene), belongs to the immunoglobulin (Ig) superfamily and harbors an extracellular Ig variable (IgV) domain that binds its ligands and an intracellular tyrosine kinase phosphorylation motif that mediates its inhibitory function. In addition to T cells, TIM-3 is expressed on multiple other immune populations, including NK cells, DCs, B cells, macrophages, and monocytes⁵². In RCC, a mass cytometry-based study by Chevrier et al. found that TIM-3 is expressed in fully exhausted CD8⁺ T cells (“T-0”), in precursor partially exhausted CD8⁺ T cells (“T-1”), and in PD-1⁺ CD4⁺ T cells (“T-18”)⁴⁵. TIM-3 expression on PD-1⁺ CD8⁺ T cells has been associated with a poor prognosis in RCC⁵³. Overall, while TIM-3 does not appear to be as ubiquitously expressed as PD-1 on CD8⁺ T cells^{24,46}, its expression on the most exhausted subsets of CD8⁺ T cells suggests that it could be a potential target in RCC. In addition, *in vitro* inhibition of TIM-3 has been found to increase CD8⁺ and CD4⁺ T cell proliferation and IFN γ production in RCC⁵⁴. Clinically, combinations of anti-TIM-3 antibodies with PD-1 axis inhibitors have been investigated in other tumor types and found to have an acceptable safety profile and promising anti-tumor activity^{55,56}. TIM-3 inhibitor-based combinations are currently being evaluated in clinical trials, including patients with RCC⁵⁶.

Lymphocyte activation gene-3 (LAG-3) is another immune checkpoint that holds promise in RCC. This protein binds its ligands through extracellular Ig-like domains and exerts its intracellular inhibitory function mainly through a KIEELE motif^{52,57}. Interestingly, LAG-3 can also bind human leukocyte antigen (HLA) class II molecules and activate DCs, thus increasing antigen presentation to CD8⁺ T cells⁵⁸. LAG-3 has been found to be expressed on T cells, NK cells, B cells, and DCs⁵². In RCC, LAG-3 appears to be less frequently expressed on CD8⁺ T cells than TIM-3^{45,46} (although this has been disputed⁵⁹), and its expression may be context specific⁴⁵. Further, it should be noted that a minority of infiltrating T cells co-express PD-1 with either TIM-3 or LAG-3^{46,59}. In RCC, *in vitro* blockade of PD-1 has been shown to increase LAG-3, but not TIM-3, and the concurrent blockade of PD-1 and LAG-3 (but not of PD-1 and TIM-3) has been shown to stimulate IFN γ production⁵⁹. Clinically, efitagimod alpha (IMP321) has been tested in a phase I clinical trial as a recombinant soluble LAG-3 Ig fusion protein that agonizes HLA class II proteins on APCs, with the goal of activating APCs to increase antigen presentation to CD8⁺ T cells. This agent was found to induce activated and effector memory CD8⁺ T cells, along with some limited anti-tumor activity in RCC (7 of 8 patients in the high dose group experienced a best overall response of stable disease [SD], compared with 3 of 11 patients in the lower dose group)⁶⁰. Clinical trials evaluating LAG-3 inhibitors (designed to counter-act the LAG-3 inhibitory effects on T cells) in combination with PD-1 axis blockade in RCC are currently ongoing.

T cell immunoglobulin and ITIM domain (TIGIT) is a protein with an extracellular IgV domain that binds its ligands and an intracellular domain that mediates direct and indirect inhibition of immune cells. TIGIT is primarily expressed on T and NK cells⁵². In RCC, this inhibitory checkpoint has been found to be expressed on exhausted CD8⁺ T cell subsets²⁴. In phase Ia/Ib⁶¹ and randomized phase II clinical trials⁶², tiragolumab (an anti-TIGIT antibody) showed a tolerable safety profile and promising efficacy (most notably in non-small cell lung cancer), and clinical trials of TIGIT inhibitors in RCC are ongoing.

Other immune checkpoints, such as killer-cell immunoglobulin-like receptors (KIRs) on NK cells, and Human endogenous retrovirus-H long terminal repeat-associating protein 2 (HHLA2) and V-domain immunoglobulin suppressor of T-cell activation (VISTA) that inhibit T cells, have also shown promise as targets for inhibition in RCC, with a trial of an oral inhibitor of VISTA currently ongoing⁶³.

ii. Stimulatory immune checkpoints: 4-1BB (or CD137) is part of the tumor necrosis family receptor superfamily (TNFRSF) of proteins. Upon ligand binding, 4-1BB provides a CD28-independent co-stimulation signal in T cells⁶⁴. 4-1BB is expressed on T cells, NK cells, DCs, monocytes, and neutrophils⁶⁴ and its expression is upregulated during hypoxia through HIF1 α ⁶⁵. In RCC, 4-1BB is mainly co-expressed with exhaustion markers on the most exhausted subsets of CD8⁺ T cells⁴⁵. A phase Ib clinical trial of utomilumab (a 4-1BB agonist) combined with pembrolizumab showed that the combination was safe and had promising anti-tumor efficacy across multiple cancer types, including two objective responses (one CR) in five patients with RCC⁶⁶. Agonists of 4-1BB continue to be investigated in RCC in combination with PD-1 axis inhibitors.

OX40 is another co-stimulatory immune checkpoint that is also a member of the TNFRSF and is primarily expressed by T cells⁶⁷. In RCC, OX40 is expressed on CD4⁺ T cells and other T cell subsets⁴⁵. OX40 stimulation is thought to deplete regulatory T cells and stimulate effector T cell activity, thus improving anti-tumor response⁶⁷. Multiple clinical trials, with some including patients with RCC, have suggested that OX40 agonists are safe and have single agent anti-tumor activity^{68–71}. Trials evaluating agonistic antibodies against OX40 and other immune stimulatory pathways, such as CD40, and CD27, and the STING pathway are ongoing in RCC.

2) Renewed optimism in cytokine therapy—Before the current era of immune checkpoint inhibition, high-dose IL-2 (HD-IL-2) had formed the cornerstone of RCC immunotherapy. However, the toxicity associated with this regimen limits its use to select patients and selected institutions⁷². IL-2 can physiologically bind to receptors containing the IL-2R α , IL-2R β , and IL-2R γ subunits of the IL-2 receptor. Binding to IL-2R β / γ receptors mediates the anti-tumor activity of IL-2 through activation of memory effector CD8⁺ T cells, whereas binding to the IL-2R α -containing receptors can preferentially stimulate regulatory T cells⁷³. NKTR-214 is a pegylated form of IL-2 that has been suggested to improve the pharmacokinetic and pharmacodynamic properties of IL-2. Pegylation favors binding to IL-2R β / γ over IL-2R α , thereby improving CD8⁺ T cell activation and immune responsiveness, and at lower doses than those used with un-pegylated IL-2. In addition, pegylation improved the pharmacokinetic properties of IL-2, obviating the need for the high doses of IL-2 that were linked to its toxicity⁷³. Clinically, NKTR-214 has been evaluated in a phase I/II study in combination with nivolumab, showing a tolerable safety profile with only 11% grade 3 treatment-related adverse events in 162 patients. Among 24 patients with RCC, the objective response rate (ORR) was 54%, demonstrating significant anti-tumor activity⁷⁴. NKTR-214 is currently being evaluated in multiple clinical trials in RCC, including a phase III trial of NKTR-214 plus nivolumab versus cabozantinib or sunitinib ([NCT03729245](#)).

Interleukin-12 (IL-12) is another pro-inflammatory cytokine that is being investigated in RCC. IL-12 is a key stimulator of anti-tumor immune responses that links innate and adaptive immunity and stimulates antigen presenting, T, NK, and NKT cells⁷⁵. However, pharmaceutical development of human recombinant IL-12 had been hampered by its low therapeutic index and severe toxicities⁷⁶. NHS-IL12 (M9241) is a necrosis-targeted IL-12 immunocytokine that fuses IL-12 to the NHS76 antibody, a fully human IgG1 DNA/histone-binding antibody that binds to necrotic regions (which are frequently found in tumors), and therefore directs IL-12 to tumors⁷⁶. NHS-IL12 has been evaluated as a single agent⁷⁷ and in combination with avelumab (a PD-L1 inhibitor)⁷⁸ in multiple cancers including RCC, showing a tolerable safety profile (0–20% grade 3–4 treatment related adverse events) and anti-tumor activity in ICI-refractory patients when given in combination with avelumab. NHS-IL12 is currently being evaluated in combination with other agents in clinical trials, including patients with RCC.

Beyond pro-inflammatory cytokines, inhibitors of anti-inflammatory cytokines or cytokine receptors can decrease immunosuppressive mediators and favorably alter the tumor microenvironment. X4P-001, a CXCR4 inhibitor that results in blockade of the CXCR4/

CXCL12 axis, can increase T cell infiltration and effector function within the tumor microenvironment⁷⁹. X4P-001 was evaluated in combination with axitinib in heavily pretreated patients (48% with ≥ 3 prior lines, including 48% receiving prior ICI and 91% receiving prior VEGF TKI), and had an encouraging ORR of 29%. Other agents that also inhibit anti-inflammatory cytokines or cytokine receptors are under investigation in RCC.

3) Targeting immune metabolic pathways—The tryptophan–kynurenine–aryl hydrocarbon receptor pathway is a potential immune metabolic target in patients with RCC. Tryptophan is an essential amino acid that can be metabolized by three rate-limiting enzymes; IDO (indoleamine 2,3-dioxygenase) 1 or 2 and TDO (tryptophan 2,3-dioxygenase)⁸⁰. This metabolization leads to T cell cycle arrest and anergy because of the decrease in tryptophan levels within T cells and the increase in kynurenine levels that bind to the aryl hydrocarbon receptor^{81–83}. Across tumor types, *IDO1* expression correlates with *PDCD1* (PD-1) expression⁸², and circulating kynurenine levels increase upon PD-1 inhibition⁸⁴. Patients with the greatest levels of increase of the kynurenine-to-tryptophan ratio (a metric indicating conversion of tryptophan to kynurenine) have the worst outcomes on ICI⁸⁴.

