PD-1/PD-L1 inhibitors-based treatment for advanced renal cell carcinoma: Mechanisms affecting efficacy and combination therapies

Lei Ding | Hui yu Dong | Tian ren Zhou | Yu hao Wang | Tao Yan | Jun chen Li | Zhong yuan Wang | Jie Li | Chao Liang

Department of Urology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China

Abstract
With the widespread use of PD-1/PD-L1 monoclonal antibodies (mAbs) in the treatment of multiple malignant tumors, they were also gradually applied to advanced renal cell carcinoma (aRCC). Nowadays, multiple PD-1/PD-L1 mAbs, such as nivolumab, avelumab, and pembrolizumab, have achieved considerable efficacy in clinical trials. However, due to the primary, adaptive, and acquired resistance to these mAbs, the efficacy of this immunotherapy is not satisfactory. Theories also vary as to why the difference in efficacy occurs. The alterations of PD-L1 expression and the interference of cellular immunity may affect the efficacy. These mechanisms demand to be revealed to achieve a sustained and complete objective response in patients with aRCC. Tyrosine kinase inhibitors have been proven to have synergistic mechanisms with PD-1/PD-L1 mAb in the treatment of aRCC, and CTLA-4 mAb has been shown to have a non-redundant effect with PD-1/PD-L1 mAb to enhance efficacy. Although combinations with targeted agents or other checkpoint mAbs have yielded enhanced clinical outcomes in multiple clinical trials nowadays, the potential of PD-1/PD-L1 mAbs still has a large development space. More potential mechanisms that affect the efficacy demand to be developed and transformed into the clinical treatment of aRCC to search for possible combination regimens. We elucidate these mechanisms in RCC and present existing combination therapies applied in clinical trials. This may help physicians’ select treatment options for patients with refractory kidney cancer.

Keywords
advanced renal cell carcinoma, cellular immunity, combination therapy, fundamental mechanisms, PD-1, PD-L1 inhibitor
1 | INTRODUCTION

Kidney cancer is the sixth and eighth most common cancer in men and women in 2020, accounting for approximately 5% and 3%, respectively, according to estimates by the American Cancer Society. In accordance with tumor histology and chromosomal alterations, kidney cancer is mainly classified into clear cell renal cell carcinoma (ccRCC), chromophobe RCC, papillary RCC, translocation RCC, and other rare subtypes of the renal unit or collection system. CcRCC is the main type, accounting for approximately 70%. The 5-year survival rate for localized kidney and renal pelvic cancer was 92.6%, and that for regional cancer was only 66.7%. This rate even fell to 11.7% in patients with extensive metastatic cancer. Therefore, the development of effective and safe agents for aRCC is urgent.

Through these years, therapies for patients with aRCC who are ineligible for partial or radical nephrectomy have undergone a series of revolutions, from the initial radiotherapy, chemotherapy, to nonspecific immunotherapy, such as interleukin-2 and interferon, and then to targeted therapies, such as vascular endothelial growth factor (VEGF) inhibitors and mTOR inhibitors. Despite the impressive progress, the objective response rates (ORRs) and safety of these agents remain unsatisfactory. Due to the high immunogenicity and strong T cell infiltration of RCC, programmed cell death-1/programmed cell death-ligand 1 (PD-1/PD-L1) monoclonal antibodies (mAbs), which can strengthen cellular immunity, have been gradually applied in the treatment of aRCC as monotherapy recently and achieved considerable efficacy and acceptable safety, especially in ccRCC. However, its ORRs as first-line therapy were only around 16%–37%, which still could not bring clinical benefits to most patients. In this instance, the combination of multiple checkpoint inhibitors or PD-1/PD-L1 mAbs combined with antiangiogenic agents (AAs) has emerged, whose efficacy was superior to the above targeted agents, and no statistical difference exists between them in terms of safety. In these clinical trials, the ORRs of the combination regimens could increase to approximately 59%. However, a proportion of patients still has no response, and safety issues could not be ignored.

Here, the underlying mechanisms affecting the efficacy of PD-1/PD-L1 mAbs in aRCC; the therapies that could be combined with PD-1/PD-L1 mAbs, including chemotherapy, radiotherapy, and vaccine; and the existing related clinical trials were reviewed to develop novel combined therapeutic targets and potential predictive markers for efficacy. This review may be helpful for the development of new combination therapies for aRCC.

2 | PRECLINICAL STUDIES OF PD-1/PD-L1 MABS AND ITS LANDSCAPE IN THE TREATMENT OF ARCC

PD-1 is a 288 amino acid (aa) type I transmembrane glycoprotein encoded by PDCD1 on human chromosome 2, whose cytoplasmic domain includes an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif. As for PD-L1, the ligand for PD-1 is a 290 aa type I transmembrane glycoprotein encoded by CD274 on human chromosome 9. After the activation of T cells, PD-1 expression is upregulated within 12–36 h to interact with PD-L1 to inhibit the function of T cells via various mechanisms and prevent indiscriminate killing of excessive activated T cells to normal cells. However, PD-L1 is not only expressed on lymphocytes, myeloid, and endothelial cells but also on tumor cells, tumor infiltrating lymphocytes (TILs), macrophages, and other immune cells in the tumor microenvironment (TME). Therefore, PD-1/PD-L1 axis has also been revealed to participate in mediating antitumor immunity, and the overexpression of PD-1/PD-L1 signaling pathway could influence the cytolytic activity of T cells and thus promote occurrence and invasiveness of tumors.

The interaction between PD-1 and PD-L1 could trigger the phosphorylation of tyrosine residues in ITIM from PD-1 and promote the recruitment of protein tyrosine phosphatases (PTPs), such as SHP2 and PP2A. These PTPs dephosphorylate TCR; costimulatory molecules, such as CD28, on the surface of T cells; and stimulant molecules downstream of related signaling pathways, resulting in decreased activation of transcription factors, such as activating protein 1 and NF-xB. Blocking the costimulatory effect of CD28 could downregulate the downstream PI3K/Akt/mTORC1 signaling pathway, which not only inhibits glycolysis, but also induces the formation of intracellular mitochondrial crest and impairs oxidative phosphorylation, thereby inhibiting the metabolic activity of CD8+ T cells. Moreover, the PD-L1 expressed on antigen-presenting cells (APCs) could cis-bind to CD80, a costimulatory molecule on the surface of these cells, thus blocking PD-1 ligation and the stimulation of CD28/CD80 to T cells. The expression of BATF, a transcription factor that inhibits T-cell activation, could also be induced by PD-1. In addition, the engagement of PD-1 could enhance the migration ability of T cells and reduce the contact time between T cells and the major histocompatibility complex (MHC)–antigen peptide complex on interacting cells. Ultimately, the above mechanisms block the function of T cells by antagonizing T-cell activation, proliferation, and effector function (Figure 1).
The mechanisms by which these tumors resist endogenous tumor-specific T cell killing via the PD-1/PD-L1 axis could be divided into two types: intrinsic and adaptive immune resistance, which are compatible and could coexist within the same TME. Intrinsic immune resistance is associated with alterations at the level of genes or certain signaling pathways in tumor cells, which could induce constitutive PD-L1 expression. For example, in RCC, 9p24.1 amplification could directly stimulate constitutive PD-L1 expression or indirectly stimulate it through the activation of the JAK2 signaling pathway. Activation of Akt and STAT3 signaling has also been shown to induce constitutive PD-L1 expression in multiple tumors and participate in immune resistance. Adaptive resistance is mainly induced by cytokines secreted by tumor cells and other cells in TME, especially IFN-γ. This mechanism is demonstrated as the manifestation of tumors to the stimulation of the immune microenvironment, and it induces adaptive PD-L1 expression.

Given the above preclinical mechanism, researchers have revealed various methods, such as PD-1/PD-L1 mAbs, vaccination, and adoptive immunotherapy, to reverse tumor immune escape by targeting the PD-1/PD-L1 axis or interfering with the PD-1 signaling pathway. In the present review, ClinicalTrials.gov was searched for clinical trials with PD-1/PD-L1 mAb monotherapy as an intervention. As shown in Table 1, multiple PD-1/PD-L1 mAbs as monotherapy have shown impressive efficacy in clinical trials related to RCC; however, most patients still do not have any immune response, and their security issues should not be underestimated. Some patients also experienced drug resistance during treatment, further eroding the overall efficacy. The underlying mechanism of drug resistance should be identified to address the problem of low ORRs.

