Current Urology Reports (2019) 20: 1
https://doi.org/10.1007/s11934-019-0866-8

KIDNEY DISEASES (G CIANCIO, SECTION EDITOR)

A Critical Insight into the Clinical Translation of PD-1/PD-L1 Blockade Therapy in Clear Cell Renal Cell Carcinoma

Caroline E. Nunes-Xavier[1,2] · Javier C. Angulo[3,4] · Rafael Pulido[1,5] · José I. López[1,6,7]

Published online: 15 January 2019

Abstract

Purpose of Review Targeting PD-1/PD-L1 immune checkpoints is a new therapeutic tool in patients with locally advanced and metastatic clear cell renal cell carcinoma (CCRCC). The purpose of this review is to offer clinicians an updated translational insight into the current status of a therapeutic alternative that may impact significantly patient's life.

Recent Findings Immune checkpoint inhibition has recently demonstrated promising results in selected CCRCC patients with respect to tumor progression and survival. The decision to treat these patients with immune checkpoint inhibitors (ICI) relies on the immunohistochemical detection of PD-1/PD-L1 positivity in inflammatory cells in the tumor, which makes the role of the pathologist crucial, but clinical concern upon the reliability to use immunohistochemistry (IHC) to predict therapeutic response is increasing.

Summary We review the state of the art of the immune checkpoint inhibition in CCRCC, from the basic science and its fundamentals to the daily application in clinical routine.

Keywords Clear cell renal cell carcinoma · Immune checkpoint inhibition · PD-1 · PD-L1 · Immunohistochemistry · Sampling protocols · Targeted therapy

Introduction

Renal cancer is a common malignancy ranking in the top-ten lists of the most frequent neoplasms in Western countries. More than 64,000 new cases (up to 42,000 cases in males and 22,000 in females) are expected in 2018 in the USA [1]. Clear cell renal cell carcinoma (CCRCC) is the most common histologic variant, accounting for more than 70% of the total cases [2]. CCRCC is a complex disease. On the one hand, it has been traditionally resistant to radiotherapy and chemotherapy and only surgery has demonstrated a significant impact on survival [3]. On the other, CCRCC is a paradigmatic example of inter- and intratumor heterogeneity (ITH) [4, 5], which makes its clinical evolution unpredictable. Very recent whole genomic studies in large series of CCRCC have demonstrated up to seven different evolutionary routes [6••] and two different patterns of metastatic seed [7••] thus confirming at the molecular level the high complexity of this disease.

This article is part of the Topical Collection on *Kidney Diseases*

✉ José I. López
jilpath@gmail.com

Caroline E. Nunes-Xavier
caroliten@gmail.com

Javier C. Angulo
jangulo@futurnet.es

Rafael Pulido
rpulidomurillo@gmail.com

[1] Biomarkers in Cancer Unit, Biocruces-Bizkaia Health Research Institute, Barakaldo, Bizkaia, Spain

[2] Department of Tumor Biology, Institute for Cancer Research, Oslo University Hospital Radiumhospitalet, Oslo, Norway

[3] Department of Urology, University Hospital of Getafe, Getafe, Madrid, Spain

[4] Clinical Department, European University of Madrid, Laureate Universities, Madrid, Spain

[5] IKERBASQUE, The Basque Foundation for Science, Bilbao, Spain

[6] Department of Pathology, Cruces University Hospital, Plaza de Cruces s/n, 48903 Barakaldo, Bizkaia, Spain

[7] Department of Medical-Surgical Specialties, University of the Basque Country (UPV/EHU), Leioa, Bizkaia, Spain

This high genomic variability is responsible for many therapeutic failures in CCRCC. Immune checkpoint inhibitors (ICI), alone or in combination with drugs promoting anti-angiogenesis, i.e., tyrosine kinase inhibitors (TKI), are promising new options for patients with advanced disease [8]. In brief, *VHL* gene malfunction, the hallmark of CCRCC genesis, increases the angiogenic activity via overexpression of the hypoxia inducible factor-α (HIF-α) in concert with checkpoint inhibition. Both *VHL* gene inactivation [9] and HIF-α silencing [10] have shown to inhibit the expression of the checkpoint programmed death ligand 1 (PD-L1). Other authors, however, suggest that PD-L1 expression correlates with non-inactivated *VHL* gene [11], what depicts a complex and still not well understood scenario in the regulation of PD-L1 in CCRCC. Although ICI are showing great promise in advanced renal cancer treatment, not all patients benefit from these therapies. Taking into consideration that the incidence of CCRCC is predicted to increase during the next decade mainly due to obesity and aging, finding effective predictive biomarkers in these tumors is a priority in modern oncology [12, 13•].