Clinical development of agents targeting this pathway was initially spurred by encouraging clinical data in phase I and II trials in multiple tumors, including in RCC. In fact, the combination of a selective IDO1 inhibitor (epacadostat) with pembrolizumab led to an ORR of 47% in previously untreated patients with RCC⁸⁵. These encouraging results led to further clinical development with the launch of the phase III ECHO-302 clinical trial in RCC (pembrolizumab plus epacadostat vs. pazopanib or sunitinib). However, this trial was terminated before its conclusion ([NCT03260894](#)) following the negative results of the ECHO-301 phase III trial in melanoma (pembrolizumab plus epacadostat vs. pembrolizumab plus placebo)⁸⁶. Despite this clinical setback, agents targeting this pathway continue to be developed in RCC because of the strong biological rationale for targeting the tryptophan–kynurenine–aryl hydrocarbon receptor pathway (particularly in the anti-PD-1 resistant population) in this disease. These include alternative epacadostat-based combinations, more potent IDO inhibitors such as linrodostat, and the long-acting IDO1 inhibitor KHK2455⁸².

The CD39–CD73–adenosine 2A receptor (A2AR) pathway is another immune metabolic pathway that could be targeted in patients with RCC. Extracellular adenosine triphosphate (ATP) can be generated as a result of hypoxia, tissue injury, and inflammation, and represents a signal of immunogenic cell death, leading to immune cell chemotaxis and stimulation of APCs⁸⁷. Extracellular ATP can be metabolized in the tumor microenvironment by CD39 and CD73 into adenosine, which can then bind multiple receptors, including A2AR⁸⁸. Binding of adenosine to A2AR (expressed on DCs, macrophages, T and NK cells) inhibits the immune response⁸⁹. The expression of CD39 and CD73 correlates with poor prognosis across multiple cancer types, including RCC^{90,91}, and this relationship is thought to be driven not only by the enzymatic activity of these proteins (increasing production of adenosine) but also by their direct pro-oncogenic, non-catalytic activity that promotes angiogenesis and tumor invasion⁹⁰. Moreover, increased adenosine associates with poor clinical outcomes in patients with RCC treated with nivolumab⁹².

ADORA2A (A2AR) and *NT5E* (CD73) are also highly expressed in RCC compared to other tumors⁹³ and a subset of inflamed, but CD8⁺ T cell-low, RCC tumors appears to have the highest expression of *ADORA2A* and *NT5E*, suggesting that the adenosine pathway could be playing a crucial role in limiting CD8⁺ T cell infiltration in a subset of RCC tumors³³.

Clinically, ciforadenant (CPI-444), an A2AR inhibitor, demonstrated single agent anti-tumor activity in the treatment of RCC with a disease control rate at 6 months of 17% versus 39% when combined with atezolizumab (a PD-L1 inhibitor)⁹³. The patients who seemed to benefit most from ciforadenant were those that had a high adenosine-related RNA expression signature and demonstrated increased CD8⁺ T cell infiltration in tumors after treatment with ciforadenant⁹³. Multiple other agents targeting the adenosine pathway are currently being evaluated in clinical trials of patients with RCC, including agents targeting A2AR, CD39, and CD73.

4) Immunotherapy-based combinations beyond ICI / anti-VEGF for RCC—

Combination of ICI with VEGF TKI therapy are now established first-line treatment for advanced RCC^{2,3}, with anti-VEGF agents providing well documented effects on T cell trafficking and infiltration into tumors, and direct immunomodulatory effects on tumor-infiltrating T lymphocytes (TILs) and myeloid cell populations⁹⁴. While this combination has been transformative for many patients with metastatic RCC, unfortunately the majority of patients do not experience durable responses. Moving forward, combination strategies should attempt to improve the depth of response (which has been associated with durable long-term survival)⁹⁵ and directly address emerging mechanisms of PD-1 blockade resistance in RCC^{16,17}.

The rational design of novel immunotherapy-based combinations for RCC will require a framework for effectively choosing therapeutic agents. First, the combination of two more agents should be non-antagonistic and ideally have a favorable interaction (i.e. additive or even synergistic activity). There can still be an observed benefit to combinations of independently acting drugs because of patient-to-patient variability in response⁹⁶. However, two drugs without any interaction could potentially be given sequentially with similar efficacy and decreased toxicity.

Second, classic principles of drug combinations, learned from cytotoxic chemotherapy and other targeted therapies⁹⁷, likely still apply. For improved efficacy, combinations should ideally target multiple immunologic pathways (or different steps in the same pathway). Emerging evidence also suggest that complementary strategies to reduce tumor burden and increase immunogenicity may work well to improve ICI efficacy, by releasing tumor antigens (and other pro-immunogenic molecules) and decreasing tumor-related immunosuppressive effects⁹⁸. In RCC, initial studies of radiation therapy in combination with ICI have shown tolerability and anti-tumor activity. It is unclear if these findings represent a true abscopal effect versus disease control from one or the other (or both) of the treatment modalities.^{99,100} Other combination approaches could include therapies that decrease tumor burden independently of ICI, such as inhibitors of HIF2 α ^{101,102}. For tolerability, the drugs in combination should ideally have non-overlapping toxicity, and care should be taken to minimize the possibility of synergistic toxicity (for instance, the

seemingly synergistic rates of ICI-induced hepatitis seen with the combination of nivolumab plus ipilimumab¹⁰³).

Finally, a conceptual framework for rationally selecting agents to target multiple steps in the cancer-immunity cycle is critical¹¹. In Table 1 and S1, we outline examples of recent and ongoing immunotherapy clinical trials in RCC, highlighting which steps in the RCC immunity cycle (Figure 1) are targeted by these combination strategies.

Section 3. The Potential for Precision Immune Therapy.

Although ICI has transformed the treatment landscape for advanced RCC, it is still, fundamentally, a non-specific approach that aims to broadly re-invigorate the immune response with the hope that such activity will have beneficial anti-tumor effects. Current immunotherapy approaches do not explicitly make use of a key feature of the adaptive immune response – target specificity – and may therefore (i) be less efficacious (as reinvigorated immune cells might still fail to specifically target tumor cells), and (ii) lead to more immune-related adverse-effects (from an activated immune response that may also be directed against normal tissue). More recently, advances in genomics, T cell engineering, and fundamental immunology have now enabled precision medicine approaches to immune therapy¹⁰⁴. Moving forward, precision immunotherapy aims to directly target tumor antigens, utilizing “off-the-shelf” and personalized approaches (Figure 3). The goal of this approach is to effectively “steer” the immune system towards the tumor, rather than simply “releasing the brakes”¹⁰⁵.

A critical step in precision immunotherapy will be choosing the right RCC target antigens. Broadly, target antigens can include cell surface molecules that are uniquely (or highly) expressed on RCC cells. This approach has been particularly successful in hematologic malignancies, with chimeric antigen receptor (CAR) T cell therapies targeting CD19 in lymphoid malignancies and BCMA in multiple myeloma^{106,107}. An alternative approach involves targeting peptides that are derived from proteins processed within a cell and presented on HLA class I molecules, which can be recognized by cognate T cell receptors (TCRs). This HLA-peptide-TCR interaction lies at the heart of T cell-mediated antigen-specific immunity, and therapeutic approaches could therefore leverage this native immune process for anti-tumor efficacy.

1) Targeting surface antigens—Overexpressed proteins on the surface of tumor cells have been effectively targeted for decades, first with monoclonal antibodies (initially against HER2 in a subtype of breast cancer¹⁰⁸) and more recently with CAR T cell therapy. In this section, we review prior and ongoing attempts to target surface antigens in RCC.

i. CAR T cells: Although CAR T cell therapy has shown tremendous efficacy within hematologic malignancies, there has unfortunately been much less success for solid tumors. For CAR T cells to be successful in solid tumors, they must (i) be designed against suitable target antigens (highly expressed on tumor cells but not on normal cells), (ii) hone to and successfully infiltrate the tumor, and (iii) persist and carry out effector function in an immunosuppressive microenvironment¹⁰⁹. These requirements may explain why CAR T cell therapy has had limited success in RCC.

The first CAR T cell study in RCC used a single-chain variable fragment (scFv) from the G250 monoclonal antibody (girentuximab) targeted against carbonic anhydrase IX (CAIX), a hypoxia-inducible factor (HIF)-regulated gene¹¹⁰ that is frequently upregulated in ccRCC¹¹¹. Patients did not receive any lymphodepleting chemotherapy, and were administered up to 10 infusions of the first generation anti-CAIX CAR T cells (with CD3 ζ , but lacking CD28 or 4-1BB co-stimulatory domains) together with IL-2^{112–114}. In the first cohort, two of three patients developed dose-limiting liver toxicity (grade 3–4) due to on-target effects on CAIX-expressing bile duct epithelial cells. Similar high-grade liver toxicity was observed in two of five patients in a second cohort. Interestingly, in a third cohort of four patients, subjects were pre-treated with low-dose of the G250 monoclonal antibody prior to CAR T cell infusion with the goal of “blocking” target antigen on bile duct cells, and consequently no high grade liver toxicity (grade 2) was observed in these patients. As was common with first generation constructs, these CAR T cells had very limited persistence (typically less than 30 days), and no clinical responses were observed. Similarly, a clinical trial of VEGFR2-targeting CAR T cells (NCT01218867) was terminated because of no objective response. Multiple alternative targets are currently being explored in RCC, including CD70¹¹⁵ (NCT02830724), c-MET (NCT03638206), AXL, and ROR2 (NCT03393936).