3 | MECHANISMS OF PD-L1 EXPRESSION ALTERATIONS IN RCC

Researchers initially found that the high level of PD-L1 expression on tumor cells or TILs in TME was often accompanied by increased TMN stage and cell atypia in RCC, which also indicates increased risk of disease progression and worsened prognosis. In two clinical trials, CheckMate214 and KEYNOTE426, no significant difference was demonstrated in OS between PD-L1-negative patients and PD-L1-positive patients. In Javelin Renal 101, no statistically significant difference was found in PFS between these two subgroups. Thus, PD-1/PD-L1 mAbs may be occupied by PD-L1 expressed on normal cells, such as lymphocytes, myeloid cells, and endothelial cells, and this hypothesis is also the possible reason...
| NCT, study       | Phase | Setting                          | Experimental arm (pts,agents) | Control arm (pts,agents) | Primary endpoints | ORR(%) | Serious AEs (exp,%) |
|------------------|-------|---------------------------------|-------------------------------|--------------------------|-------------------|--------|-------------------|
| NCT00730639 CA209-003 | I     | First line; CRPC, RCC, MM, NSCLC. | 18 (1.0 mg/kg, arm1) 16 (10.0 mg/kg, arm2) nivolumab | None                   | Number of participants With SAEs, TRAEs | NA     | NA |
| NCT01354431 CA209-010 | II    | Second line; aRCC, mRCC         | 60 (0.3 mg/kg, arm1) 54 (2.0 mg/kg, arm2) 54 (10.0 mg/kg, arm3) nivolumab | None                   | PFS               | arm1:20.0, 80%CI13.4–28.2 arm2:22.2, 80%CI15.0–31.1 arm3:20.4, 80%CI13.4–29.1 | arm1: 45.76% arm2: 61.11% arm3: 40.74% |
| NCT01358721 CA209-009 | I     | First or second line; RCC       | Previously-treated: 22 (0.3 mg/kg, arm1) 22 (2.0 mg/kg, arm2) 23 (10.0 mg/kg, arm3) Treatment-naive: 24 (10.0 mg/kg, arm4) nivolumab | None                   | Percent change from baseline in Activated and memory T Cells | arm1: 9.1% arm2: 18.2% arm3: 21.7% arm4: 4.2% | arm1: 59.09% arm2: 50.00% arm3: 52.17% arm4: 54.17% |
| NCT01668784 CheckMate 025 | III   | Second line; aRCC, mRCC         | 410 (arm1) nivolumab            | 411 (arm2) everolimus   | OS, PFS, TRAEs (%) | arm1: 25.1, 95%CI21.0–29.6 arm2: 5.4, 95%CI3.4–8.0 | arm1: 47.78% arm2: 43.58% |
| NCT02212730 KEYNOTE 031 | I     | First line; RCC                 | 6 (arm1, pre-resection) pembrolizumab | 4 (arm2, post-resection) pembrolizumab | AEs during the neoadjuvant pembrolizumab regimen (number) | None | arm1: 33.33% arm2: 0.00% |
| NCT02596035 CheckMate 374 | IV    | Second line; aRCC, mRCC         | 142 nivolumab                  | None                   | 3 or higher grade IMAEs (%) | None | 41.55% |
| NCT03444766 CA209-887 | IV    | Second line; aRCC, mRCC, NSCLC  | 100 (overall) nivolumab        | None                   | TRAEs (number) | None | 30.00% |
| NCT01772004 JAVELIN Solid Tumor | I     | First or second line; RCC       | 62 (line1, arm1) 20(line2, arm2) avelumab | None                   | OS, PFS             | arm1: 16.1, 95%CI8.0–27.7 arm2: 10.0, 95%CI1.2–31.7 | arm1: 22.6% arm2: 35.0% |
| NCT02836795 Junshi-JS001- BJZL-1 | I     | First or second line; RCC, M, UC | 6 (RCC) toripalimab           | None                   | TRAEs (all grade, 3 or higher grade), ORR | 33.3% (RCC) | 13.89%(overall) |

(Continues)
For the occurrence of immune-related adverse events. Therefore, more targets of PD-L1-positive patients could show enhanced clinical benefits. Although the application of PD-L1 expression has many limitations, such as temporal and spatial heterogeneity, measurement techniques, and uncertain positive cutoff value, the mechanisms of alterations in its expression are still worth exploring. Based on these potential preclinical mechanisms, some agents with strong antitumor effect could enhance the efficacy of PD-1/PD-L1 mAbs. Some agents could enhance the expression of PD-L1 on tumors and lead to immunosuppression. The non-overlapping effect of PD-1/PD-L1 inhibitors and these agents could enhance the antitumor effect.

### 3.1 Intrinsic factors of PD-L1 expression alteration

#### 3.1.1 Genetic alterations

The biallelic von Hippel Lindau (VHL) gene inactivation is the most characteristic genetic alteration in ccRCC, as it causes decreased expression of VHL protein (pVHL). Together with Elongin B and Elongin C, pVHL could form the VBC complex and regulate the stability of hypoxia-inducible factor-α (HIF-α). In ccRCC, HIF-2α targets hypoxic-response elements in the proximal promoter of PD-L1; thus, the accumulation of HIF-2α caused by VHL inactivation could directly cause constitutive PD-L1 overexpression.

### TABLE 1 (Continued)

| NCT, study | Phase | Setting (pts,agents) | Control arm (pts,agents) | Primary endpoints | ORR(%) | Serious AEs (exp,%) |
|------------|-------|----------------------|-------------------------|------------------|--------|------------------|
| NCT01375842 PCD4989g | I | First or second line; aRCC, mRCC | 70 atezolizumab None | TRAEs(all grade, 3 or higher grade) | 15%, 95% CI 7–26% | NA |
| NCT00729664 CA210-001 | I | First or second line; RCC, NSCLC, etc. | 17 (RCC) MDX 1105 (PD-1 mAb) None | TRAEs(all grade, 3 or higher grade), ORR | 12%, 95% CI 2–36 (RCC) | 5%(overall) |
| NCT02853344 KEYNOTE-427 | II | First line; RCC | 110 (arm1: ccRCC) 165 (arm2: nccRCC) pembrolizumab Nnoe | ORR | arm1: 36.4%, 95% CI 27.4–46.1; arm2: 26.7%, 95% CI 20.1–34.1 | High-grade TRAEs: arm1: 30% arm2: 17% |

Clinical trials with PD-1/PD-L1 mAb monotherapy as an intervention that had results and enrolled more than 5 patients with RCC. The characteristics and partial results of these clinical trials are presented in the table.

Abbreviations: aRCC, advanced renal cell carcinoma; ccRCC, clear cell renal cell carcinoma; CI, confidence interval; CRPC, castration resistant prostate cancer; DCR, disease control rate; DOR, duration of response; M, melanocytoma; MM, malignant melanocytoma; mRCC, metastatic renal cell carcinoma; NA, not available; NE, not estimate; NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progression free survival; pts, patients; SAEs, serious adverse events; TRAEs, treatment-related adverse events; UC, urothelial cancer

| Abbreviations: aRCC, advanced renal cell carcinoma; ccRCC, clear cell renal cell carcinoma; CI, confidence interval; CRPC, causation resistant prostate cancer; DCR, disease control rate; DOR, duration of response; M, melanocytoma; MM, malignant melanocytoma; mRCC, metastatic renal cell carcinoma; NA, not available; NE, not estimate; NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progression free survival; pts, patients; SAEs, serious adverse events; TRAEs, treatment-related adverse events; UC, urothelial cancer |

*Table continued...*
the loss of miR-339, thus upregulating PD-L1 expression and weakening antitumor immunity.61 Another study that included 121 patients with RCC, suggested that RCC cells overexpressed BUB1B in approximately 25% of patients, and these cells had poor response to nivolumab. Data analysis showed that BUB1B overexpression was positively associated with PD-L1, IFN-γ, and CD8+ T cell exhaustion signals and resulted in upregulation of CD44, phosphorylation of p53, and chromosomal instability. The high IFN-γ expression and DNA damage caused by chromosomal instability may be the reasons for the upregulation of PD-L1 expression.62,63

Genes encoding classical complement pathway protein were confirmed to be highly expressed in ccRCC, and the C1q encoded by these genes was positively correlated with PD-L1/PD-L2 expression.64 C1q is mainly produced by the M2 subtype of tumor-associated macrophages in ccRCC, and it induced macrophages to polarize to immunosuppressive phenotype (M2) in vitro.64,65 This interaction is possibly related to the upregulation of PD-L1/PD-L2. Amplification of JAK2, PD-L1, and PD-L2 at 9p24.1 could often be found in the sarcomatoid tissue of chromophobe or ccRCC.66,67 It could directly cause PD-L1/PD-L2 overexpression and the upregulation of the JAK2/STAT3 pathway,34 which stimulates PD-L1 overexpression in multiple other tumors by binding its downstream molecule IRF1 to the PD-L1 promoter.66,69

3.1.2 | Epigenetic alterations

The microRNA (miRNA) network plays an important role in the regulation of PD-L1 expression in aRCC, although the underlying mechanisms are not fully understood. A study comparing miRNA expression in 23 patients with metastatic ccRCC before and after treatment with nivolumab showed a negative association between miR-22, miR-24, and soluble PD-L1 expression.70 Among patients with persistent response to immunotherapy, those with high miR-339 expression had better PFS,70 and this finding is consistent with that of the aforementioned study.61 Another study found that in RCC, miR-497-5p could directly bind to the 3′ UTR of PD-L1 mRNA to inhibit PD-L1 expression at the protein level.71 Su Zeng et al. demonstrated that the miR-224-5p expression in urinary extracellular vesicles was abnormally elevated in patients with RCC, and this elevation could inhibit the gene CCND1 encoding cyclin D1 and thereby downregulate the proteolytic hydrolysis of PD-L1 mediated by the downstream cyclin D1/SPOP signaling pathway.72

Genetic alterations induced by the modification of histone methylation could affect PD-L1 expression by upregulating the expression of immunogenic endogenous retroviruses (πERVs) in RCC, especially ERV3-2.73 Researchers also found that high vimentin expression in RCC was accompanied by high PD-L1 expression.74
Previous studies suggested that high vimentin expression represented more epithelial–mesenchymal transformation (EMT), which could induce the demethylation of PD-L1 promoter by upregulating DNA methyl-transferase 1 (DNMT1). Vimentin could also interact with deacetylated PD-L1 to facilitate its nuclear translocation via cytoskeleton, thus promoting the formation of positive feedback of PD-L1 expression. These findings may be the reasons why vimentin is positively correlated with PD-L1 in RCC.