At present, the assessment of immune checkpoint status relies on the immunohistochemical identification by the pathologist of one of the ligands of programmed death-1 (PD-1) in the mononuclear inflammatory cells inside the tumor, or in the tumor cells. However, this assessment involves several concerns considering the high ITH that some CCRCC display. In this regard, very recent studies have demonstrated a high variability in the regional PD-L1 expression in CCRCC [14, 15] and in non-small cell lung cancer [16], what makes the extent of tumor sampling and immune-testing a crucial issue. An insufficient sampling and/or testing might be among the causes of the non-expected good response to anti-PD-L1 drugs observed in up to 17% of patients whose tumors apparently did not express PD-L1 [12]. Although the contrary also may happen, with some authors pointing to a possible association between intracellular metabolic factors as responsible for the resistance to anti-PD-1 therapy in patients who do express PD-L1 by IHC [17].

This review offers to the clinicians a translational update of the state of the art of ICI in CCRCC, from the basic science to the clinical application and future trends in the field.

Molecular and Cellular Aspects of PD-1/PD-L1 Axis in CCRCC

Human PD-1 (official gene name *PDCD1*; 288 amino acids in its longer form) is a type I transmembrane glycoprotein that belongs to the CD28/CTLA-4 immune checkpoint receptor family. PD-1 is monomeric and contains a single immunoglobulin-like variable (IgV) domain in its N-terminal extracellular region, which mediates PD-1 binding to its ligands PD-L1 and PD-L2 [18–21]. PD-1 intracellular region encompasses about 95 amino acids, and contains two immunoreceptor tyrosine-based (ITIM and ITSM) regulatory motifs, which undergo tyrosine phosphorylation and mediate the binding to the SH2-domain containing tyrosine phosphatases PTPN6 (SHP1) and PTPN11 (SHP2), favoring the inhibition of T and B cell antigen receptor-mediated signaling [22–25] (Fig. 1a). A serum soluble variant of PD-1 (PD-1Δex3; sPD-1) has been found [26], although its relevance in CCRCC remains to be determined.

Human PD-L1 (also known as B7-H1; official gene name *CD274*; 290 amino acids in its longer form) is a type I transmembrane glycoprotein that belongs to the B7 family of immune checkpoint proteins. PD-L1 contains one IgV-like and one immunoglobulin-like constant (IgC) domain in its extracellular PD-1 binding region, and a short C-terminal intracellular region of about 30 amino acids, which is important for the transmission of cell survival signals [24, 27, 28] (Fig. 1a). Human PD-L2 (also known as B7-DC, official gene name *PDCD1LG2*; 273 amino acids in its longer form) is the other PD-1 ligand and the closer related protein to PD-L1, with a similar arrangement of Ig-like domains and about 40% amino acid identity in its extracellular region, but divergent in its short intracellular C-terminal portion [20]. PD-L1 and PD-L2 shorter isoforms have been described which lack Ig-like domains or the transmembrane and intracellular regions, but their physiologic role is unclear [29, 30]. Both PD-L1 and PD-L2 are expressed in kidney epithelial cells under basal conditions and are upregulated in an inflammatory-dependent-induced manner [31–33] (Fig. 1a). A soluble form of PD-L1 has been detected in the serum of CCRCC patients in association with tumor aggressiveness [34], although its clinical correlation was not confirmed in a separate study [10].

PD-1 expression is well known in hematopoietic tissues and cells, including T cells, B cells, NK cells, monocytes/macrophages, and dendritic cells, although recent findings have revealed PD-1 expression in several non-hematopoietic cancer cell types [35]. PD-1 expression is rapidly induced upon T or B cell antigen stimulation, or upon lymphoid cell activation conditions, such as lipopolysaccharide or proinflammatory cytokine stimulation [24, 25, 36]. Major transcription factors regulating positively PD-1 gene expression include NFAT, STATs, NF-κB, and IRFs [25, 28]. In RCC patients, PD-1 is highly expressed on the surface of both activated tumor-infiltrating mononuclear immune cells and peripheral blood mononuclear cells, in association with higher tumor staging and poor outcome [37, 38].