Moving forward, numerous strategies have been devised to attempt to address the specific challenges in solid tumors, including CAR T cells engineered with logic gates to target multiple antigens¹¹⁶, with drug-inducible “suicide switches” for safety¹¹⁷, with CAR construct or genetic alterations that improve persistence¹¹⁸, or with the ability to produce immunomodulatory molecules to “armor” themselves against the immunosuppressive microenvironment¹¹⁹. Within RCC, these strategies include newer generation CAR constructs that include co-stimulatory domains¹²⁰ and are specific for novel targets like HLA-G¹²¹, dual-targeting CAR T cells specific to both CAIX and CD70¹²², and CAR T cells engineered to secrete an anti-PD-L1 antibody¹²³, which are all in pre-clinical development.

ii. Antibody-based therapies: Monoclonal antibodies directed against tumor surface antigens have shown efficacy across a broad array of hematologic and solid tumors, typically causing direct cytotoxicity or leading to immune-mediated destruction of tumor cells via antibody-dependent cytotoxicity via NK cells (ADCC), antibody-dependent phagocytosis by macrophages (ADP), or complement-dependent cytotoxicity (CDC)¹²⁴. Unfortunately, this approach has not yet been successful in RCC. In the largest study to date, a phase III trial of the anti-CAIX antibody girentuximab as adjuvant therapy for high-risk resected ccRCC failed to improve disease-free (DFS) or overall survival (OS) compared to placebo¹²⁵. Of note, in tumors with the highest CAIX expression score (200 or greater, with scoring based on intensity of CAIX staining and number of positive cells), patients treated with girentuximab trended towards an improved DFS compared to placebo (hazard ratio, 0.75; 65% confidence interval, 0.55 – 1.04, $P = 0.08$).

Monoclonal antibodies can also be used to deliver high doses of a cytotoxic “payload” directly to tumor cells by conjugating the antibodies to either radionuclides or to chemotherapeutic agents¹²⁶. A small phase II study of girentuximab conjugated to the

radionuclide lutetium-177 in patients with previously treated metastatic ccRCC did have modest efficacy (57% of patients with SD and 7% of patients with partial response [PR]), but most patients experienced high grade myelotoxicity¹²⁷.

Antibody-drug conjugates (ADCs) have shown efficacy in hematologic malignancies, in breast cancer, and in bladder cancer¹²⁸. A phase I trial of an ADC targeting CD70 (SGN-CD70A) showed modest activity in heavily pre-treated patients with metastatic ccRCC (median of 4 lines of prior therapy), with 13 of 18 (72%) patients experiencing SD, and 1 patient (6%) achieving a PR¹²⁹. Notably, high CD70 expression was not an inclusion criterion for this trial. Similarly, a phase I trial of CDX-014, an ADC targeting TIM-1 (also known as KIM-1), showed some anti-tumor activity in metastatic RCC, with a clinical benefit rate (CR, PR, or SD greater than 6 months) of 31%¹³⁰. Intriguingly, the two patients with the highest TIM-1 expression on tumor cells had the most tumor shrinkage. Finally, an ADC targeting ENPP3 (AGS-16C3F) showed promising activity in a phase I trial of patients with previously treated metastatic RCC¹³¹. While high grade keratopathy was seen at higher doses, at a lower dose level of 1.8mg/kg, 3 of 13 (23%) patients experienced durable PR lasting at least 100 weeks. A randomized phase II study of AGS-16C3F versus axitinib has finished accrual, with results pending. Overall, these studies demonstrate the feasibility of antibody-conjugates in RCC, but also highlight the need to carefully select patients who express high levels of target surface antigen for these studies.

2) Targeting HLA-restricted antigens—Although RCC cells express some antigens on the cell surface, many potential tumor-specific protein targets may reside **inside** the cell and are not amenable to CAR T cell or antibody-based therapies. These tumor-specific proteins are typically cleaved into small peptides and presented by HLA class I molecules on the surface of tumor cells, enabling recognition by the cognate TCR on T cells. These HLA-restricted antigens (i.e. tumor peptides that can only be presented on a specific HLA allele) may arise from overexpressed proteins relative to normal tissue (tumor-associated antigens), expression of proteins typically only expressed in germ cells (cancer-testis antigens), novel peptides derived from nonsynonymous somatic mutations (i.e. neoantigens), or aberrant expression of ERVs. HLA-restricted antigens may be expressed on many patients that share the same HLA type (“public” antigens), or many be completely unique to an individual tumor, requiring personalized therapy (“personal” antigens). Here, we review previous and ongoing efforts to target HLA-restricted antigens using adoptive cell therapy (ACT) and vaccination approaches.

i. T cell therapies: T cells are primary mediators of anti-tumor immunity, and therefore administration of both endogenous and genetically altered T cells have been used in cancer therapy. Early ACT involved infusion of TILs that were *ex vivo* expanded and administered with IL-2 in patients with metastatic melanoma¹³². These autologous TILs, administered with IL-2 and after lymphodepleting chemotherapy, were able to induce durable complete regressions (lasting longer than 3 years) in 19 of 93 (20%) heavily pre-treated patients with advanced metastatic melanoma^{133,134}. While initial studies of TIL therapy were performed only at highly specialized facilities capable of cell manipulation, more recently, the development of centralized, commercial good manufacturing practice (GMP) facilities has

enabled TIL therapy to be delivered more broadly. Lifileucel (LN-144), an autologous TIL therapy administered with IL-2 following lymphodepletion, showed an ORR of 36.4% in patients with metastatic melanoma that had progressed on prior PD-1 blockade¹³⁵. While an initial study of TIL therapy (with IL-2 but without lymphodepletion) in RCC did demonstrate some benefit, with 5 of 55 (9.1%) of treated patients achieving a CR¹³⁶, later studies revealed difficulties in expanding TILs from RCC tumors. In a phase III study in RCC, TILs could not be expanded to sufficient quantities in 33 of 72 patients (41%)¹³⁷. Overall, there was no significant improvement in response with TIL therapy compared to IL-2 alone. More recently, the development of optimized “rapid expansion protocols” for TILs have enabled successful T cell expansion from more than 90% of RCC tumor specimens, but although these TILs demonstrated tumor-reactivity, the immune response was generally weaker than in melanoma¹³⁸. Overall, this therapy needs further development to optimize the expansion, strength, and anti-tumor specificity of TILs in RCC.

Whereas TIL therapy relies on the ability of endogenous T cells to recognize tumor antigens, engineered TCR approaches involve transduction of patient T cells with a specific TCR known to recognize a specific antigen (in the context of a specific HLA allele), with the goal of more directly targeting the infused T cells against the tumor. This therapeutic strategy is just beginning to emerge in RCC, stemming from thorough immunologic analysis of metastatic RCC patients treated with allogeneic HSCT¹³⁹. Using patient-derived primary RCC cell lines, Childs, Takahashi and colleagues were able to confirm the presence of RCC-reactive CD8⁺ T cells in 4 recipients of allogeneic HSCT¹⁴⁰. For one patient with a durable PR lasting over 3 years, they were able to clone the RCC-reactive TCR and identify the target antigen, which was an HLA-A11—restricted peptide derived from an aberrantly expressed ERV, HERV-E (also known as ERVE-4). Subsequent work established HERV-E as selectively expressed in ccRCC^{19,141}, and a phase I trial of autologous T cells transduced with the TCR against HERV-E in HLA-A11:01 patients with metastatic ccRCC is ongoing ([NCT03354390](#)).

While this work is promising, prior experience in other tumor types suggests that caution is still needed with this approach. First, the selection of truly tumor-specific targets is critical. An engineered TCR therapy against the cancer-testis antigen MAGE-A3 (aberrantly expressed in melanoma) led to severe neurotoxicity and death in two patients, attributed to the use of a TCR that also recognized MAGE-A12, which was subsequently shown to be expressed at low levels within the human brain¹⁴². Second, any attempt to alter the native TCR to enhance affinity must be done with careful consideration of off-target effects. In another study of engineered TCR therapy against an HLA-A01—restricted peptide derived from MAGE-A3, investigators used site-directed mutagenesis to enhance the affinity of a native TCR, with the goal of improving anti-tumor efficacy¹⁴³. Unfortunately, the first two patients on study developed fatal cardiogenic shock, despite the absence of MAGE-A3 expression in cardiomyocytes. Subsequent investigation demonstrated that the affinity-enhanced TCR also recognized a peptide derived from the protein titin, which is widely expressed in the human heart. These unfortunate situations should by no means dissuade future investigation into this promising therapeutic modality, but rather suggest that substantial caution is required to select truly tumor-specific antigens, and to robustly screen

for off-target antigen recognition (now enabled by the development of methods for systematically screening TCRs against libraries of HLA-presented peptides¹⁴⁴).

ii. Therapeutic vaccines: Therapeutic cancer vaccines have the potential to direct the immune system against tumor-specific targets, with the goal of inducing durable immune responses (by establishing memory against tumor antigens) and minimizing off-target immune-related toxicity. An initial vaccine effort in RCC attempted to target “tumor-associated peptides” that are overexpressed in (but not truly specific for) ccRCC tumor cells. Using a combination of gene expression profiling, mass spectrometry of HLA ligands, and T cell reactivity assays, investigators identified nine HLA-A02—restricted tumor-associated antigens (and one HLA-DR—restricted antigen)¹⁴⁵. A peptide vaccine formulation of these 10 peptides (termed IMA901), together with GM-CSF (to improve T cell responses) and following a dose of cyclophosphamide (to deplete regulatory T cells) was evaluated in early phase clinical trials and showed that most patients had a T cell response to at least one peptide^{145,146}. However, in the pivotal phase III IMPRINT trial, IMA901 combined with sunitinib did not demonstrate any progression-free survival (PFS) or OS benefit compared to sunitinib alone¹⁴⁷. Of note, the magnitudes of CD8⁺ T cell responses to vaccine peptides were three-fold lower than in prior studies, suggesting that sunitinib could have negatively impacted immune responses (potentially by impairing T cell priming). A subsequent study incorporated HLA class II-restricted peptides into a multi-epitope vaccine, including a CAIX-derived peptide¹⁴⁸. The vaccine was well tolerated in a phase I/II trial (as adjuvant therapy following metastasectomy, and was associated with HLA class I and II peptide response *in vitro* and favorable overall survival (compared to a similar but independent cohort of patients on observation. This work highlights the potential for also targeting HLA class II epitopes.

Rather than pre-selecting antigenic targets, an alternative personalized vaccine approach attempts to incorporate all potential antigens from an individual own patient’s tumors. In the phase III ADAPT trial, amplified tumor RNA was co-electroporated (together with CD40L RNA) into autologous monocyte-derived DCs (called rocapuldencel-T or AGS-003) and administered as a therapeutic vaccine in combination with sunitinib (vs. sunitinib alone). Unfortunately, there was no significant difference in PFS or OS between the two arms. This result was at least partially confounded by an unusually long median OS with sunitinib (32.4 months), and the concurrent development of multiple effective salvage therapies during the course of the trial. Encouragingly, an association between immune response and improved OS was noted. This DC vaccine (now known as CMN-001) is scheduled to be tested in combination with nivolumab and ipilimumab in an upcoming trial ([NCT04203901](#)).