3.2 Extrinsic factors of PD-L1 expression alteration

The cytokines present in TME could affect PD-L1 expression, and their high expression is mainly due to the stimulation of RCC by the immune microenvironment. Agents, some other therapies, and metabolic imbalance are also associated with the expression of PD-L1 in the TME of RCC.

3.2.1 Cytokines

IFN-γ has been demonstrated to induce PD-L1 expression in multiple tumors. It is consistent with the positive relationship between PD-L1 and IFN-γ in RCC. IFN-γ could induce PD-L1 overexpression at the transcriptional level by promoting STAT1 phosphorylation, which is also the mechanism of IL-27 and IL-32g inducing PD-L1 overexpression. Researchers revealed that IL-10 and IL-1α could promote the phosphorylation of STAT3 and tumor suppressor gene p65, respectively, to upregulate PD-L1 expression. In melanocytes, the agents that inhibit eukaryotic cell initiation factor 4A could downregulate the transcription level of STAT1 and indirectly downregulate PD-L1 expression to induce tumor regression. This mechanism may also be applied in the treatment of RCC to enhance the efficacy of anti-PD-1/PD-L1.

3.2.2 Therapies

Traditional targeted therapy plays an important role in the treatment of RCC. Eric et al. demonstrated that sunitinib and bevacizumab increased the infiltration level of CD8+ T cells in TME and induced their secretion of IFN-γ in RCC, thus upregulating PD-L1 expression. Studies on thymus-free nude mice with renal carcinoma have also shown that these AAs could upregulate the PD-L1 protein levels independent of CD8+ T cells, and experiments in vitro suggested that this mechanism is not affected by HIF-1/2α. These findings indicated that AAs may directly induce PD-L1 expression. However, pazopanib, another AA, downregulates the PD-L1 expression of dendritic cells (DCs) in aRCC. The influence of AAs on PD-L1 expression is still not fully understood. MTOR inhibitors, another type of classic targeted agents, have been revealed to be involved in the regulation of PD-L1 expression. They could induce the nuclear translocation of transcription factor EB (TFEB), the main target of mTORC1, and upregulate its expression. TFEB could bind with PD-L1 promoter, stimulate PD-L1 expression, and ultimately inhibit the function of tumor infiltrating CD8+ T cells.

Some untargeted agents also regulate PD-L1 expression in the TME of RCC. For instance, honokiol, a natural product of bisphenol, could inhibit the phosphorylation of downstream molecule Akt and PD-L1 expression by inhibiting the c-Met related signaling pathway, thus enhancing the antitumor immune response. RCC cells treated with the bromine domain inhibitor JQ1 showed reduced proliferation and reduced PD-L1/PD-L2 expression, although the exact mechanism is unknown. In addition, after neoadjuvant stereotactic radiotherapy (Neo-SABR), the PD-L1 expression in the tumor thrombus of patients with RCC and the tumor thrombus of the inferior vena cava increased, which may be attributed to the induction of immune-related inflammatory cytokines.

3.2.3 Metabolic factors

High-grade RCC tends to represent more metabolic reprogramming, with glucose disorders being the most common. Glucose deficiency could reduce the energy produced by glycolysis pathway and promote downstream ERK/c-Jun phosphorylation by activating EGFR, thereby enhancing PD-L1 expression. In high-grade RCC, glucose-converted glutamine is used to alleviate oxidative stress via the glutathione pathway, and the grade of RCC was positively correlated with the expression of glutamine depletion signature. Glutamine deprivation has been demonstrated to induce PD-L1 expression by stimulating the EGFR/ERK/c-Jun signaling pathway. Epidermal growth factor could induce glutamine deprivation to promote PD-L1 expression. Inhibitors of EGFR/ERK/c-Jun have been shown to reverse these effects, which may enhance the efficacy of PD-1/PD-L1 mAbs. Besides, tryptophan metabolism is related to the regulation of PD-L1 expression. Kynurenine is synthesized by the catabolism of tryptophan by indoleamine 2,3-dioxygenase/tryptophan 2,3-dioxygenase (IDO/TDO). Researchers revealed that after the treatment of nivolumab in some patients with aRCC, kynurenine was
upregulated by enhancing the activity of IDO/TDO; thus, PD-L1 expression could be induced to counteract the immune stimulation caused by PD-1 blockade.94 Inhibition of this metabolic pathway could improve tumor immune resistance.95

4 | MECHANISMS AFFECTING CELLULAR IMMUNITY IN RCC

The antitumor immune process mainly involves cellular immunity, which could be summarized as follows: antigen presentation, activation, and migration of immune cells and recognition and killing of tumor cells by immune cells. In addition to PD-L1 expression, interference with cellular immunity could affect the efficacy of PD-1/ PD-L1 mAbs. A thorough understanding of these mechanisms may be helpful in enhancing the efficacy of PD-1/ PD-L1 mAbs.

4.1 | Antigen presentation

Higher tumor mutation burden (TMB) usually means that more tumor neoantigens are produced to present to T cells by MHC proteins.96 However, several retrospective analyses showed no significant association between TMB and immunotherapy response in RCC.97 The results of a retrospective analysis of 34 patients with aRCC who received PD-1/PD-L1 mAbs were consistent with the above conclusions. They also revealed that heterozygosity loss of MHC-I class genes associated with antigen presentation occurred by as high as 33% in the progression disease group, leading to antigen presentation limitation. In the disease control group, 68.8% of patients were found to have enrichment of DNA repair gene mutations, especially homologous recombinant repair-related genes, which may be related to the increase in tumor neoantigens and thus enhance antigen presentation.98 In ccRCC, the low intratumor heterogeneity, which is the genetic diversity of subclones in a single tumor, could promote the antigen presentation to T cells by enhancing the immune activity of new tumor antigens, the abundance of DCs, and the expression of HLA class I gene, and ultimately improve the response to PD-1 blockade.99 Although these mechanisms are difficult to interfere with, they may serve as biomarkers for predicting the efficacy. Another single-cell analysis of aRCC tissues treated with atezolizumab showed that in RCC cells and DC subsets with high IL-8 expression, the expression of genes involved in antigen presentation and processing, including HLA-C and IFIT3, decreased, and the overexpression of IL-8 was associated with poor ORR.100

Marianna Nuti et al. found that pazopanib could upregulate the HLA-DR, CD40, and CCR7 expression levels of DCs in aRCC tissues, inhibit their endocytosis, and ultimately promote the activation and enhance the antigen presentation function.95 It could also downregulate PD-L1 expression on DCs and inhibit the secretion of IL-10 to enhance the stimulation of T cells, thus inducing Th1 type immune response and promoting the increase in circulating CD137+CD44+ T cells,83,101 which may be partially attributed to the inhibition of p-ERK/β-catenin signals expressed by DCs.83,102,103 This phenomenon may be the theoretical basis for improving the response to PD-1/PD-L1 mAbs. Previous studies also showed that intestinal microbiota and its products have cross reactivity with tumor neoantigens, which could enhance antigen presentation and stimulate T-cell activation,104,105 while the use of TAB could disrupt intestinal microbiota and was associated with worsened prognosis in patients with aRCC receiving PD-1/PD-L1 mAbs.106

4.2 | Direct effects on immune cells actively involved in cellular immunity

During the activation of T cells, alterations in the expression of costimulatory/coinhibitory molecules on the surface could significantly affect the activity of T cells. In studies related to RCC, researchers demonstrated that CD28 co-stimulation significantly improved the glycolysis and mitochondrial metabolism of CD8+ TLs, thereby improving their mitochondrial and effector functions, possibly by upregulating GLUT3.107 Besides, Joel LeMaoul et al. found two mutually exclusive subgroups in the TME of RCC: CD8+ILT2+PD-1+ TILs and CD8+ILT2- PD-1+ TILs. CD8+ILT2+PD-1+ TILs highly express CD51, perforin (PRF1), and Granzyme-β (GZMB) and secrete more IFN-γ, which has strong cytotoxicity.108 The use of PD-1/ PD-L1 mAbs may promote the differentiation of CD8+PD-1+ TILs toward highly cytotoxic ILT2+ TILs.41 However, the interaction of HLA-G expressed by tumor cells and ILT2 could significantly inhibit the effector function of TILs, and ILT2 blockade could rescue this inhibition,108 indicating the possibility of the combination of HLA-G/ ILT2 blockade and PD-1/PD-L1 blockade. The largest proportion of CD4+ and CD8+ T cells isolated from RCC was that expressing PD-1 and LAG-3, and PD-1 blockade could significantly upregulate LAG-3 expression. In-vitro experiments showed that the dual blockade of LAG-3 and PD-1 could more effectively induce T cells to secrete IFN-γ and improve the function of TILs in TME.109 Moreover, signal lymphocyte activation molecule (SLAM)F7 expressed on tumor-associated macrophages (TAMs) could activate the self-ligand SLAMF7 on T cells in RCC, which
could promote the phosphorylation of STAT1/3 and up-regulate the expression of costimulatory molecules, such as PD-1 and T cell exhaustion-related transcription factors. Eventually, the transformation of CD8+ T cells to terminal exhaustion phenotype is induced. SLAMF7 inhibition may synergistically enhance the blocking effect of PD-1 mAbs, thus promoting cellular immunity.