The expression pattern of PD-L1 is wider, displaying both constitutive and inducible expression in lymphoid, myeloid, and endothelial cells [24, 27, 39]. PD-L1 expression is high in many human cancers, both in the tumor-infiltrating immune cells and in the tumor cells [25, 40, 41]. The PD-L1 gene promoter harbors functional hypoxia response elements which

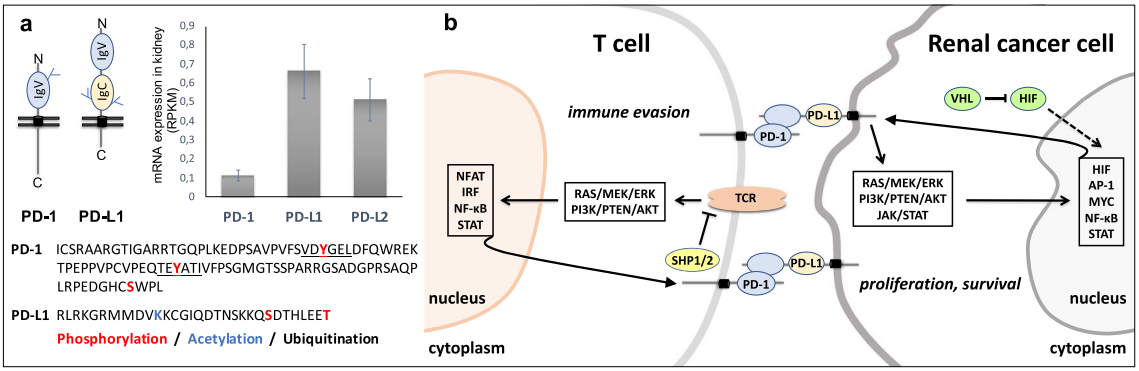

Fig. 1 PD-1/PD-L1 axis in renal cancer. **a.** Top left, a schematic depiction of the domain composition of PD-1 and PD-L1, including their transmembrane regions (black boxes), is shown. C, C-terminal intracellular region; N, N-terminal extracellular region; IgV, immunoglobulin-like variable; IgC, immunoglobulin-like constant. Glycosylation at the extracellular regions is indicated with branched lines. Bottom, the amino acid sequences (one-letter code) of the human PD-1 and PD-L1 intracellular regions are indicated. The ITIM (sequence VDYGEL) and ITSM (sequence TEYATI) motifs in PD-1 are underlined. Residues which can be phosphorylated are in red, and residues which can be acetylated are in blue (https://www.phosphosite.org/; and references in the text). PD-L1 can also be ubiquitinated, but the affected residues have not been identified. Top right, mRNA expression in kidney of PD-1, PD-L1, and PD-L2 is shown, from NCBI Gene Resource (https://www.ncbi.nlm.nih.gov/; RPKM, reads per kilo base per million mapped reads). **b.** Schematic of cell signaling mediating PD-1/PD-L1 expression, immune evasion, and pro-tumorigenesis in T cells and renal cancer cells. The square boxes in the cytoplasm illustrate relevant signal transduction pathways activated through the T cell receptor (TCR) in T cells or, hypothetically, through PD-L1 in renal cancer cells. The tyrosine phosphatases SHP1/2 are major effectors of the attenuation in T cells of TCR-mediated signaling triggered upon PD-1/PD-L1 binding. The square boxes in the cell nucleus illustrate transcription factors that promote PD-1 and PD-L1 expression. The relevance of the VHL/HIF pathway in renal cancer cells is depicted

mediate binding to the transcription factors HIF-1α and HIF-2α enhancing PD-L1 gene expression [9, 42]. Accordingly, PD-L1 expression in CCRCC has been correlated with *VHL* inactivation and HIF-2α expression [9, 43]. NF-κB, STAT1, STAT3, AP-1, and MYC also bind and transactivate PD-L1 promoter, regulating positively PD-L1 transcription in both hematopoietic and tumor cells in response to inflammatory cytokines or growth factor stimulation [25, 28] (Fig. 1b), although the specific role of these transcription factors in the regulation of PD-L1 expression in renal cancer cells remains unexplored.