A novel approach, enabled by the development of next-generation sequencing technologies and improved HLA class I epitope prediction¹⁴⁹, is the targeting of tumor neoantigens. By sequencing an individual’s tumor and predicting which mutations generate peptides likely to bind that patient’s HLA class I alleles, a personalized neoantigen vaccine can be generated. Prior studies in melanoma and glioblastoma multiforme demonstrated that the approach is safe, feasible, and capable of generating neoantigen-specific immune responses^{150–153}. This promising therapeutic strategy is currently being investigated in RCC in the adjuvant setting in combination with locally administered ipilimumab in an RCC-specific trial,

[NCT02950766](#). Within the metastatic setting, preliminary reports from a phase Ib study of the RNA-based neoantigen vaccine RO7198457 plus atezolizumab demonstrated an ORR of 22% in an ICI-naïve cohort¹⁵⁴. While immune monitoring results for RCC were not presented, encouragingly the vaccine elicited strong CD8⁺ T cell responses against a neoantigen in a patient with triple-negative breast cancer.

3) Challenges and opportunities in precision immunotherapy—While precision immunotherapy carries tremendous promise in RCC, there has unfortunately been limited success thus far. Moving forward, it will be critical to (i) choose the right combination therapies, and (ii) pick the right tumor antigens. Both the IMPRINT and ADAPT studies utilized sunitinib in combination with their respective vaccine, with investigators considering whether the VEGF TKI may have had an adverse effect on T cell priming¹⁴⁷. While it is tempting to combine these precision therapies with PD-1 blockade, recent studies have demonstrated how the timing of PD-1 inhibition can negatively impact T cell priming¹⁵⁵ and the ability to form CD8⁺ T cell memory¹⁵⁶. Therefore, the agents for and timing of combination therapies will have to be carefully selected in future studies.

While a limited number of specific RCC antigens have been targeted in prior studies, a systematic discovery effort for antigenic targets in RCC is needed to expand the list of potential targets in this disease. The rapid development of immunogenomic tools, incorporating mass spectrometry and single cell sequencing technologies, will now enable a more systematic discovery of RCC tumor antigens. Such efforts could broadly define the repertoire of RCC surface antigens (the *surfaceome*) that could serve as targets for CAR T cells or antibody-based strategies. For HLA-restricted antigens, tools for antigen prediction and detection¹⁴⁹ allow for robust identification of potential T cell targets, and when coupled with workflows for TCR reconstruction and specificity testing¹⁵⁷, enables the direct linking of TCRs with target antigen. The goal of these efforts should be the rapid identification of tumor-specific antigen targets in RCC and translation to novel, antigen-directed therapies.

Conclusions

The field of immunotherapy for RCC is rapidly expanding and remains extraordinarily promising. Conventional ICI and ICI-based combinations have dramatically improved clinical outcomes for patients with RCC. To truly move the field forward with the goal of providing durable clinical benefit for the *majority* of patients with RCC, rational combinations of novel immune therapy approaches beyond PD-L1 and CTLA-4 will be needed. Beyond standard strategies for combining therapeutic agents (see **Immunotherapy-based combinations beyond ICI / anti-VEGF for RCC**), we should prioritize agents address unmet clinical needs, by either improving the depth of response in the first-line setting, demonstrating efficacy in the post-PD-1 setting, or enabling prolonged periods of treatment-free survival.

“Deep” responses, defined as CR or PR with a high degree of tumor shrinkage (variably defined as at least 60–80%, depending on the study) is historically associated with a better prognosis in advanced RCC¹⁵⁸. Of critical importance, with anti-PD-1 therapies, depth of response has been associated with long-term survival in metastatic RCC. In pivotal phase III

trials of anti-PD-1—based combination therapies, the vast majority of patients who had CR (or, in some studies deep PR) experienced long-term survival^{95,159–161}. Therefore, agents that cause substantial tumor shrinkage in early clinical studies are prime candidates for investigation in combination with first-line PD-1 blockade.

Unfortunately, the majority of RCC tumors do ultimately develop resistance to first-line anti-ICI-based therapy. While there is emerging evidence that “re-challenge” with anti-PD-1—based therapies likely provide some benefit^{162,163}, most patients do not receive durable benefit from salvage ICI therapy in this setting. Therefore, agents that lead to response and improve survival in this post-PD-1 setting should be prioritized.

Finally, for patients living with metastatic disease, it is important to also try to improve the *quality* and not just quantity of life. Recently, investigators demonstrated in melanoma that PD-1 blockade can lead to prolonged treatment-free survival (TFS), a period of time free from treatment or treatment-related adverse effects¹⁶⁴. TFS is now being actively explored as an endpoint in RCC¹⁶⁵ and should be included in clinical trials evaluating novel immunotherapies.

In the coming years, by understanding the role of potential therapeutic agents in the context of the RCC-specific cancer-immunity cycle, by systematically identifying antigenic targets in RCC, and by focusing on areas of unmet clinical need, physicians and scientists can develop more effective and more precise immunotherapies. These approaches will help to overcome primary and acquired resistance to ICI, and ultimately improve the lives of patients with RCC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. McKay RR, Boss D & Choueiri TK Evolving Systemic Treatment Landscape for Patients With Advanced Renal Cell Carcinoma. (2018). doi:10.1200/JCO.2018.79.0253
2. Motzer RJ et al. Avelumab plus Axitinib versus Sunitinib for Advanced Renal-Cell Carcinoma. *N. Engl. J. Med* 380, 1103–1115 (2019). [PubMed: 30779531]
3. Rini BI et al. Pembrolizumab plus Axitinib versus Sunitinib for Advanced Renal-Cell Carcinoma. *N. Engl. J. Med* NEJMoa1816714 (2019). doi:10.1056/NEJMoa1816714
4. Motzer RJ et al. Nivolumab plus Ipilimumab versus Sunitinib in Advanced Renal-Cell Carcinoma. *N. Engl. J. Med* 378, 1277–1290 (2018). [PubMed: 29562145]
5. McDermott DF et al. The High-Dose Aldesleukin “Select” Trial: A Trial to Prospectively Validate Predictive Models of Response to Treatment in Patients with Metastatic Renal Cell Carcinoma. *Clin. Cancer Res.* 21, 561–568 (2015). [PubMed: 25424850]
6. Maruschke M Spontaneous regression of renal cell carcinoma: Reality or myth? *World J. Clin. Urol.* 3, 201 (2014).
7. Janiszewska AD, Poletajew S & Wasiuty ski A Spontaneous regression of renal cell carcinoma. *Wspolczesna Onkologia* 17, 123–127 (2013). [PubMed: 23788977]
8. Takahashi Y et al. Regression of human kidney cancer following allogeneic stem cell transplantation is associated with recognition of an HERV-E antigen by T cells (Journal of Clinical Investigation (2008) 118, (1099–1109) DOI: 10.1172/JCI34409). *J. Clin. Invest.* 118, 1584 (2008).
9. Signoretti S, Flaifel A, Chen YB & Reuter VE Renal cell carcinoma in the era of precision medicine: From molecular pathology to tissue-based biomarkers. *J. Clin. Oncol.* 36, 3553–3559 (2018).
10. Albiges L, Flippot R, Rioux-Leclercq N & Choueiri TK Non-clear cell renal cell carcinomas: From shadow to light. *J. Clin. Oncol.* 36, 3624–3631 (2018).
11. Chen DS & Mellman I Oncology Meets Immunology: The Cancer-Immunity Cycle. *Immunity* 39, 1–10 (2013). [PubMed: 23890059]
12. Vigneron N Human Tumor Antigens and Cancer Immunotherapy. (2015). doi:10.1155/2015/948501
13. Samstein RM et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nature Genetics* 51, 202–206 (2019). [PubMed: 30643254]
14. FDA approves pembrolizumab for adults and children with TMB-H solid tumors | FDA. Available at: <https://www.fda.gov/drugs/drug-approvals-and-databases/fda-approves-pembrolizumab-adults-and-children-tmb-h-solid-tumors>. (Accessed: 18th July 2020)
15. Yarchoan M, Hopkins A & Jaffee EM Tumor mutational burden and response rate to PD-1 inhibition. *New England Journal of Medicine* 377, 2500–2501 (2017).
16. McDermott DF et al. Clinical activity and molecular correlates of response to atezolizumab alone or in combination with bevacizumab versus sunitinib in renal cell carcinoma. *Nat. Med* 24, 749–757 (2018). [PubMed: 29867230]
17. Braun DA et al. Interplay of somatic alterations and immune infiltration modulates response to PD-1 blockade in advanced clear cell renal cell carcinoma. *Nat. Med* 26, 909–918 (2020). [PubMed: 32472114]
18. Turajlic S et al. Insertion-and-deletion-derived tumour-specific neoantigens and the immunogenic phenotype: a pan-cancer analysis. *Lancet Oncol.* 18, 1009–1021 (2017). [PubMed: 28694034]
19. Rooney MS, Shukla SA, Wu CJ, Getz G & Hacohen N Molecular and genetic properties of tumors associated with local immune cytolytic activity. *Cell* 160, 48–61 (2015). [PubMed: 25594174]
20. SOGA N et al. Limited expression of cancer-testis antigens in renal cell carcinoma patients. *Mol. Clin. Oncol* 1, 326–330 (2013). [PubMed: 24649170]