The cytokines in TME are also involved in cellular immunity. Bempagaldesleukin (NKTR-214), a CD122 (IL-2 receptor β chain) type IL-2 agonist, binds primarily to the heterodimer IL-2βγ and continues to stimulate the IL-2βγ receptor pathway to provide a sustained stimulus signal, thereby prioritizing the stimulation of effector T cells. In combination with nivolumab, it could significantly up-regulate the expression of genes related to CD45+ lymphocytes, CD8+ T cells, macrophages, and natural killer (NK) cells and promote the TME infiltration of CD8+ T cells. Streptavidin-IL-2 surface-modified tumor cell vaccine could enhance the killing effect of specific antitumor T cells in RCC, but it could also induce high PD-1 expression on these cells and upregulate PD-L1 expression in TME. PD-1/PD-L1 mAbs could reverse this immune escape, promote IFN-γ and IL-12 expression, and further enhance the cytotoxicity of specific antitumor T cells. Moreover, glutamine-addicted ccRCC depletes extracellular glutamine and then upregulates HIF-1α expression, thereby inducing tumor-infiltrating macrophages to secrete IL-23. It could promote IL-10 and TGF-β expression and then decrease the IFN-γ, GZMB, and PRF1 expression levels on CD8+ T cells to inhibit their cytotoxicity; thus, IL-23 inhibitors also have a synergistic effect with PD-1 mAbs. However, the IL-10 receptor agonist pegolodecaclin could stimulate CD8+ T cell activity and induce its expansion, and its combination with PD-1 mAbs exhibited stronger antitumor efficacy. This finding suggested that IL-10 may have immunosuppressive and immunostimulatory effects under different conditions in RCC. The specific mechanism still needs to be further explored.

In addition, radiation therapy could positively affect cellular immunity in RCC. SABR irradiation could induce the proliferation of tumor-responsive PD-1+CD11ahighCD8+ T cells in irradiated sites and drainage lymphoid tissues, and PD-1 blockade could improve their antitumor immunity. Their combination induces the regression of unirradiated secondary tumors, known as abscopal effect, which may be caused by the migration of the abovementioned T cells with antitumor activity to the unirradiated site. Except for affecting the activation, proliferation, and function of immune cells, migration is also an important part of cellular immunity. Mavorixafor, an allosteric CXCR4 chemokine receptor inhibitor, improves immune response in patients with RCC who have no response to nivolumab. Researchers found that all patients receiving the combination therapy had elevated levels of CXCL9 chemokine expression, which could promote T-cell activation and migration into TME. Analysis of feces from patients with RCC treated with PD-1/PD-L1 mAb showed that enrichment of A. muciniphila and mucinogen was significantly associated with favorable prognosis. Experiments in vivo showed that fecal microbiota transplantation using respondent feces promoted the aggregation of CXCRT3+CD4+ T cells into tumor tissues and improved the antitumor activity of PD-1 blockade. In RCC, the low expression of adenosine and adenosine 2A receptor (A2AR) was associated with enhanced response to PD-1 mAbs. Previous studies have shown that adenosine inhibited the activity of various antitumor immune cells by binding to A2AR on the surface of immune cells. Studies related to RCC suggested that A2AR inhibitors broadened the circulating T cell pool and promoted the recruitment of CD8+ T cells in TME.

Some therapies could improve T cell activity and induce T cell recruitment simultaneously. DR-BCAT, an RNA interference trigger, targets the CTNNBI gene that encodes β-catenin. Inhibition of CTNNBI expression could promote an increase in the CI4 transcription level, thereby upregulating the expression of marker genes for DCs and CTLs and ultimately promoting the recruitment and cytotoxicity of CD8+ T cells. Acarbose, an alpha-glucoisidase inhibitor, could improve the efficacy of PD-1 mAbs in the mouse model of aRCC. It could promote the recruitment of CD8+ T cells in the TME, and the activation of CD8+ T cells could be enhanced by increasing the proportion of DCs in the tumor and upregulating the expression of the costimulatory ligand CD86 on them. Vascular-targeted photodynamic therapy, which rapidly blocks the associated blood vessels that supply tumor nutrients and leads to tumor necrosis and eradication, also improved cellular immunity in an in-situ RENCA mouse model of lung metastatic RCC through promoting the infiltration of T cells in the metastatic site and increasing the proportion of CD8+ T cells and CD4+FOXP3+T cells. Furthermore, cryoablation could improve T cell activity by upregulating IFN-γ, IL-10, and GZMB expression and promoting the infiltration of CD8+ T cells at the early stage of RCC, indicating that the combination of cryoablation and anti-PD-1 exhibited strong efficacy.

### 4.3 Alterations of immunosuppressive cytokines and cells

The increase in immunosuppressive cytokines and the aggregation and activation of suppressive immune cells
could form an “immune desert” microenvironment, which weakens the efficacy of PD-1/PD-L1 mAbs.\textsuperscript{126–128} Overexpression of IL-1β in TME could drive tumor immune resistance.\textsuperscript{129} RCC-related studies showed that anti-IL-1β could participate in the formation of an immunostimulatory TME through multiple mechanisms, including remodeling the medullary compartment, reducing the infiltration of polymorphonuclear myeloid-derived suppressor cells (MDSCs) in TME, inducing macrophages to polarize to M1-type TAMs and promoting an increase in M1-like tumor necrosis factor, and downregulating the expression of IL-6, CXCL8 and other immunosuppressive cytokines, in which the decrease in CXCL8 may be the potential mechanism of inhibiting the recruitment of MDSCs.\textsuperscript{130,131} IL-1β blockade combined with PD-1 blockade could upregulate IFN-γ, TNF-α, and other inflammatory cytokines and enhance the above antitumor immune response.\textsuperscript{131} In the early stage of PD-1 mAb treatment, obese mice with RCC also showed increased IL-1β and more MDSC infiltration, and this finding is consistent with the above conclusion.\textsuperscript{132} Overexpression of IL-23 in glutamine-addicted renal carcinoma could enhance the proliferative ability and function of regulatory cells (Tregs) and thus improve the efficacy of PD-1 blockade.\textsuperscript{113}

The expression of signal regulatory protein α (SIRPα) expressed on macrophages, as proven to interact with CD47 on target cells to inhibit the phagocytosis of tumor cells by macrophages in breast cancer, was also abnormally increased in RCC.\textsuperscript{133,134} Fortunately, SIRPα mAb (MY-1) could block the above phagocytosis inhibition, induce macrophages to polarize to M1 type, and promote the accumulation of CD8+ T cells and NK cells to impair the proliferation of RENCA cells. Experiments using a colon cancer mouse suggested that MY-1 and PD-1 mAb have synergistic effects that could also be applied in RCC.\textsuperscript{135} The dysfunction of PBRM1 in approximately 41% of patients with RCC also significantly induced the enrichment of IL-6/JAK-STAT and immune-stimulating signals, which could upregulate IFN-γ expression and may promote the formation of immune-stimulating microenvironment, thereby improving the response to PD-1/ PD-L1 mAbs.\textsuperscript{135,136}