As mentioned above, the major effect of PD-1/PD-L1 interaction accounting for immune evasion relies on the negative signaling on the antigen receptor complexes, transduced through SHP1/2 tyrosine phosphatases, which triggers the reversal of CD3-complex tyrosine phosphorylation and the decline in cytokine production and lymphocyte proliferation [19, 23, 44]. In consequence, critical lymphocyte activation-, cell cycle-, and pro-survival pathways, including PI3K/PTEN/AKT and RAS/MEK/ERK pathways, are attenuated through PD-1-facilitated signaling [45, 46] (Fig. 1b). Negative regulation by PD-1 of PI3K/AKT signaling in T cells has also been proposed on the basis of its positive effect on PTEN phosphatase activity via CK2-mediated phosphorylation [47], in line with the observed association between PTEN loss and resistance to PD-1-based immunotherapy in several human cancers [48, 49]. Of interest, PTEN protein loss has been associated with renal carcinogenesis, although its prognostic value is unclear [50–52]. PD-L1 also plays pro-tumorigenic reverse signaling roles in cancer cells upon binding to its receptors in hematopoietic cells, which results in activation of proliferative and survival signaling pathways. This further fuels the expression of PD-L1, and subsequent tumor progression [53] (Fig. 1b). In addition, PD-L1 has been shown to exert non-immune proliferative roles in a variety of tumor cell types [54–56]. Noticeably, in renal carcinoma cells, PD-L1 induced epithelial to mesenchymal transition (EMT) and stem cell-like phenotypes [57], suggesting the existence of PD-L1 intrinsic pathways promoting renal cancer progression, which could be clinically relevant in the response to PD-1/PD-L1 immunotherapies.

Pathological Approach to PD-1/PD-L1 Blockade in CCRCC

Many CCRCC are aggressive neoplasms with different molecular profiles influencing treatment response [58]. Clinical aggressiveness in these neoplasms has been recently correlated with specific mutational statuses [6••]. In particular, it has been reported that a subset of CCRCC characterized by high chromosome complexity and losses of 9p and 14q has an increased metastatic potential [7••]. Targeting immune checkpoints in renal cancer is being extensively analyzed in CCRCC in the last years. As an example, at least two meta-analyses [59, 60] and some series [61–63] have confirmed the adverse effects of PD-L1 expression in the prognosis and survival of CCRCC.

The next horizon to treat advanced CCRCC has been very recently reviewed [8]. TKI are being used in combination with ICI with positive results in CCRCC due to the fact that PD-L1 downregulation is related to HIF-α inhibition [10]. In this

sense, the monoclonal antibody (mAb) bevacizumab, inhibiting the binding of vascular endothelial growth factor (VEGF) to its receptors, and the mammalian target of rapamycin (mTOR) inhibitor temsirolimus have improved both progression-free and overall survivals of CCRCC patients [64•, 65]. Some evidences also hypothesize that ICI and some genes involved in the EMT process may have a combined synergic effect on tumor progression and metastatic potential [66].

Since US Food and Drug Administration (FDA) approved anti-CTLA-4 ipilimumab for the treatment of advanced melanoma in 2011, other neoplasms have been subsequently approved for diverse anti-CTLA-4 or anti-PD-L1 drugs in particular clinical settings [11]. Nivolumab, an anti-PD-1 mAb, was approved for advanced renal cell carcinoma treatment in second line in 2015 [11] and atezolizumab, an anti-PD-L1 mAb, is in the process of clinical validation for advanced renal cell carcinoma [8, 67].

In modern oncology, histological, immunohistochemical, and molecular studies, as well as biomarker detection, are performed in tumor samples previously selected from the surgical specimens. Pathologists do perform this tumor selection worldwide. Small tumors (< 3 cm) can be totally sampled, but larger tumors cannot, so pathologists perform a tumor selection following internationally accepted protocols (the so-called routine sampling). Recent studies, however, have shown that routine protocols may be insufficient to unveil with reliability the hidden ITH that many tumors develop [4, 5, 68–70]. To overcome this problem, a new tumor sampling strategy has been recently developed: the multisite tumor sampling (MSTS) [71•, 72]. This sampling method, based on the divide and conquer algorithm, follows the rationale *the more you sample the more you find*, a strategy successfully followed in physics [73] and medicine [74]. Interestingly, MSTS improves ITH detection in large tumors while keeping the cost fixed.