21. Attermann AS, Bjerregaard AM, Saini SK, Grønbæk K & Hadrup SR Human endogenous retroviruses and their implication for immunotherapeutics of cancer. *Annals of Oncology* 29, 2183–2191 (2018). [PubMed: 30239576]
22. Smith CC et al. Endogenous retroviral signatures predict immunotherapy response in clear cell renal cell carcinoma. *J. Clin. Invest.* 128, 4804–4820 (2018). [PubMed: 30137025]
23. Giraldo NA et al. Orchestration and prognostic significance of immune checkpoints in the microenvironment of primary and metastatic renal cell cancer. *Clin. Cancer Res.* 21, 3031–3040 (2015). [PubMed: 25688160]
24. Jansen CS et al. An intra-tumoral niche maintains and differentiates stem-like CD8 T cells. *Nature* 576, 465–470 (2019). [PubMed: 31827286]
25. Ricketts CJ et al. The Cancer Genome Atlas Comprehensive Molecular Characterization of Renal Cell Carcinoma. *Cell Rep.* 23, 313–326.e5 (2018). [PubMed: 29617669]
26. Thorsson V et al. The Immune Landscape of Cancer. *Immunity* 48, 812–830.e14 (2018). [PubMed: 29628290]
27. Creighton CJ et al. Comprehensive molecular characterization of clear cell renal cell carcinoma. *Nature* 499, 43–49 (2013). [PubMed: 23792563]
28. Iliopoulos O, Levy AP, Jiang C, Kaelin WG & Goldberg MA Negative regulation of hypoxia-inducible genes by the von Hippel-Lindau protein. *Proc. Natl. Acad. Sci. U. S. A.* 93, 10595–10599 (1996). [PubMed: 8855223]
29. Jubb AM et al. Expression of vascular endothelial growth factor, hypoxia inducible factor 1 α , and carbonic anhydrase IX in human tumours. *J. Clin. Pathol* 57, 504–512 (2004). [PubMed: 15113858]
30. Khan KA & Kerbel RS Improving immunotherapy outcomes with anti-angiogenic treatments and vice versa. *Nature Reviews Clinical Oncology* 15, 310–324 (2018).
31. enbabao lu Y et al. Tumor immune microenvironment characterization in clear cell renal cell carcinoma identifies prognostic and immunotherapeutically relevant messenger RNA signatures. *Genome Biol.* 17, 231 (2016). [PubMed: 27855702]
32. Wang T et al. An empirical approach leveraging tumorgrafts to dissect the tumor microenvironment in renal cell carcinoma identifies missing link to prognostic inflammatory factors. *Cancer Discov.* 8, 1142–1155 (2018). [PubMed: 29884728]
33. Clark DJ et al. Integrated Proteogenomic Characterization of Clear Cell Renal Cell Carcinoma Resource Integrated Proteogenomic Characterization of Clear Cell Renal Cell Carcinoma. *Cell* 179, 964–983 (2019). [PubMed: 31675502]
34. Yuen KC et al. High systemic and tumor-associated IL8 correlates with reduced clinical benefit of PD-L1 blockade. *Nat. Med* (2020).
35. Schalper KA et al. Elevated serum interleukin 8 is associated with enhanced intratumor neutrophils and reduced clinical benefit with immune checkpoint inhibitors. *Nat. Med* (2020).
36. Bakouny Z & Choueiri TK IL-8 and cancer prognosis on immunotherapy. *Nat. Med* 26, 650–651 (2020). [PubMed: 32405061]
37. Bakouny Z et al. Integrative Molecular Characterization of Sarcomatoid and Rhabdoid Renal Cell Carcinoma Reveals Determinants of Poor Prognosis and Response to Immune Checkpoint Inhibitors. *bioRxiv* 2020.05.28.121806 (2020). doi:10.1101/2020.05.28.121806
38. Rini BI et al. Pembrolizumab (pembro) plus axitinib (axi) versus sunitinib as first-line therapy for metastatic renal cell carcinoma (mRCC): Outcomes in the combined IMDC intermediate/poor risk and sarcomatoid subgroups of the phase 3 KEYNOTE-426 study. *J. Clin. Oncol* 37, 4500–4500 (2019).
39. McDermott DF et al. CheckMate 214 post-hoc analyses of nivolumab plus ipilimumab or sunitinib in IMDC intermediate/poor-risk patients with previously untreated advanced renal cell carcinoma with sarcomatoid features. *J. Clin. Oncol* 37, 4513–4513 (2019).
40. Choueiri TK et al. Efficacy and biomarker analysis of patients (pts) with advanced renal cell carcinoma (aRCC) with sarcomatoid histology (sRCC): Subgroup analysis from the phase III JAVELIN renal 101 trial of first-line avelumab plus axitinib (A + Ax) vs sunitinib (S). *Ann. Oncol* 30, v361 (2019).

41. Rini BI et al. Atezolizumab plus bevacizumab versus sunitinib in patients with previously untreated metastatic renal cell carcinoma (IMmotion151): a multicentre, open-label, phase 3, randomised controlled trial. *Lancet* 393, 2404–2415 (2019). [PubMed: 31079938]
42. Rini BI et al. Atezolizumab (atezo) + bevacizumab (bev) versus sunitinib (sun) in pts with untreated metastatic renal cell carcinoma (mRCC) and sarcomatoid (sarc) histology: IMmotion151 subgroup analysis. *J. Clin. Oncol* 37, 4512–4512 (2019).
43. Fridman WH, Zitvogel L, Sautès-Fridman C & Kroemer G The immune contexture in cancer prognosis and treatment. *Nature Reviews Clinical Oncology* 14, 717–734 (2017).
44. Giraldo NA et al. Tumor-infiltrating and peripheral blood T-cell immunophenotypes predict early relapse in localized clear cell renal cell carcinoma. *Clin. Cancer Res.* 23, 4416–4428 (2017). [PubMed: 28213366]
45. Chevrier S et al. An Immune Atlas of Clear Cell Renal Cell Carcinoma. *Cell* 169, 736–749.e18 (2017). [PubMed: 28475899]
46. Pignon JC et al. Irrecist for the evaluation of candidate biomarkers of response to nivolumab in metastatic clear cell renal cell carcinoma: Analysis of a phase II prospective clinical trial. *Clin. Cancer Res.* 25, 2174–2184 (2019). [PubMed: 30670497]
47. Siska PJ et al. Mitochondrial dysregulation and glycolytic insufficiency functionally impair CD8 T cells infiltrating human renal cell carcinoma. *JCI insight* 2, (2017).
48. Beckermann K et al. Targeting metabolic dysregulation of T cells in kidney cancer. *J. Clin. Oncol* 38, 722–722 (2020).
49. Miao D et al. Genomic correlates of response to immune checkpoint therapies in clear cell renal cell carcinoma. 359, 801–806 (2018).
50. Hegde PS, Karanikas V & Evers S The where, the when, and the how of immune monitoring for cancer immunotherapies in the era of checkpoint inhibition. *Clin. Cancer Res.* 22, 1865–1874 (2016). [PubMed: 27084740]
51. Mariathasan S et al. TGF β attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature* 554, 544–548 (2018). [PubMed: 29443960]
52. Marhelava K, Pilch Z, Bajor M, Graczyk-Jarzynka A & Zagodzón R Targeting negative and positive immune checkpoints with monoclonal antibodies in therapy of cancer. *Cancers* 11, (2019).
53. Granier C et al. Tim-3 expression on tumor-infiltrating PD-1+CD8+ T cells correlates with poor clinical outcome in renal cell carcinoma. *Cancer Res.* 77, 1075–1082 (2017). [PubMed: 27872087]
54. Cai C et al. Tim-3 expression represents dysfunctional tumor infiltrating T cells in renal cell carcinoma. *World J Urol* 34, 561–567 (2016). [PubMed: 26253654]
55. Mach N et al. 202P Phase (Ph) II study of MBG453 1 spartalizumab in patients (pts) with non-small cell lung cancer (NSCLC) and melanoma pretreated with anti-PD-1/L1 therapy. 30, (2019).
56. Harding JJ et al. A phase Ia/Ib study of an anti-TIM-3 antibody (LY3321367) monotherapy or in combination with an anti-PD-L1 antibody (LY3300054): Interim safety, efficacy, and pharmacokinetic findings in advanced cancers. *J. Clin. Oncol* 37, 12–12 (2019). [PubMed: 30379624]
57. Workman CJ & Vignali DAA The CD4-related molecule, LAG-3 (CD223), regulates the expansion of activated T cells. *European Journal of Immunology* 33, 970–979 (2003). [PubMed: 12672063]
58. Andrae S, Buisson S & Triebel F MHC class II signal transduction in human dendritic cells induced by a natural ligand, the LAG-3 protein (CD223). *Blood* 102, 2130–2137 (2003). [PubMed: 12775570]
59. Zelba H et al. PD-1 and LAG-3 dominate checkpoint receptor-mediated T-cell inhibition in renal cell carcinoma. *Cancer Immunol. Res.* 7, 1891–1899 (2019). [PubMed: 31484656]
60. Brignone C, Escudier B, Grygar C, Marcu M & Triebel F A phase I pharmacokinetic and biological correlative study of IMP321, a novel MHC class II agonist, in patients with advanced renal cell carcinoma. *Clin. Cancer Res. An Off. J. Am. Assoc. Cancer Res.* 15, 6225–6231 (2009).
61. Phase Ia/Ib dose-escalation study of the anti-TIGIT antibody tiragolumab as a single agent and in combination with atezolizumab in patients with advanced solid tumors. Available at: <https://www.abstractsonline.com/pp8/#!/9045/presentation/11341>. (Accessed: 13th July 2020)
62. Rodriguez-Abreu D et al. Primary analysis of a randomized, double-blind, phase II study of the anti-TIGIT antibody tiragolumab (tira) plus atezolizumab (atezo) versus placebo plus atezo as