Posttranslational modification is also involved in cellular immunity. Entinostat, a selective class I histone deacetylase inhibitor, significantly inhibited the immunosuppressive function of MDSCs and Treg infiltration in TME in a mouse model of renal cancer, thereby enhancing the efficacy of PD-1 mAbs. Entinostat could significantly reduce the levels of arginase-1 (Arg1), iNOS, and COX-2 in MDSC to inhibit its function.\textsuperscript{137} Arg1 could promote L-arginine metabolism in the circulation to induce the cell-cycle arrest of cytotoxic T cells.\textsuperscript{138}
| Type                      | Therapy        | Brief description                                                                 | NCTs                                                      |
|---------------------------|----------------|------------------------------------------------------------------------------------|-----------------------------------------------------------|
| **Drug therapies**        |                |                                                                                    |                                                           |
| Cytokines or their        | IL−2           |                                                                                    | NCT03111901, NCT03260504, NCT03991130                      |
| agonists/inhibitors       | ALKS 4230      | Binding the intermediate affinity IL−2 receptor complex                            | NCT02799095                                              |
|                           | NKTR−214       | A CD122 IL−2 agonist                                                               | NCT02983045, NCT03435640, NCT03729245, NCT04540705      |
|                           | Canakinumab    | IL−1β mAb                                                                          | NCT04028245                                              |
|                           | Gevokizumab    | IL−1β mAb                                                                          | NCT03798626                                              |
|                           | rhIL−15        | Recombinant human IL−15                                                            | NCT04150562                                              |
|                           | SO−C101        | IL−15 receptor alpha recombinant protein                                           | NCT04234113                                              |
|                           | N−803          | IL−15 antagonist                                                                    | NCT03228667                                              |
|                           | GITR           | Glucocorticoid-induced TNF receptor-associated proteins                            | NCT03126110, NCT03277352                                 |
|                           | PegIFN−2β      | Pegylated Interferon Alfa−2β                                                       | NCT02089685                                              |
|                           | NIS793         | TGF−β inhibitor                                                                     | NCT02947165                                              |
|                           | Mogamulizuma   | CCR4 mAb                                                                           | NCT02946671                                              |
|                           | X4P−001        | CXCR4 inhibitor                                                                     | NCT02923531                                              |
|                           | IRX−2          | A multitarget biologic agent containing physiological quantities of IL−1β, IL−2, IFNγ, TNFa, etc. | NCT03758781                                              |
| **Co-inhibitory/co-       | Varililumab    | Anti-CD27 mAb                                                                       | NCT02335918, NCT02543645                                 |
| stimulatory molecules     | BMS−986315     | Anti-NKG2A mAb                                                                     | NCT04349267                                              |
|                           | MBG453         | Tim−3 mAb                                                                          | NCT02608268                                              |
|                           | Relatlimab     | LAG−3 mAb                                                                           | NCT02996110                                              |
|                           | LAG525         | LAG−3 mAb                                                                           | NCT02460224                                              |
|                           | INCAGN01949    | OX−40 mAb                                                                           | NCT03241173                                              |
|                           | APX005 M       | CD40 agonist                                                                        | NCT03502330, NCT04495257                                 |
|                           | CDX−1140       | CD40 agonist                                                                        | NCT03329950                                              |
|                           | INBRX−106      | OX40 agonist                                                                        | NCT04198766                                              |
| **Metabolism-related      | Ciforadenant   | Inhibitor of adenosine A2AR                                                         | NCT02655822                                              |
| molecules                 | NIR178         | Inhibitor of adenosine A2AR                                                         | NCT03207867                                              |
|                           | Etrumadenan    | Inhibitor of adenosine A2AR                                                         | NCT03629756                                              |
|                           | LYT3475070     | Inhibit CD73 to reduce adenosine production                                         | NCT04148937                                              |
|                           | CPI−006        | Inhibit CD73 to reduce adenosine production                                         | NCT03454451                                              |
|                           | Oleclumab      | Inhibit CD73 to reduce adenosine production                                         | NCT04262375                                              |
|                           | CB−839         | Glutaminase inhibitor                                                               | NCT02771626                                              |
| **Genetic alterations**   | PT2385         | HIF−2α inhibitor                                                                    | NCT02293980                                              |
|                           | Itacitinib     | PI3Kδ inhibitor                                                                     | NCT02646748, NCT02899078                                 |
|                           | Savolitinib    | c-MET inhibitor                                                                     | NCT02819596                                              |
|                           | APL−101        | c-MET inhibitor                                                                     | NCT03656113                                              |
|                           | Sitravatinib   | Target multiple RTKs, including c-Kit, c-Met, etc.                                  | NCT04518046, NCT03015740, NCT03680521, NCT03680521       |
|                           | Olaparib       | PARP inhibitor                                                                       | NCT03741426                                              |

(Continues)
| Type                        | Therapy         | Brief description                                                                 | NCTs                           |
|-----------------------------|-----------------|-----------------------------------------------------------------------------------|--------------------------------|
| **Type Therapy**            |                 |                                                                                  |                                |
| Niraparib                   | Selective PARP1 and PARP2 inhibitor | NCT04779151                                                                      |
| Denosumab                   | Receptor activator of NF-κB ligand mAb | NCT03280667                                                                      |
| XmAb*18087                  | A bispecific antibody that recruits T cells via CD3 to kill SSTR2-expressing tumor cells | NCT03849469                      |
| ARRY−614                    | p38-MAPK dual inhibitor | NCT04074967                                                                      |
| Epigenetic alterations      | Guadecitabine   | DNA methyltransferase inhibitor                                                   | NCT03308396                    |
| Posttranslation modification| Vorinostat      | HDAC inhibitor                                                                    | NCT02619253                    |
|                             | Entinostat      | HDAC inhibitor                                                                    | NCT03024437, NCT03552380       |
|                             | HBI−8000        | HDAC inhibitor                                                                    | NCT02718066                    |
| Chemotherapy                | Irinotecan      | DNA topoisomerase I inhibitor                                                     | NCT02423954                    |
|                             | Gemcitabine     | DNA synthesis inhibitor                                                           | NCT03483883                    |
|                             | Cyclophosphamide| Cell cycle specific alkylation agent acting on the S phase                        | NCT04262427                    |
| Affect immunosuppressive cells | FPA008          | CSF1R antibody, causing TAMs exhaustion                                           | NCT02526017                    |
|                             | AZD8701         | Restrict Treg function by inhibiting FOXP3                                        | NCT04504669                    |
|                             | INCB001158      | Arg−1 inhibitor                                                                   | NCT02903914                    |
|                             | KY1044          | kill Tregs that were highly expressed in ICOs by ADCC                            | NCT03829501                    |
|                             | Eganelisib      | PI3K-γ inhibitor targeting M2 type macrophages                                    | NCT03961698                    |
|                             | GB1275          | A molecule modulator of CD11B inhibiting the infiltration of TAMs                 | NCT04060342                    |
| Non-drug therapies          | Radiation therapy | Radiation therapy                                                                | NCT02318771, NCT02962804, NCT02978404 |
|                             | SBRT/SABR       | Focusing radiotherapy of small irradiation field is realized by stereotactic and positioning technology | NCT02599779, NCT02781506, NCT02855203, NCT03693014, NCT04235777, NCT02992912, NCT03065179, NCT03115801, NCT03149159, NCT03511391 |
|                             | Hypofractionated radiation therapy | Increase the dose per exposure and reduce the total number of exposures | NCT03050060                    |
| Vaccine                     | Ankara vaccine  | Modified vaccinia virus vaccine expressing p53                                    | NCT02432963                    |
|                             | RO7198457       | mRNA-based vaccines customized based on sequencing results                         | NCT03289962                    |
|                             | DSP−7888        | WT1 peptide vaccine, inducing WT1-specific CTLs and helper T cells                 | NCT0331133                     |
|                             | GEN−009 adjuvanted vaccine | A tailored vaccine customized by using autologous T cells to identify tumor neoantigens | NCT03633110                    |
| Physical ablation           | Cryoablation    | Laser interstitial thermal therapy                                                | NCT03189186                    |
|                             | LITT            | Laser interstitial thermal therapy                                                | NCT04187872                    |
Combination Therapy Regimens

On the basis of these potential mechanisms, several combination therapeutic regimens have been applied in fundamental experiments or clinical trials related to RCC. Here, therapies that could be combined with PD-1/PD-L1 mAbs and the related clinical trials in RCC were summarized. This summary could be helpful in the selection of treatment options for patients with RCC who have no response to PD-1/PD-L1 mAbs.

5.1 | Classic treatment regimens

AAs or CTLA-4 mAb plus PD-1/PD-L1 mAb are classic treatment regimens, some of which have been approved by the Food and Drug Administration for the treatment of aRCC. Pan-cancer studies demonstrated that VEGF could make vascular abnormalities reduce tumor perfusion and then promote acidosis and hypoxia to form an immunosuppressive TME; it could also downregulate the expression of adhesion molecules on endothelial cells, increase interstitial fluid pressure, and upregulate PD-1 expression on T cells to inhibit the activity and infiltration of T cells.\textsuperscript{139} CTLA-4 could weaken the activity of T cells in lymph nodes and tissues by limiting the co-stimulation of CD28 and inhibit the activity of DC cells by Treg.\textsuperscript{140} Not all of these mechanisms have been demonstrated in RCC, but according to the results in Table 2, these two combination therapies exhibited superior antitumor efficacy over monotherapy, although their safety remains questionable.