PD-L1 expression pattern is quite diverse across different tumor regions, as it has been proved in a recent analysis of 39 CCRCC [14], so its identification depends largely on the deepness of tumor sampling and on the number of blocks immunostained. In this sense, MSTS has demonstrated a superior performance compared with routine sampling in detecting PD-L1 expression in 22 CCRCC. MSTS detected, in this study [15], more PD-L1-positive cases than routine sampling. Thus, an insufficient sampling could be a plausible explanation for the unexpected positive response to anti-PD-L1 therapy that has been observed in a subset of PD-L1-negative CCRCC [12]. In addition, a different PD-L1 immunostaining associated with the use of different anti-mAb recognizing different epitopes of PD-L1 increases the level of uncertainty in the interpretation of the results. Even more, the possibility exists that the reactivity of these different mAb can be affected by PD-L1 post-translational modifications [75] (Fig. 1a). Finally, whether the expression of PD-L2 in the tumor or in the tumor-infiltrating lymphocytes could also account for the response to anti-PD-1 therapy of PD-L1-negative CCRCC deserves investigation [76].

The variety of anti-PD-L1 clones commercially available on the market is a specific problem to achieve a reliable immunohistochemical evaluation. Thus, up to four different anti-PD-L1 in two different platforms are available in the market: 22C3 and 28-8 on the Dako Link 48 platform (Dako, Carpinteria, CA, USA), and SP-142 and SP-263 on the Ventana Benchmark Ultra platform (Ventana Medical Systems, Tucson, AZ, USA), so the results may vary depending on the antibody tested and the platform used. Several studies performed in non-small cell lung cancer focus on the problem of interpretation derived from this variability [77–79] and claim for an accurate definition of the criteria to determine the PD-L1 status in a given tumor [80].

Another problematic issue needing solution is the interobserver immunohistochemical variability detected in the evaluation of these antibodies in daily practice, a point that has been recently analyzed in lung carcinomas [81] and in genitourinary and head/neck carcinomas [82]. An additional difficulty comes from the fact that the cutoff between a positive and a negative result varies from one tumor type to another, and from one antibody to another. For example, while the cutoff required to administrate atezolizumab in advanced urothelial carcinoma is 5% of positive cells (SP-142) [83], this figure decreases to 1% in current trials for advanced CCRCC (SP-142) [84••]. The cells in which the immunostaining must be assessed also vary depending on the tumor type and the clone used. Actually, only positive immunostaining of mononuclear intratumor inflammatory cells must be considered in CCRCC [14] and urothelial carcinoma (SP-142) (recommendations made by the manufacturer), whereas positivity in both inflammatory and neoplastic cells must be taken into account in lung carcinomas (SP-263, 22C3, 28-8) [77].

Present and Future of PD-1/PD-L1 Blockade in the Treatment of Advanced CCRCC

Precedent paragraphs have shown to what extent the molecular biology underlying renal cancer is a complex field of continuous ongoing research. Deeper understanding of the immunology of T cell activation has led to development of ICI, one of the major goals in the fight against renal cancer in the very recent years. The use of monoclonal antibodies that inhibit PD-1/PD-L1 and CTLA-4 axes thus releasing the inhibition of T cell activation has opened a new era for immunotherapy in patients with advanced CCRCC [85•].

A phase II randomized trial with previously treated metastatic RCC randomized 168 patients to receive intravenous nivolumab 0.3, 2, and 10 mg/kg every 3 weeks. Median