- first-line (1L) treatment in patients with PD-L1-selected NSCLC (CITYSCAPE). *J. Clin. Oncol* 38, 9503–9503 (2020).
63. Albacker A et al. 141PD CA-170, a first in class oral small molecule dual inhibitor of immune checkpoints PD-L1 and VISTA, demonstrates tumor growth inhibition in pre-clinical models and promotes T cell activation in Phase 1 study. *Abstr. B. 42nd ESMO Congr. (ESMO 2017)* 8–12 Sept. 2017, Madrid, Spain 28, v405–v406 (2017).
 64. Chester C, Sanmamed MF, Wang J & Melero I Immunotherapy targeting 4–1BB: Mechanistic rationale, clinical results, and future strategies. *Blood* 131, 49–57 (2018). [PubMed: 29118009]
 65. Palazón A et al. The HIF-1 α hypoxia response in tumor-infiltrating T lymphocytes induces functional CD137 (4–1BB) for immunotherapy. *Cancer Discov.* 2, 608–623 (2012). [PubMed: 22719018]
 66. Tolcher AW et al. Phase Ib Study of Utomilumab (PF-05082566), a 4–1BB/CD137 Agonist, in Combination with Pembrolizumab (MK-3475) in Patients with Advanced Solid Tumors. *Clin. Cancer Res. An Off. J. Am. Assoc. Cancer Res.* 23, 5349–5357 (2017).
 67. Alves Costa Silva C, Facchinetti F, Routy B & Derosa L New pathways in immune stimulation: Targeting OX40. *ESMO Open* 5, (2020).
 68. Glisson BS et al. Phase 1 study of MEDI0562, a humanized OX40 agonist monoclonal antibody (mAb), in adult patients (pts) with advanced solid tumors. *Ann. Oncol* 27, 1052PD (2016).
 69. Curti BD et al. OX40 is a potent immune-stimulating target in late-stage cancer patients. *Cancer Res.* 73, 7189–7198 (2013). [PubMed: 24177180]
 70. El-Khoueiry AB et al. The relationship of pharmacodynamics (PD) and pharmacokinetics (PK) to clinical outcomes in a phase I study of OX40 agonistic monoclonal antibody (mAb) PF-04518600 (PF-8600). *J. Clin. Oncol.* 35, 3027–3027 (2017).
 71. Safety, tolerability, and pharmacokinetics of the OX40 agonist ABBV-368 in patients with advanced solid tumors - *Annals of Oncology*. Available at: [https://www.annalsofoncology.org/article/S0923-7534\(19\)49606-3/fulltext](https://www.annalsofoncology.org/article/S0923-7534(19)49606-3/fulltext). (Accessed: 13th July 2020)
 72. Klapper JA et al. High-dose interleukin-2 for the treatment of metastatic renal cell carcinoma: A retrospective analysis of response and survival in patients treated in the Surgery Branch at the National Cancer Institute between 1986 and 2006. *Cancer* 113, 293–301 (2008). [PubMed: 18457330]
 73. Charych DH et al. NKTR-214, an Engineered Cytokine with Biased IL2 Receptor Binding, Increased Tumor Exposure, and Marked Efficacy in Mouse Tumor Models. *Clin. Cancer Res. An Off. J. Am. Assoc. Cancer Res.* 22, 680–690 (2016).
 74. Diab A et al. NKTR-214 (CD122-biased agonist) plus nivolumab in patients with advanced solid tumors: Preliminary phase 1/2 results of PIVOT. *J. Clin. Oncol.* 36, 3006–3006 (2018).
 75. Tugues S et al. New insights into IL-12-mediated tumor suppression. *Cell Death Differ.* 22, 237–246 (2015). [PubMed: 25190142]
 76. Fallon J et al. The immunocytokine NHS-IL12 as a potential cancer therapeutic. *Oncotarget* 5, 1869–1884 (2014). [PubMed: 24681847]
 77. Strauss J et al. First-in-Human Phase I Trial of a Tumor-Targeted Cytokine (NHS-IL12) in Subjects with Metastatic Solid Tumors. *Clin Cancer Res* (2018). doi:10.1158/1078-0432.CCR-18-1512
 78. Phase 1b, open-label, dose-escalation study of M9241 (NHS-IL12) plus avelumab in patients (pts) with advanced solid tumors | *OncologyPRO*. Available at: <https://oncologypro.esmo.org/meeting-resources/esmo-2019-congress/Phase-1b-open-label-dose-escalation-study-of-M9241-NHS-IL12-plus-avelumab-in-patients-pts-with-advanced-solid-tumors>. (Accessed: 14th July 2020)
 79. McDermott DF et al. Safety and Efficacy of the Oral CXCR4 Inhibitor X4P-001 + Axitinib in Advanced Renal Cell Carcinoma Patients: An Analysis of Subgroup Responses by Prior Treatment. *Ann. Oncol* 30, v475–v532 (2019).
 80. Badawy AA-B Kynurenine Pathway of Tryptophan Metabolism: Regulatory and Functional Aspects. *Int. J. Tryptophan Res.* 10, 1178646917691938 (2017). [PubMed: 28469468]
 81. Eleftheriadis T, Pissas G, Antoniadis G, Liakopoulos V & Stefanidis I Indoleamine 2,3-dioxygenase depletes tryptophan, activates general control non-repressible 2 kinase and down-regulates key enzymes involved in fatty acid synthesis in primary human CD4⁺ T cells. *Immunology* 146, 292–300 (2015). [PubMed: 26147366]

82. Labadie BW, Bao R & Luke JJ Reimagining IDO pathway inhibition in cancer immunotherapy via downstream focus on the tryptophan-kynurenine-aryl hydrocarbon axis. *Clin. Cancer Res.* 2, clincanres.2882.2018 (2018).
83. Gandhi R et al. Activation of the aryl hydrocarbon receptor induces human type 1 regulatory T cell-like and Foxp3(+) regulatory T cells. *Nat. Immunol* 11, 846–53 (2010). [PubMed: 20676092]
84. Li H et al. Metabolomic adaptations and correlates of survival to immune checkpoint blockade. *Nat. Commun* 10, 1–6 (2019). [PubMed: 30602773]
85. Lara P et al. Epacadostat plus pembrolizumab in patients with advanced RCC: Preliminary phase I/II results from ECHO-202/KEYNOTE-037. *J. Clin. Oncol* 35, 4515–4515 (2017).
86. Long GV et al. Epacadostat plus pembrolizumab versus placebo plus pembrolizumab in patients with unresectable or metastatic melanoma (ECHO-301/KEYNOTE-252): a phase 3, randomised, double-blind study. *Lancet Oncol.* 20, 1083–1097 (2019). [PubMed: 31221619]
87. Kroemer G, Galluzzi L, Kepp O & Zitvogel L Immunogenic Cell Death in Cancer Therapy. *Annu. Rev. Immunol* 31, 51–72 (2013). [PubMed: 23157435]
88. Allard B, Longhi MS, Robson SC & Stagg J The ectonucleotidases CD39 and CD73: Novel checkpoint inhibitor targets. *Immunol. Rev* 276, 121–144 (2017). [PubMed: 28258700]
89. Allard D, Turcotte M & Stagg J Targeting A2 adenosine receptors in cancer. *Immunol. Cell Biol.* 95, 333–339 (2017). [PubMed: 28174424]
90. Leone RD & Emens LA Targeting adenosine for cancer immunotherapy. *J. Immunother. Cancer* 6, 57 (2018). [PubMed: 29914571]
91. Song L et al. Ecto-5'-nucleotidase (CD73) is a biomarker for clear cell renal carcinoma stem-like cells. *Oncotarget* 8, 31977–31992 (2017). [PubMed: 28404888]
92. Giannakis M et al. Metabolomic correlates of response in nivolumab-treated renal cell carcinoma and melanoma patients. *J. Clin. Oncol* 35, 3036–3036 (2017).
93. Fong L et al. Adenosine 2A receptor blockade as an immunotherapy for treatment-refractory renal cell cancer. *Cancer Discov.* 10, 40–53 (2020). [PubMed: 31732494]
94. Hirsch L, Flippot R, Escudier B & Albiges L Immunomodulatory Roles of VEGF Pathway Inhibitors in Renal Cell Carcinoma. *Drugs* (2020). doi:10.1007/s40265-020-01327-7
95. Plimack ER et al. Pembrolizumab plus axitinib versus sunitinib as first-line therapy for advanced renal cell carcinoma (RCC): Updated analysis of KEYNOTE-426. *J. Clin. Oncol* 38, 5001–5001 (2020).
96. Palmer AC & Sorger PK Combination Cancer Therapy Can Confer Benefit via Patient-to-Patient Variability without Drug Additivity or Synergy. *Cell* 171, 1678–1691.e13 (2017). [PubMed: 29245013]
97. Kwak EL, Clark JW & Chabner B Targeted agents: The rules of combination. *Clinical Cancer Research* 13, 5232–5237 (2007). [PubMed: 17875749]
98. Zappasodi R, Merghoub T & Wolchok JD Emerging Concepts for Immune Checkpoint Blockade-Based Combination Therapies. *Cancer Cell* 33, 581–598 (2018). [PubMed: 29634946]
99. Hammers HJ et al. Combination of dual immune checkpoint inhibition (ICI) with stereotactic radiation (SBRT) in metastatic renal cell carcinoma (mRCC) (RADVAX RCC). *J. Clin. Oncol* 38, 614–614 (2020).
100. Masini C et al. Nivolumab (NIVO) in combination with stereotactic body radiotherapy (SBRT) in pretreated patients (pts) with metastatic renal cell carcinoma (mRCC): First results of phase II NIVES study. *J. Clin. Oncol* 38, 613–613 (2020). [PubMed: 31829907]
101. Jonasch E et al. A First-in-Human Phase 1/2 Trial of the Oral HIF-2a Inhibitor PT2977 in Patients with Advanced RCC. *Ann. Oncol* 30, v356–v402 (2019).
102. Choueiri TK & Kaelin WG Targeting the HIF2–VEGF axis in renal cell carcinoma. *Nat. Med* 26, 1519–1530 (2020). [PubMed: 33020645]
103. Reynolds K, Thomas M & Dougan M Diagnosis and Management of Hepatitis in Patients on Checkpoint Blockade. *Oncologist* 23, 991–997 (2018). [PubMed: 29853659]
104. Braun DA & Wu CJ Antigen discovery and therapeutic targeting in hematologic malignancies. *Cancer Journal (United States)* 23, 115–124 (2017).