5.2 | Other possible combination therapy options

In addition to classic combination therapies, multiple therapies could be combined with PD-1/PD-L1 mAbs to treat aRCC (Table 3). The mechanisms of enhancing the antitumor immunity by some therapies were mentioned above, but more potential preclinical mechanisms must be explored. Encouragingly, IL-2 and its agonist, SABR, and inhibitors targeting multiple receptor tyrosine kinases have shown a strong potential to improve the efficacy of PD-1/PD-L1 mAbs, and they have been used in multiple phase I clinical trials presented in Table 3. These treatments may hold the promise for patients with refractory aRCC.
6 | CONCLUSIONS

This article focused on the existing mechanisms affecting the efficacy of PD-1/PD-L1 mAbs in aRCC. Biomarkers, such as PBRM1 and intratumoral heterogeneity, that may predict the response to PD-1/PD-L1 mAbs were also discussed. According to the existing mechanisms affecting PD-L1 expression in RCC, constitutive PD-L1 overexpression and PD-L1 overexpression induced by long-term tumor neoantigen stimulation may be associated with poor response, while tumor cells stimulated by the immune microenvironment promote the secretion of inflammatory cytokines, such as IFN-γ and IL-1β, thus inducing adaptive PD-L1 overexpression, which is related to enhanced response. Inflammatory cytokines may help distinguish these three situations and predict the curative effect. For patients with refractory aRCC, the clinical transformation of these fundamental mechanisms is particularly important, and the efficacy of combination therapies still needs to be further explored. Although numerous combination therapies based on PD-1/PD-L1 mAbs have been applied in clinical trials, their clinical prevalence still has a long way to go.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in references or can be found in clinicaltrials.gov.

ORCID

Jie Li https://orcid.org/0000-0003-2269-9310

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70:7-30.
2. Jonash E, Gao J, Rathmell WK. Renal cell carcinoma. BMJ. 2014;349: g4797.
3. American Cancer Society. Cancer facts & figures 2017. Available at: https://www.cancer.org/content/dam/cancer-org/research/cancerfacts-and-statistics/annual-cancer-facts-and-figures/2017/cancer-factsand-figures-2017.pdf. Accessed November 1, 2017.
4. Barata PC, Rini BI. Treatment of renal cell carcinoma: current status and future directions. CA Cancer J Clin. 2017;67:507-524.
5. Prattichizzo C, Gigante M, Pontrelli P, et al. Establishment and characterization of a highly immunogenic human renal carcinoma cell line. Int J Oncol. 2016;49:457-470.
6. Senbabaoglu Y, Gejman RS, Winer AG, et al. Tumor immune microenvironment characterization in clear cell renal cell carcinoma identifies prognostic and immunotherapeutically relevant messenger RNA signatures. Genome Biol. 2016;17:231.
7. Atkins MB, Tannir NM. Current and emerging therapies for first-line treatment of metastatic clear cell renal cell carcinoma. Cancer Treat Rev. 2018;70:127-137.
8. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366:2443-2454.
9. Motzer RJ, Escudier B, McDermott DF, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. N Engl J Med. 2015;373:1803-1813.
10. Tang B, Yan X, Sheng X, et al. Safety and clinical activity with an anti-PD-1 antibody JS001 in advanced melanoma or urologic cancer patients. J Hematol Oncol. 2019;12:7.
11. Vaishampayan U, Schoffski P, Ravaud A, et al. Avelumab monotherapy as first-line or second-line treatment in patients with metastatic renal cell carcinoma: phase IIb results from the JAVELIN Solid Tumor trial. J Immunother Cancer. 2019;7:275.
12. McDermott DF, Huseni MA, Atkins MB, 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. 2018;24:749-757.
13. Rini BI, Plimack ER, Stus V, et al. Pembrolizumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. N Engl J Med. 2019;380:1116-1127.
14. Rini BI, Powles T, Atkins MB, 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. The Lancet. 2019;393:2404-2415.
15. Choueiri TK, Motzer RJ, Rini BI, et al. Updated efficacy results from the JAVELIN Renal 101 trial: first-line avelumab plus axitinib versus sunitinib in patients with advanced renal cell carcinoma. Ann Oncol. 2020;31:1030-1039.
16. Motzer RJ, Escudier B, McDermott DF, 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. 2020;8: e000891.
17. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol. 2008;26:677-704.
18. Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. Int Immunol. 2007;19:813-824.
19. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J. 1992;11:387-3895.
20. Noguchi T, Ward JP, Gubin MM, et al. Temporally distinct PD-L1 expression by tumor and host cells contributes to immune escape. Cancer Immunol Res. 2017;5:106-117.
21. Kong H, Zhu G, Tamada K, Chen L. B7–H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. Nat Med. 1999;5:1365-1369.
22. Freeman GJ, Long AJ, Iwai Y, et al. Engagement of the PD-1 immune-inhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med. 2000;192:1027-1034.
23. Yokosuka T, Takamatsu M, Kobayashi-Imanishi W, Hashimoto-Tane A, Azuma M, Saito T. Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphatase SHP2. J Exp Med. 2012;209:1201-1217.
24. Hui E, Cheung J, Zhu J, et al. T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition. Science. 2017;355:1428-1433.
25. Arm JP, Nwankwo C, Austen KF. Molecular identification of a novel family of human Ig superfamily members that possess immunoreceptor tyrosine-based inhibition motifs and homology to the mouse gp49B1 inhibitory receptor. J Immunol. 1997;159:2342-2349.

26. Daeron M, Jaeger S, Du Pasquier L, Vivier E. Immunoreceptor tyrosine-based inhibition motifs: a quest in the past and future. Immuno Rev. 2008;224:11-43.

27. Vely F, Olivero S, Olcse L, et al. Differential association of phosphatases with hematopoietic co-receptors bearing immunoreceptor tyrosine-based inhibition motifs. Eur J Immunol. 1997;27:1994-2000.

28. Klein Geltink RI, O'Sullivan D, Corrado M, et al. Mitochondrial priming by CD28. Cell. 2017;171(385-397):e311.

29. Ogando J, Saez ME, Santos J, et al. PD-1 signaling affects cristae morphology and leads to mitochondrial dysfunction in human CD8(+) T lymphocytes. J Immunother Cancer. 2019;7:151.

30. Sugii D, Maruhashi T, Okazaki IM, et al. Restriction of PD-1 function by cis-PD-L1/CD80 interactions is required for optimal T cell responses. Science. 2019;364:558-566.

31. Zhao Y, Lee CK, Lin CH, et al. PD-L1/B7H-1 inhibits the function by cis-PD-L1/CD80 interactions is required for optimal T cell responses. Science. 2019;364:558-566.

32. Quigley M, Pereyra F, Nilsson B, et al. Transcriptional analysis of HIV-specific CD8+ T cells shows that PD-1 inhibits T cell function by upregulating BATF. Nat Med. 2010;16:1147-1151.

33. Honda T, Egen JG, Lammermann T, Kastenmüller W, Torabi-Parizi P, Germain RN. Tuning of antigen sensitivity by T cell receptor-dependent negative feedback controls T cell effector function in inflamed tissues. Immunity. 2014;40:235-247.

34. Gupta S, Cheville JC, Junghluth AA, et al. JAK2/PD-L1/PD-L2 (9p24.1) amplifications in renal cell carcinomas with sarcomatoid transformation: implications for clinical management. Mod Pathol. 2019;32:1344-1358.

35. Marzec M, Zhang Q, Goradia A, et al. Oncogenic kinase NPM/ALK induces through STAT3 expression of immunosuppressive protein CD274 (PD-L1, B7-H1). Proc Natl Acad Sci U S A. 2008;105:20852-20857.

36. Parsa AT, Waldron JS, Panner A, et al. Loss of tumor suppressor PTEN function increases B7–H1 expression and immunoresistance in glioma. Nat Med. 2007;13:84-88.

37. Chen S, Crabill GA, Pritchard TS, et al. Mechanisms regulating PD-L1 expression on tumor and immune cells. J Immunother Cancer. 2019;7:305.

38. Blank C, Brown I, Peterson AC, et al. PD-L1/B7H-1 inhibits the effector phase of tumor rejection by T cell receptor (TCR) transgenic CD8+ T cells. Cancer Res. 2004;64:1140-1145.

39. He YF, Zhang GM, Wang XH, et al. Blocking programmed death-1 ligand-PD-1 interactions by local gene therapy results in enhancement of antitumor effect of secondary lymphoid tissue chemokine. J Immunol. 2004;173:4919-4928.

40. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366:2455-2465.

41. Choueiri TK, Fishman MN, Escudier B, et al. Immunomodulatory activity of nivolumab in metastatic renal cell carcinoma. Clin Cancer Res. 2016;22:5461-5471.

42. McDermott DF, Solson JA, Szol M, et al. Atezolizumab, an anti-programmed death-ligand 1 antibody, in metastatic renal cell carcinoma: long-term safety, clinical activity, and immune correlates from a phase 1a study. J Clin Oncol. 2016;34:833-842.

43. Motzer RJ, Rini BI, McDermott DF, et al. Nivolumab for metastatic renal cell carcinoma: results of a randomized phase II trial. J Clin Oncol. 2015;33:1430-1437.

44. McDermott DF, Lee JL, Zibro M, et al. Open-label, single-arm, phase II study of pembrolizumab monotherapy as first-line therapy in patients with advanced non-clear cell renal cell carcinoma. J Clin Oncol. 2021;39(9):1029-1039.

45. McDermott DF, Lee JL, Bjarnason GA, et al. Open-label, single-arm phase II study of pembrolizumab monotherapy as first-line therapy in patients with advanced clear cell renal cell carcinoma. J Clin Oncol. 2021;39(9):1020-1028.

46. Thompson RH, Tong H, Kwon ED. Implications of B7–H1 expression in clear cell carcinoma of the kidney for prognostication and therapy. Clin Cancer Res. 2007;13:709s-715s.