overall survival was 18.2, 25.5, and 24.7 months, respectively [86]. The CheckMate 025 phase III trial evaluated nivolumab (3 mg/kg dosage every 2 weeks) versus everolimus (10 mg/once daily) and included 821 patients with metastatic CRRCC previously treated with one or two lines of VEGF-directed therapy. Median overall survival was 25 months in the group treated with nivolumab and 19.6 months in the group treated with everolimus (HR = 0.73, $p = 0.02$), and overall response rate was 25% for nivolumab and 5% for everolimus (OR = 5.98, $p < 0.002$). Progression-free survival was not different among groups, 4.6 months for nivolumab versus 4.4 months for everolimus (HR = 0.88, $p = 0.11$). Nivolumab also proved to be less toxic, with 19% grade 3–4 adverse events versus 37% in the everolimus group. Very interestingly, the PD-L1-positive patients had worse survival in both treatment arms, and also nivolumab showed clinical benefit in both PD-L1-positive and PD-L1-negative patients [87••]. Post hoc studies from CheckMate 025 also revealed that patients on nivolumab previously treated with sunitinib, pazopanib, or IL-2 also had survival benefit [86, 88]. In these terms, nivolumab has been approved for second-line treatment of advanced RCC after failure of VEGF-targeted therapy with the overall survival still to be defined but with an already proved survival benefit previously unequaled for a second-line therapy in RCC [89•, 90].

The treatment landscape in advanced and metastatic RCC is moving from TKI and the mTOR inhibitors to specific immuno-oncology agents among which ICI prevail today, although their use as first-line therapy needs further exploration. ICI can be used as monotherapy or in combination, either synergic combination of different immuno-oncology agents (synergic ICI) or combining ICI with TKI. RCC therapeutics are expected to move in the direction of combination therapies extending overall survival as a benchmark for new drug approvals and biomarker validation for improved selection of patients for specific therapies [91]. Ongoing phase III trials with emphasis not only on efficacy but also on safety of different combinations tested will define future therapeutic guidelines in RCC.

Dual immune checkpoint blockade through inhibition of PD-1 (nivolumab 3 mg/kg) and CTLA-4 (ipilimumab 1 mg/kg) has been co-administered four times every week and followed by nivolumab every 2 weeks until progression in first-line clinical setting treatment-naïve advanced or metastatic CCRCC compared to sunitinib (50 mg p.o. 4 weeks-on, 2-weeks-off) in the CheckMate 214 phase III clinical trial. Overall survival was significantly improved in the group with synergic combination of ICI (HR = 0.63, $p < 0.001$). Median overall survival has not been reached in the nivolumab and ipilimumab group and is 32.9 months in the sunitinib arm. Notoriously, overall response rate in the group of intermediate risk and poor risk was 42% for nivolumab and ipilimumab combination versus 27% with sunitinib. Interestingly, the risk of grade 3–5 adverse events was higher in the sunitinib arm, but treatment-related adverse events leading to discontinuation was 15% for nivolumab and ipilimumab, and 7% for sunitinib. The influence of PD-L1 expression was also analyzed and progression-free survival in patients with PD-L1 positivity (expressed as >1%) was 22.8 months for combined ICI versus 5.9 months for sunitinib (HR = 0.48, $p = 0.0003$) in the intermediate-risk and poor-risk group [92].

Many phase II and phase III clinical trials are now being conducted to evaluate the efficacy of different ICI (nivolumab, ipilimumab, atezolizumab, avelumab, pembrolizumab) together with cabozantinib, becacizumab, axitinib, everolimus, or epacadostat. Future status of immuno-oncology therapy within the next coming years will surely be determined by the results and strategies derived from them (Table 1). The overview of possible combinations is enormous and surely these exploratory trials will lead to set a firm clinical basis to develop a new era of immunotherapy in renal cancer [67, 89•]; not only with better survival, but also with less adverse effects and better patient perception of quality of life. Trials evaluating TKI and vaccines, such as AGS-003 or IMA901, have given initial negative results [89•, 93, 94] and maybe this is another reason for which more resources have been directed for the development of different strategies incorporating ICI.

The establishment of valid predictors of treatment response to immuno-oncology must become a priority, as a substantial proportion of patients treated with ICI have little or no benefit, and also these treatments are expensive and might bring associated toxicities [95]. Compared with chemotherapy, ICI have a significantly lower risk of all- and high-grade fatigue, sensory neuropathy, diarrhea and hematologic toxicities, all-grade anorexia, nausea, and constipation, any all- and high-grade AEs, and treatment discontinuation [96]. However, increased risk of all-grade rash, pruritus, colitis, aminotransferase elevations, hypothyroidism and hyperthyroidism, and all- and high-grade pneumonitis with PD-1/PD-L1 therapies can be expected [96, 97]. Urologists should start getting used to this peculiar security profile as their patients will be more often involved in clinical trials with ICI. Also, utmost concern must be kept in the combination of different agents as some of these potential therapies may show prohibitive toxicity.