105. Ott PA & Wu CJ Cancer vaccines: Steering t cells down the right path to eradicate tumors. *Cancer Discov.* 9, 476–481 (2019). [PubMed: 30862723]
106. Maude SL, Teachey DT, Porter DL & Grupp SA CD19-targeted chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. *Blood* 125, 4017–4023 (2015). [PubMed: 25999455]
107. D'Agostino M & Raje N Anti-BCMA CAR T-cell therapy in multiple myeloma: can we do better? *Leukemia* 34, 21–34 (2020). [PubMed: 31780814]
108. Slamon DJ et al. Use of chemotherapy plus a monoclonal antibody against her2 for metastatic breast cancer that overexpresses HER2. *N. Engl. J. Med.* 344, 783–792 (2001). [PubMed: 11248153]
109. Martinez M & Moon EK CAR T cells for solid tumors: New strategies for finding, infiltrating, and surviving in the tumor microenvironment. *Front. Immunol* 10, (2019).
110. Kaluz S, Kaluzová M, Liao SY, Lerman M & Stanbridge EJ Transcriptional control of the tumor- and hypoxia-marker carbonic anhydrase 9: A one transcription factor (HIF-1) show? *Biochimica et Biophysica Acta - Reviews on Cancer* 1795, 162–172 (2009).
111. Tostain J, Li G, Gentil-Perret A & Gigante M Carbonic anhydrase 9 in clear cell renal cell carcinoma: A marker for diagnosis, prognosis and treatment. *Eur. J. Cancer* 46, 3141–3148 (2010). [PubMed: 20709527]
112. Lamers CHJ et al. Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 24, (2006).
113. Lamers CHJ et al. Treatment of metastatic renal cell carcinoma with CAIX CAR-engineered T cells: Clinical evaluation and management of on-target toxicity. *Mol. Ther.* 21, 904–912 (2013). [PubMed: 23423337]
114. Lamers CHJ, Klaver Y, Gratama JW, Sleijfer S & Debets R Treatment of metastatic renal cell carcinoma (mRCC) with CAIX CAR-engineered T-cells - A completed study overview. *Biochem. Soc. Trans* 44, 951–959 (2016). [PubMed: 27284065]
115. Wang QJ et al. Preclinical evaluation of chimeric antigen receptors targeting CD70-expressing cancers. *Clin. Cancer Res.* 23, 2267–2276 (2017). [PubMed: 27803044]
116. Roybal KT et al. Precision Tumor Recognition by T Cells with Combinatorial Antigen-Sensing Circuits. *Cell* 164, 770–779 (2016). [PubMed: 26830879]
117. Diaconu I et al. Inducible Caspase-9 Selectively Modulates the Toxicities of CD19-Specific Chimeric Antigen Receptor-Modified T Cells. *Mol. Ther* 25, 580–592 (2017). [PubMed: 28187946]
118. Fraietta JA et al. Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells. *Nature* 558, 307–312 (2018). [PubMed: 29849141]
119. Weber EW, Maus MV & Mackall CL The Emerging Landscape of Immune Cell Therapies. *Cell* 181, 46–62 (2020). [PubMed: 32243795]
120. Li H et al. CAIX-specific CAR-T Cells and Sunitinib Show Synergistic Effects Against Metastatic Renal Cancer Models. *J. Immunother* 43, 16–28 (2020). [PubMed: 31574023]
121. Loustau M First CAR-T cell immunotherapy against HLA-G: Targeting a unique ICP and TAA - ScienceDirect. *Ann. Oncol* 30, xi12 (2019).
122. Wang Y et al. Abstract 3179: Design and activity of 2 nd generation, dual-targeted CAR T cell factories against ccRCC. in *Cancer Research* 79, 3179–3179 (American Association for Cancer Research (AACR), 2019).
123. Suarez ER et al. Chimeric antigen receptor T cells secreting anti-PD-L1 antibodies more effectively regress renal cell carcinoma in a humanized mouse model. *Oncotarget* 7, 34341–34355 (2016). [PubMed: 27145284]
124. Scott AM, Allison JP & Wolchok JD Monoclonal antibodies in cancer therapy. *Cancer Immun.* 12, (2012).
125. Chamie K et al. Adjuvant Weekly Girentuximab Following Nephrectomy for High-Risk Renal Cell Carcinoma: The ARISER Randomized Clinical Trial. *JAMA Oncol.* 3, 913–920 (2017). [PubMed: 27787547]
126. Diamantis N & Banerji U Antibody-drug conjugates - An emerging class of cancer treatment. *British Journal of Cancer* 114, 362–367 (2016). [PubMed: 26742008]

127. Muselaers CHJ et al. Phase 2 Study of Lutetium 177-Labeled Anti-Carbonic Anhydrase IX Monoclonal Antibody Girentuximab in Patients with Advanced Renal Cell Carcinoma. *Eur. Urol* 69, 767–770 (2016). [PubMed: 26706103]
128. Coats S et al. Antibody-drug conjugates: Future directions in clinical and translational strategies to improve the therapeutic index. *Clinical Cancer Research* 25, 5441–5448 (2019). [PubMed: 30979742]
129. Pal SK et al. A phase 1 trial of SGN-CD70A in patients with CD70-positive, metastatic renal cell carcinoma. *Cancer* 125, 1124–1132 (2019). [PubMed: 30624766]
130. McGregor BA et al. Safety and efficacy of CDX-014, an antibody-drug conjugate directed against T cell immunoglobulin mucin-1 in advanced renal cell carcinoma. *Invest. New Drugs* (2020). doi:10.1007/s10637-020-00945-y
131. Thompson JA et al. Phase I trials of anti-ENPP3 antibody–drug conjugates in advanced refractory renal cell carcinomas. *Clin. Cancer Res.* 24, 4399–4406 (2018). [PubMed: 29848572]
132. Rosenberg SA et al. Observations on the Systemic Administration of Autologous Lymphokine-Activated Killer Cells and Recombinant Interleukin-2 to Patients with Metastatic Cancer. *N. Engl. J. Med* 313, 1485–1492 (1985). [PubMed: 3903508]
133. Dudley ME et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science* (80-.). 298, 850–854 (2002).
134. Rosenberg SA et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. *Clin. Cancer Res.* 17, 4550–4557 (2011). [PubMed: 21498393]
135. Sarnaik A et al. Long-term follow up of lifileucel (LN-144) cryopreserved autologous tumor infiltrating lymphocyte therapy in patients with advanced melanoma progressed on multiple prior therapies. *J. Clin. Oncol* 38, 10006–10006 (2020).
136. Figlin RA et al. Treatment of Metastatic Renal Cell Carcinoma With Nephrectomy, Interleukin-2 and Cytokine-Primed or CD8(+) Selected Tumor Infiltrating Lymphocytes from Primary Tumor. *J. Urol* 158, 740–745 (1997). [PubMed: 9258071]
137. Figlin RA et al. Multicenter, randomized, phase III trial of CD8+ tumor-infiltrating lymphocytes in combination with recombinant interleukin-2 in metastatic renal cell carcinoma. *J. Clin. Oncol* 17, 2521–2529 (1999). [PubMed: 10561318]
138. Andersen R et al. T-cell responses in the microenvironment of primary renal cell carcinoma-implications for adoptive cell therapy. *Cancer Immunol. Res.* 6, 222–235 (2018). [PubMed: 29301752]
139. Childs R et al. Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. *N. Engl. J. Med* 343, 750–758 (2000). [PubMed: 10984562]
140. Takahashi Y et al. Regression of human kidney cancer following allogeneic stem cell transplantation is associated with recognition of an HERV-E antigen by T cells. *J. Clin. Invest* 118, 1099–1109 (2008). [PubMed: 18292810]
141. Cherkasova E et al. Detection of an immunogenic HERV-E envelope with selective expression in clear cell kidney cancer. *Cancer Res.* 76, 2177–2185 (2016). [PubMed: 26862115]
142. Morgan RA et al. Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy. *J. Immunother* 36, 133–151 (2013). [PubMed: 23377668]
143. Linette GP et al. Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. *Blood* 122, 863–871 (2013). [PubMed: 23770775]
144. Kula T et al. T-Scan: A Genome-wide Method for the Systematic Discovery of T Cell Epitopes. *Cell* 178, 1016–1028.e13 (2019). [PubMed: 31398327]
145. Walter S et al. Multi-peptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival. *Nat. Med* 18, 1254–1261 (2012). [PubMed: 22842478]
146. Kirner A, Mayer-Mokler A & Reinhardt C IMA901: A multi-peptide cancer vaccine for treatment of renal cell cancer. *Hum. Vaccines Immunother.* 10, 3179–3189 (2014).
147. Rini BI et al. IMA901, a multi-peptide cancer vaccine, plus sunitinib versus sunitinib alone, as first-line therapy for advanced or metastatic renal cell carcinoma (IMPRINT): a multicentre,

- open-label, randomised, controlled, phase 3 trial. *Lancet Oncol.* 17, 1599–1611 (2016). [PubMed: 27720136]
148. Rausch S et al. Results of a Phase 1/2 Study in Metastatic Renal Cell Carcinoma Patients Treated with a Patient-specific Adjuvant Multi-peptide Vaccine after Resection of Metastases. *Eur. Urol. Focus* 5, 604–607 (2019). [PubMed: 28988765]
 149. Sarkizova S et al. A large peptidome dataset improves HLA class I epitope prediction across most of the human population. *Nat. Biotechnol* 38, 199–209 (2020). [PubMed: 31844290]
 150. Ott PA et al. An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature* 547, 217–221 (2017). [PubMed: 28678778]
 151. Sahin U et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature* 547, 222–226 (2017). [PubMed: 28678784]
 152. Keskin DB et al. Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. *Nature* 565, 234–239 (2019). [PubMed: 30568305]
 153. Hilf N et al. Actively personalized vaccination trial for newly diagnosed glioblastoma. *Nature* 565, 240–245 (2019). [PubMed: 30568303]
 154. Lopez JS et al. Abstract CT301: A phase Ib study to evaluate RO7198457, an individualized Neoantigen Specific immunoTherapy (iNeST), in combination with atezolizumab in patients with locally advanced or metastatic solid tumors. in *Bioinformatics, Convergence Science, and Systems Biology* 80, CT301–CT301 (American Association for Cancer Research, 2020).
 155. Verma V et al. PD-1 blockade in subprimed CD8 cells induces dysfunctional PD-1+CD38hi cells and anti-PD-1 resistance. *Nat. Immunol* 20, 1231–1243 (2019). [PubMed: 31358999]
 156. Pauken KE et al. The PD-1 Pathway Regulates Development and Function of Memory CD8+ T Cells following Respiratory Viral Infection. *Cell Rep.* 31, (2020).
 157. Hu Z et al. A cloning and expression system to probe T-cell receptor specificity and assess functional avidity to neoantigens. *Blood* 132, 1911–1921 (2018). [PubMed: 30150207]
 158. Grünwald V et al. Depth of remission is a prognostic factor for survival in patients with metastatic renal cell carcinoma. *Eur. Urol* 67, 952–958 (2015). [PubMed: 25577718]
 159. Voss MH et al. Depth of response (DepOR) analysis and correlation with clinical outcomes from JAVELIN Renal 101. *J. Clin. Oncol* 38, 690–690 (2020).
 160. Motzer RJ et al. Survival outcomes and independent response assessment with nivolumab plus ipilimumab versus sunitinib in patients with advanced renal cell carcinoma: 42-month follow-up of a randomized phase 3 clinical trial. *J. Immunother. Cancer* 8, 891 (2020).
 161. Grünwald V et al. Association between depth of response and overall survival: Exploratory analysis in patients with previously untreated advanced renal cell carcinoma (aRCC) in CheckMate 214. *Ann. Oncol* 30, v382–v383 (2019).
 162. Ravi P et al. Evaluation of the Safety and Efficacy of Immunotherapy Rechallenge in Patients with Renal Cell Carcinoma. *JAMA Oncol.* (2020). doi:10.1001/jamaoncol.2020.2169
 163. Gul A et al. Salvage Ipilimumab and Nivolumab in Patients With Metastatic Renal Cell Carcinoma After Prior Immune Checkpoint Inhibitors. *J. Clin. Oncol JCO*.19.03315 (2020). doi:10.1200/jco.19.03315
 164. Regan MM et al. Treatment-free survival: A novel outcome measure of the effects of immune checkpoint inhibition—a pooled analysis of patients with advanced melanoma. *J. Clin. Oncol* 37, 3350–3358 (2019). [PubMed: 31498030]
 165. Regan MM et al. Treatment-free survival, with and without toxicity, as a novel outcome applied to immuno-oncology agents in advanced renal cell carcinoma. *Ann. Oncol* 30, v393–v394 (2019).