47. Motzer RJ, Tannir NM, McDermott DF, et al. Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma. N Engl J Med. 2018;378:1277-1290.

48. Motzer RJ, Penkov K, Haenen J, et al. Avelumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. N Engl J Med. 2019;380:1103-1115.

49. Callea M, Albiges L, Gupta M, et al. Differential expression of PD-L1 between primary and metastatic sites in clear-cell renal cell carcinoma. Cancer Immunol Res. 2015;3:1158-1164.

50. Kluger HM, Zito CR, Turcu G, et al. PD-L1 studies across tumor types, its differential expression and predictive value in patients treated with immune checkpoint inhibitors. Clin Cancer Res. 2017;23:4270-4279.

51. Zhu J, Armstrong AJ, Friedlander TW, et al. Biomarkers of immunotherapy in urothelial and renal cell carcinoma: PD-L1, tumor mutational burden, and beyond. J Immunother Cancer. 2018;6:4.

52. Sato Y, Yoshizato T, Shiraiishi Y, et al. Integrated molecular analysis of clear-cell renal cell carcinoma. Nat Genet. 2013;45:860-867.

53. Chakraborty AA. Coalescing lessons from oxygen sensing, tumor metabolism, and epigenetics to target VHL loss in kidney cancer. Semin Cancer Biol. 2020;67:34-42.

54. Messai Y, Gad S, Noman MZ, et al. Renal cell carcinoma programmed death-ligand 1, a new direct target of hypoxia-inducible factor-2 Alpha, is regulated by von hippel-lindau gene mutation status. Eur Urol. 2016;70:623-632.

55. Ruf M, Moch H, Schraml P. PD-L1 expression is regulated by hypoxia inducible factor in clear cell renal cell carcinoma. Int J Cancer. 2016;139:396-403.

56. Miyata Y, Kanetake H, Kanda S. Presence of phosphorylated hepatocyte growth factor receptor/c-Met is associated with tumor progression and survival in patients with conventional renal cell carcinoma. Clin Cancer Res. 2006;12:4876-4881.

57. Natali PG, Prat M, Nicotra MR, et al. Overexpression of the met/HGF receptor in renal cell carcinomas. Int J Cancer. 1996;69:212-217.

58. Balan M, Mier y Teran E, Waaga-Gasser AM, et al. Novel roles of c-Met in the survival of renal cancer cells through the regulation of HO-1 and PD-L1 expression. J Biol Chem. 2015;290:8110-8120.

59. Kammerer-Jacquet SF, Medane S, Bensalah K, et al. Correlation of c-MET expression with PD-L1 expression in metastatic clear
65. Clarke EV, Weist BM, Walsh CM, Tenner AJ. Complement protein C1q bound to apoptotic cells suppresses human macrophage- and dendritic cell-mediated Th17 and Th1 T cell subset proliferation. *J Leukoc Biol*. 2015;97:147-160.

66. Casuscelli J, Weinhold N, Gundem G, et al. Genomic landscape and evolution of metastatic chromophobe renal cell carcinoma. *JCI Insight*. 2017;2:e92688.

67. Malouf GG, Ali SM, Wang K, et al. Genomic characterization of renal cell carcinoma with sarcomatoid dedifferentiation: point mutations in SMARCB1. *Cancer Immunol Res*. 2018;6:711-722.

68. Garcia-Diaz A, Shin DS, Walsh CM, Tenner AJ. Complement protein C1q bound to apoptotic cells suppresses human macrophage and dendritic cell-mediated Th17 and Th1 T cell subset proliferation. *J Leukoc Biol*. 2015;97:147-160.

69. Green MR, Monti S, Rodig SJ, et al. Interferon receptor signaling pathways regulating PD-L1 and PD-L2 expression.*Cancer Immunol Res*. 2016;7:156.

70. Incorvaia L, Fanale D, Badalamenti G, et al. A "Lymphocyte phage and dendritic cell-mediated Th17 and Th1 T cell subset proliferation.*J Leukoc Biol*. 2015;97:147-160.

71. Qu F, Ye J, Pan X, et al. MicroRNA-497-5p down-regulation increases PD-L1 expression in clear cell renal cell carcinoma. *J Drug Target*. 2019;27:67-74.

72. Qin Z, Hu H, Sun W, et al. miR-224-5p contained in urinary extracellular vesicles regulates PD-L1 expression by inhibiting cyclin D1 in renal cell carcinoma cells. *Cancers*. 2021;13(4):618.

73. Panda A, de Cubas AA, Stein M, et al. Endogenous retrovirus expression is associated with response to immune checkpoint blockade in clear cell renal cell carcinoma. *JCI Insight*. 2018;3:e121522.

74. Yao JX, Chen X, Zhu YJ, Wang H, Hu XY, Guo JM. Prognostic value of vimentin is associated with immunosuppression in metastatic renal cell carcinoma. *Front Oncol*. 2020;10:1181.

75. Asgarova A, Asgarov K, Godet Y, et al. PD-L1 expression is regulated by both DNA methylation and NF-kB during EMT signaling in non-small cell lung carcinoma. *Oncoimmunology*. 2018;7:e1423170.

76. Monteiro-Reis S, Lobo J, Henrique R, Jeronimo C. Epigenetic mechanisms influencing epithelial to mesenchymal transition in bladder cancer. *Int J Mol Sci*. 2019;20(2):297.

77. Gao Y, Nihira NT, Bu X, et al. Acetylation-dependent regulation of PD-L1 nuclear translocation dictates the efficacy of anti-PD-1 immunotherapy. *Nat Cell Biol*. 2020;22:1064-1075.

78. Asgarova A, Asgarov K, Godet Y, et al. PD-L1 expression is associated with response to immune checkpoint blockade in clear cell renal cell carcinoma. *Target Oncol*. 2020;15:377-390.

79. Cerezo M, Guemiri R, Drulliennec S, et al. Translational control of tumor immune escape via the eIF4F-STAT1-PD-L1 axis in melanoma. *Nat Med*. 2018;24:1877-1886.

80. Liu XD, Hoang A, Zhou L, et al. Resistance to antiangiogenic therapy is associated with an immunosuppressive tumor microenvironment in metastatic renal cell carcinoma. *Cancer Immunol Res*. 2015;3:1017-1029.

81. Zizzari IG, Napolitano C, Botticelli A, et al. TK inhibitor pazopanib primes DCs by downregulation of the beta-catenin pathway. *Cancer Immunol Res*. 2018;6:711-722.

82. Sabarwal A, Chakraborty S, Mahanta S, Banerjee S, Balan M, Pal S. A novel combination treatment with honokiol and Rapamycin effectively restricts c-met-induced growth of renal cancer cells, and also inhibits the expression of tumor cell PD-L1 involved in immune escape. *Cancers (Basel)*. 2020;12(7):1782.

83. Liu K, Zhou Z, Gao H, et al. JQ1, a BET-bromodomain inhibitor, inhibits human cancer growth and suppresses PD-L1 expression. *Cell Biol Int*. 2019;43:642-650.

84. Margulis V, Freifeld Y, Pop LM, et al. Neoadjuvant SAbR for renal cell carcinoma inferior vena cava tumor thrombus - safety lead-in results of a phase II trial. *Int J Radiat Oncol Biol Phys*. 2021;110(4):1135-1142.

85. Yu Y, Liang Y, Li D, et al. Glucose metabolism involved in PD-L1-mediated immune escape in the malignant kidney tumour microenvironment. *Cell Death Discov*. 2021;7:15.

86. Faiena I, Ueno D, Shuch B. Glutamine and the tumor immune microenvironment. *Eur Urol*. 2019;75:764-765.

87. Wettersten HI, Hakini AA, Morin D, et al. Grade-dependent metabolic reprogramming in kidney cancer revealed by combined proteomics and metabolomics analysis. *Cancer Res*. 2015;75:2541-2552.