The Need for a Better PD-1/PD-L1 Blockade Biomarker Strategy

New biomarker development is urgent in this new era of immuno-oncology. Tumor-associated PD-L1 expression has been proposed as a potential biomarker for PD-1 pathway expression but it is difficult to implement for reasons that have been already mentioned. In fact, there are current data to translate how the various assays compare with each other. Also, PD-L1 is an inducible molecule and many CCRCC are highly heterogeneous. Even though, targeting PD-1/PD-L1, alone or

Table 1 Main undergoing clinical trials that test combinations of immune checkpoint inhibitors in advanced/metastatic renal cell carcinoma

Combinations	Trial name	NCT number	Drugs evaluated	Primary endpoints
Immune checkpoint inhibitors	CheckMate 214	NCT02231749	Nivolumab + Ipilimumab vs. Sunitinib	PFS, OS, ORR
Immune checkpoint inhibitors	CheckMate 9ER	NCT03141177	Nivolumab + Cabozantinib ± Ipilimumab vs. Sunitinib	PFS
Immune checkpoint inhibitors with anti-VEGF mAb	IMmotion 151	NCT02420821	Atezolizumab + Bevacizumab vs Sunitinib	PFS in PD-L1 (+) patients, OS
Immune checkpoint inhibitors with VEGFR TKI	Javelin Renal 101	NCT02684006	Avelumab + Axitinib vs. Sunitinib	PFS and OS in PD-L1 (+) patients
Immune checkpoint inhibitors with VEGFR TKI	Keynote-426	NCT02853331	Pembrolizumab + Axitinib vs. Sunitinib	PFS, OS
Immune checkpoint inhibitors with GFR TKI	CLEAR	NCT02811861	Lenvatinib + Everolimus or Lenvatinib + Pembrolizumab vs. Sunitinib	PFS
Immune checkpoint inhibitors with IDO	Keynote-679	NCT03260894	Pembrolizumab + Epacadostat vs. Sunitinib or Pazopaniv	

Immune checkpoint inhibitor specificity: Nivolumab, anti-PD-1; Pembrolizumab, anti-PD-1; Atezolizumab, anti-PD-L1; Avelumab, anti-PD-L1; Ipilimumab, anti-CTLA-4

PFS progression-free survival, *OS* overall survival, *ORR* overall response rate, *VEGF* vascular endothelial growth factor, *VEGFR* vascular endothelial growth factor receptor, *GFR* growth factor receptor, *mAb* monoclonal antibody, *TKI* tyrosine kinase inhibitors, *IDO* indoleamine 2-3-dioxigenase

in combination with TKI, is a promising therapeutic tool in patients with advanced CCRCC. Several trials are ongoing trying to define real benefit, new drugs, and strict indications of use. Since ICI are being applied in advanced tumors of several topographies and different antibodies and their respective drugs are available on the market, at least four important points should be assured for an optimal routine. First, the heterogeneous distribution of positive areas across the tumor requires a reliable tumor sampling, especially in those cases in which ITH is high. The question of, "how much tumor tissue should be tested for a reliable evaluation?" needs an accurate answer. Second, the results obtained with the antibodies commercially available are not interchangeable, so the comparisons between different series may not reflect the real situation. Third, there are several difficulties in assigning the respective negative versus positive cutoffs in each tumor type, since these figures vary depending on the observer, tumor type, and the antibody used, so the interobserver results may be discordant and confusing. Fourth, the unknown expression pattern and role of PD-L2 should be defined.

A recent meta-analysis evaluates the relative efficacy of PD-1 or PD-L1 inhibitors versus conventional drugs in patients with different neoplasia (mainly lung cancer, but also renal cancer, head/neck cancer, melanoma, and urothelial cancer) that were PD-L1 positive and PD-L1 negative. PD-L1 expression alone is clearly insufficient to determine which patients should receive ICI because this therapy is beneficial in both groups. Also, no significant differences are observed in terms of overall survival between studies in which patients are randomized according to PD-L1-positive and PD-L1-negative expression, respectively [98••]. That means PD-1 or PD-L1 blockade is a preferable treatment option even for patients that are PD-L1 negative.