Key points:

- RCC differs substantially from other immunotherapy-responsive solid tumors, and therefore successful development of novel immune treatments requires knowledge of RCC-specific biology.
- RCCs are highly CD8⁺ T cell infiltrated tumors and consequently many therapeutic approaches focus on reinvigorating T cells in the tumor immune microenvironment.
- Novel immune checkpoint inhibitors, co-stimulatory pathway agonists, modified cytokine therapies, and metabolic pathway modulators are all promising approaches to remodel the RCC microenvironment and improve anti-tumor T cell responses.
- Precision immunotherapies aim to specifically target RCC tumor antigens, thereby “steering” the immune response specifically towards malignant cells.
- Cellular therapies, monoclonal antibodies, and therapeutic vaccination are promising approaches in RCC that act in an antigen-directed manner, and may improve the efficacy of current immunotherapy.

Figure 1. The Renal Cell Carcinoma Cancer-Immunity Cycle.

This cycle is based on the original cancer-immunity cycle proposed by Chen & Mellman¹¹.

The cycle steps and factors affecting ICI response are edited to reflect the specificities of clear cell renal cell carcinoma tumors.

ERV: Endogenous Retroviruses; RCC: Renal cell carcinoma; TMB: Tumor Mutational Burden; TLS: Tertiary Lymphoid Structures; APC: Antigen Presenting Cell; IL-8: Interleukin-8; VEGF: Vascular Endothelial Growth Factor; ICI: Immune Checkpoint Inhibitor

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Braun DA, Bakouny Z, Hirsch L, Flippot R, Van Allen EM, Wu CJ, Choueiri TK. Beyond conventional immune-checkpoint inhibition - novel immunotherapies for renal cell carcinoma. *Nat Rev Clin Oncol*. 2021 04; 18(4):199–214.

Figure 2. Targeting the RCC Immune Microenvironment.

(a) Stimulatory (text label in green) and inhibitory (text label in red) immune checkpoints on T cells that can be targeted by agonist and antagonist antibodies, respectively. (b) Novel cytokine therapies can activate T cell responses and stimulate the anti-tumor immune response. NHS-IL12 includes the NHS76 antibody that binds to necrotic debris and therefore directs IL-12 to tumors, where necrosis is prevalent. (c) The tryptophan–kynurenine–aryl hydrocarbon receptor and CD39–CD73–adenosine 2A receptor are immune metabolic pathways that can be targeted at multiple levels.

Ab: Antibody; TCR: T cell receptor; HLA: Human leukocyte antigen; PSGL-1: P-selectin glycoprotein ligand-1; VISTA: V-domain Ig suppressor of T cell activation; HHLA2: Human Endogenous Retrovirus-H Long Terminal Repeat-Associating Protein 2; CTLA-4: cytotoxic T-lymphocyte-associated protein 4; PD-1: Programmed cell death protein 1; PD-L1: Programmed death-ligand 1; LAG-3: Lymphocyte activation gene-3; TIM-3: T cell immunoglobulin and mucin-domain containing-3; TIGIT: T cell immunoglobulin and ITIM domain; IL12-R: Interleukin 12 receptor; IL2-R: Interleukin 2 receptor; ATP: Adenosine triphosphate; AMP: Adenosine monophosphate; A2AR: adenosine 2A receptor; AhR: Aryl hydrocarbon receptor; Tryp: Tryptophan; Kyn: Kynurenine; RCC: Renal Cell Carcinoma; TDO: Tryptophan 2,3-dioxygenase; IDO1: Indoleamine 2,3-dioxygenase 1.

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Figure 3. Strategies for precision immunotherapy in RCC.

Antigen-directed therapies can target either surface antigens or HLA-restricted antigens, and may require personalization or be available “off-the-shelf” for multiple patients. **Upper left panel**, autologous CAR T therapy targeting an RCC surface antigen, like CAIX. Novel CAR constructs will improve specificity (through logic gates), increase persistence, add safety features (“suicide switch”), and/or secrete additional immunomodulatory molecules. **Bottom left panel**, CRISPR technology may enable “off-the-shelf” allogeneic CAR T cells. Other non-personalized, surface antigen-specific therapies include monoclonal antibodies, with or without an additional conjugated cytotoxic payload (drug or radionuclide). **Upper right panel**, numerous strategies to target HLA-restricted antigens require personalized therapy, including expansion and infusion of TILs, engineered TCR therapy against one specific HLA-restricted antigen, or neoantigen vaccination therapies. **Bottom right panel**, using next generation sequencing and mass spectrometry tools, a priority moving forward is to identify shared HLA-restricted antigenic targets (such as peptides derived from aberrantly expressed ERVs) that could be administered “off-the-shelf” to multiple RCC patients. CAR – chimeric antigen receptor; TCR – T cell receptor; TIL – tumor-infiltrating T lymphocyte; ERV – endogenous retrovirus; HLA – human leukocyte antigen; RCC – renal cell carcinoma; CRISPR – clustered regularly interspaced short palindromic repeats.

NOTE: Permission for reproduction of this figure was not granted by the publisher. For Figure 3, please refer to Figure 3 in the manuscript by Braun et al.:

Braun DA, Bakouny Z, Hirsch L, Flippot R, Van Allen EM, Wu CJ, Choueiri TK. Beyond conventional immune-checkpoint inhibition - novel immunotherapies for renal cell carcinoma. *Nat Rev Clin Oncol.* 2021 04; 18(4):199–214.

Table 1.

Examples of promising recent and ongoing immunotherapy clinical trials in RCC. The mechanism of action, therapeutic combination (if applicable), and the affected steps in the cancer immunity cycle are provided.

COMPOUND	MECHANISM OF ACTION	COMBINATION	CLINICAL TRIAL ID	STEP(S) OF CANCER-IMMUNITY CYCLE
PRO-INFLAMMATORY CYTOKINES				
HD-IL-2	Pro-inflammatory cytokine (T cell growth factor)	Nivolumab	NCT02989714	Step 3: T cell priming and activation Step 6: Recognition of cancer cells by T cells Step 7: Killing of cancer cells
		Radiation therapy	NCT02306954	
NKTR-214	Pro-inflammatory cytokine (T cell growth factor)	Nivolumab +/- Ipilimumab	NCT02983045	
INHIBITORS OF ANTI-INFLAMMATORY CYTOKINES, CYTOKINE RECEPTORS OR GROWTH FACTORS				
Mavorixafor (X4P-001)	CXCR4 inhibitor	Axitinib	NCT02667886	Step 4: Trafficking of immune cells into tumors Step 5: Infiltration of immune cells into tumors
CELLULAR THERAPIES				
Anti-hCD70 CAR T cell	Recognition and killing of CD70-expressing cells	HD-IL-2	NCT02830724	Step 6: Recognition of cancer cells by T cells Step 7: Killing of cancer cells
Adoptive T cell therapy with TILs	Recognition of HLA-restricted tumor antigens	IL-2	NCT02926053	
HERV-E TCR transduced autologous T cells	Recognition of HLA-A11:01—restricted HERVE-E—derived peptide	None	NCT03354390	
CELLULAR THERAPIES				
NEOVAX	Neoantigen peptides + adjuvant	Ipilimumab (local administration)	NCT02950766	Step 2: Cancer antigen presentation Step 3: T cell priming and activation
RO7198457	Neoantigen RNA	Atezolizumab	NCT03289962	
IMMUNE CHECKPOINT INHIBITORS				
MK-7684	TIGIT inhibitor	Pembrolizumab	NCT02964013	Step 3: T cell priming and activation Step 7: Killing of cancer cells
CO-STIMULATORY IMMUNE RECEPTOR AGONISTS				
Utomilumab	4–1 BB agonist	Pembrolizumab	NCT02179918	Step 7: Killing of cancer cells
TRYPTOPHAN PATHWAY INHIBITORS				
Linrodostat	IDO1 inhibitor	Nivolumab	NCT03192943	Step 7: Killing of cancer cells (alters immunosuppressive microenvironment)
ADENOSINE PATHWAY INHIBITORS				
Ciforadenant (CPI-444)	A2AR inhibitor	None or Atezolizumab	NCT02655822	Step 7: Killing of cancer cells (alters TAMs, DCs, NK cells / immunosuppressive microenvironment)
BMS-986179	CD73 inhibitor	Nivolumab	NCT02754141	