88. Ma G, Liang Y, Chen Y, et al. Glutamine deprivation induces PD-L1 expression via activation of EGFR/ERK/c-Jun signaling in renal cancer. *Mol Cancer Res*. 2020;18:324-339.
94. Li H, Bullock K, Gurjao C, et al. Metabolomic adaptations and correlates of survival to immune checkpoint blockade. *Nat Commun.* 2019;10:4346.
95. Munn DH, Sharma MD, Lee JR, et al. Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. *Science.* 2002;297:1867-1870.
96. Jardim DL, Goodman A, de Melo GD, Kurzrock R. The challenges of tumor mutational burden as an immunotherapy biomarker. *Cancer Cell.* 2021;39:154-173.
97. Tucker MD, Rini BI. Predicting response to immunotherapy in metastatic renal cell carcinoma. *Cancers (Basel).* 2020;12(9):2662.
98. Labriola MK, Zhu J, Gupta RT, et al. Characterization of tumor mutation burden, PD-L1 and DNA repair genes to assess relationship to immune checkpoint inhibitors response in metastatic renal cell carcinoma. *J Immunother Cancer.* 2020;8(1):e000319.
99. Ran X, Xiao J, Zhang Y, et al. Low intratumor heterogeneity correlates with increased response to PD-1 blockade in renal cell carcinoma. *Ther Adv Med Oncol.* 2020;12:1758835920977117.
100. Yuen KC, Liu LF, Gupta V, et al. High systemic and tumor-associated IL-8 correlates with reduced clinical benefit of PD-L1 blockade. *Nat Med.* 2020;26:693-698.
101. Moser M, Murphy KM. Dendritic cell regulation of TH1-TH2 development. *Nat Immunol.* 2000;1:199-205.
102. Manoharan I, Hong Y, Suryawanshi A, et al. TLR2-dependent activation of beta-catenin pathway in dendritic cells induces regulatory responses and attenuates autoimmune inflammation. *J Immunol.* 2014;193:4203-4213.
103. Puig-Kroger A, Reilloo M, Fernandez-Capetillo O, et al. Extracellular signal-regulated protein kinase signaling pathway negatively regulates the phenotypic and functional maturation of monocyte-derived human dendritic cells. *Blood.* 2001;98:2175-2182.
104. Tai N, Peng J, Liu F, et al. Microbial antigen mimics activate diabetogenic CD8 T cells in NOD mice. *J Exp Med.* 2016;213:2129-2146.
105. Zitvogel L, Ayyoub M, Routy B, Kroemer G. Microbiome and anticancer immunosurveillance. *Cell.* 2016;165:276-287.
106. Routy B, Le Chatelier E, Derosa L, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science.* 2018;359:91-97.
107. Beckermann KE, Hongo R, Ye X, et al. CD28 costimulation drives tumor-infiltrating T cell glycosylation to promote inflammation. *JCI. Insights.* 2020;5.
108. Dumont C, Jacquier A, Verine J, et al. CD8(+)PD-1(-)ILT2(+)) T cells are an intratumoral cytotoxic population selectively inhibited by the immune-checkpoint HLA-G. *Cancer Immunol Res.* 2019;7:1619-1632.
109. O’Connell P, Hyslop S, Blake MK, Godbehere S, Amalfitano A, Aldhamen YA. SLAMF7 signaling reprograms T cells toward exhaustion in the tumor microenvironment. *J Immunol.* 2021;206:193-205.
110. Charych DH, Hoch U, Langowski JL, 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.* 2016;22:680-690.
111. Diab A, Tannir NM, Bentebibel SE, et al. Bempagolidesleukin (NKTR-214) plus nivolumab in patients with advanced solid tumors: phase I dose-escalation study of safety, efficacy, and immune activation (PIVOT-02). *Cancer Discov.* 2020;10:1158-1173.
112. Zhu X, Shi X, Li J, et al. Combination immunotherapy with interleukin-2 surface-modified tumor cell vaccine and programmed death receptor-1 blockade against renal cell carcinoma. *Cancer Sci.* 2019;110:31-39.
113. Fu Q, Xu L, Wang Y, et al. Tumor-associated macrophage-derived interleukin-23 interlinks kidney cancer glutamine addiction with immune evasion. *Eur Urol.* 2019;75:752-763.
114. Naing A, Wong DJ, Infante JR, et al. Pegilodecakin combined with pembrolizumab or nivolumab for patients with advanced solid tumours (IVY): a multicentre, multicohort, open-label, phase 1b trial. *Lancet Oncol.* 2019;20:1544-1555.
115. Park SS, Dong H, Liu X, et al. PD-1 restrains radiotherapy-induced abscessal effect. *Cancer Immunol Res.* 2015;3:610-619.
116. Bedognetti D, Spivey TL, Zhao Y, et al. CXCR3/CCR5 pathways in metastatic melanoma patients treated with adoptive therapy and interleukin-2. *Br J Cancer.* 2013;109:2412-2423.
117. Choueiri TK, Atkins MB, Rose TL, et al. A phase 1b trial of the CXCR4 inhibitor mavrixavor and nivolumab in advanced renal cell carcinoma patients with no prior response to nivolumab monotherapy. *Invest New Drugs.* 2021;39(4):1019-1027.
118. Kamai T, Kijima T, Tsuzuki T, et al. Increased expression of adenosine 2A receptors in metastatic renal cell carcinoma is associated with poorer response to anti-vascular endothelial growth factor agents and anti-PD-1/Anti-CTLA4 antibodies and shorter survival. *Cancer Immunol Immunother.* 2021;70(7):2009-2021.
119. Ohta A, Gorelik E, Prasad SJ, et al. A2A adenosine receptor protects tumors from antitumor T cells. *Proc Natl Acad Sci U S A.* 2006;103:13132-13137.
120. Fong L, Hotson A, Powderly JD, et al. Adenosine 2A receptor blockade as an immunotherapy for treatment-refractory renal cell cancer. *Cancer Discov.* 2020;10:40-53.
121. Ganesh S, Shui X, Craig KP, et al. RNAi-mediated beta-catenin inhibition promotes T cell infiltration and antitumor activity in combination with immune checkpoint blockade. *Mot Ther.* 2018;26:2567-2579.
122. Orlandella RM, Turbitt WJ, Gibson JT, et al. The antidiabetic agent acarbose improves anti-PD-1 and rapamycin efficacy in preclinical renal cancer. *Cancers (Basel).* 2020;12(10):2872.
123. Murray KS, Winter AG, Corradi RB, et al. Treatment effects of WST11 vascular targeted photodynamic therapy for urothelial cell cancer in swine. *J Urol.* 2016;196:236-243.
124. O’Shaughnessy MJ, Murray KS, La Rosa SP, et al. Systemic antitumor immunity by PD-1/PD-L1 inhibition is potentiated by vascular-targeted photodynamic therapy of primary tumors. *Clin Cancer Res.* 2018;24:592-599.
125. Zhu C, Lin S, Liang J, Zhu Y. PD-1 blockade enhances the antitumor immune response induced by cryoablation in a murine model of renal cell carcinoma. *Cryobiology.* 2019;87:86-90.
126. Dannenmann SR, Thielicke J, Stockl M, et al. Tumor-associated macrophages subvert T-cell function and correlate with reduced survival in clear cell renal cell carcinoma. *Oncoimmunology.* 2013;2:e23562.
127. Togashi Y, Shitara K, Nishikawa H. Regulatory T cells in cancer immunosuppression - implications for anticancer therapy. *Nat Rev Clin Oncol.* 2019;16:356-371.
128. Weber R, Fleming V, Hu X, et al. Myeloid-derived suppressor cells hinder the anti-cancer activity of immune checkpoint inhibitors. Front Immunol. 2018;9:1310.

129. Rider P, Carmi Y, Gutman O, et al. IL-1alpha and IL-1beta recruit different myeloid cells and promote different stages of sterile inflammation. J Immunol. 2011;187:4835-4843.

130. Lopez-Bujanda ZA, Haffner MC & Chaimowitz MG. Castration-mediated IL-8 promotes myeloid infiltration and prostate cancer progression. Cold Spring Harbor Lab. 2019. https://doi.org/10.1101/651083.

131. Aggen DH, Ager CR, Obradovic AZ, et al. Blocking IL1 beta promotes tumor regression and remodeling of the myeloid compartment in a renal cell carcinoma model: multidimensional analyses. Clin Cancer Res. 2021;27:608-621.

132. Boi SK, Orlandella RM, Gibson JT, et al. Obesity diminishes response to PD-1-based immunotherapies in renal cancer. J Immunother Cancer. 2020;8.

133. Yanagita T, Murata Y, Tanaka D, et al. Anti-SIRPalpha antibodies as a potential new tool for cancer immunotherapy. JCI Insight. 2017;2:e89140.

134. Zhao XW, van Beek EM, Schornagel K, et al. CD47-signal regulatory protein-alpha (SIRPalpha) interactions form a barrier for antibody-mediated tumor cell destruction. Proc Natl Acad Sci U S A. 2011;108:18342-18347.

135. Miao D, Margolis CA, Gao W, et al. Genomic correlates of response to immune checkpoint therapies in clear cell renal cell carcinoma. Science. 2018;359:801-806.

136. Gerlinger M, Horswell S, Larkin J, et al. Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencing. Nat Genet. 2014;46:225-233.

137. Orillion A, Hashimoto A, Damayanti N, et al. Entinostat neutralizes myeloid-derived suppressor cells and enhances the antitumor effect of PD-1 inhibition in murine models of lung and renal cell carcinoma. Clin Cancer Res. 2017;23:5187-5201.

138. Lu T, Gabriovich DI. Molecular pathways: tumor-infiltrating myeloid cells and reactive oxygen species in regulation of tumor microenvironment. Clin Cancer Res. 2012;18:4877-4882.

139. Zhu N, Weng S, Wang J, et al. Preclinical rationale and clinical efficacy of antiangiogenic therapy and immune checkpoint blockade combination therapy in urogenital tumors. J Cancer Res Clin Oncol. 2019;145:3021-3036.

140. Rotte A. Combination of CTLA-4 and PD-1 blockers for treatment of cancer. J Exp Clin Cancer Res. 2019;38:255.

141. Rini BI, Powles T, Atkins MB, 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. 2019;393:2404-2415.