Apart from PD-L1 and PD-L2 expression, T cell density in pretreated samples, T cell receptor clonality, mutational or neoantigen burden, assessment of peripheral T cell populations, immunogen signatures, and multiplex IHC with direct assessment of both tumor and immune-cell phenotypes and their spatial relationships are some of the strategies investigated [99•]. Combining two or more methods to capture the immune status of the tumor microenvironment might be more effective as predictive biomarker. In fact, high tumor PD-L1 expression can be present even when tumor-infiltrating lymphocyte counts are low, and conversely tumors with high tumor-infiltrating lymphocyte density may not express PD-L1. Still though, ICI may be helpful but current chaos must be first clarified through a more precise characterization of tumor microenvironment immune status to guide immunotherapy with objective parameters. This point is crucial to predict treatment failures and avoid unnecessary toxicity [100].

Conclusions

ICI have started to transform the therapeutic landscape of advanced and metastatic CCRCC, both as second- or third-line therapy or as part of different immuno-oncology combinations that are currently being tested. The need to develop new biomarker strategies is evident as IHC alone for PD-L1 expression is not reliable itself to predict prognosis neither the chance of a therapeutic response. Clinicians need elements with higher positive and negative predictive value to sustain their decision to use or not these treatments. Thereof development of better markers should be primary endpoint itself in the

design of future clinical trials for checkpoint immunotherapy-based management of patients with CCRCC.

Funding This work has been partially funded by grants SAF2016-79847-R from Ministerio de Economía y Competitividad, Spain (to RP and JIL) and 239813 from The Norwegian Research Council, Norway (to CEN-X).

Compliance with Ethical Standards

Conflict of Interest Caroline E. Nunes-Xavier, Javier C. Angulo, Rafael Pulido, and José I. López each declare no potential conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018;68:7–30.
2. López JI. Renal tumors with clear cells. A review. Pathol Res Pract. 2013;209:137–46.
3. Hsieh JJ, Purdue MP, Signoretti S, Swanton C, Albiges L, Schmidinger M, et al. Renal cell carcinoma. Nat Rev Dis Primers. 2017;3:17009.
4. Zaldumbide L, Erramuzpe A, Guarch R, Cortés JM, López JI. Large (>3.8 cm) clear cell renal cell carcinomas are morphologically and immunohistochemically heterogeneous. Virchows Arch. 2015;466:61–6.
5. Guarch R, Lawrie CH, Larrinaga G, Angulo JC, Pulido R, López JI. High levels of intratumor heterogeneity characterize the expression of epithelial-mesenchymal transition markers in high grade clear cell renal cell carcinoma. Ann Diagn Pathol. 2018;34:27–30.
6.•• Turajlic S, Xu H, Litchfield K, et al. Deterministic evolutionary trajectories influence primary tumor growth: TRACERx Renal. Cell. 2018;173:595–610. **This paper describes for the first time 7 different evolutionary subtypes with impact on survival and exemplifies the complexity of intratumor heretogeneity of clear cell renal cell carcinoma.**
7.•• Turajlic S, Xu H, Litchfield K, et al. Tracking renal cancer evolution reveals constrained routes to metastases: TRACERx Renal. Cell. 2018;173:581–94. **Two different temporal patterns of metastases, early and late, are described after a genomic study in clear cell renal cell carcinoma.**
8. Atkins MB, Tannir NM. Current and emerging therapies for first-line treatment of metastatic clear cell renal cell carcinoma. Cancer Treat Rev. 2018;70:127–37.
9. Messai Y, Gad S, Noman MZ, le Teuff G, Couve S, Janji B, 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;70:623–32.
10. Ruf M, Moch H, Schaml P. PD-L1 expression is regulated by hypoxia inducible factor in clear cell renal cell carcinoma. Int J Cancer. 2016;139:396–403.
11. Kammerer-Jacquet SF, Crouzet L, Brunot A, et al. Independent association of PD-L1 expression with noninactivated *VHL* clear cell renal cell carcinoma. A finding with therapeutic potential. Int J Cancer. 2016;140:142–8.
12. Khagi Y, Kurzrock R, Patel SP. Next generation predictive biomarkers for immune checkpoint inhibition. Cancer Metastasis Rev. 2017;36:179–90.
13.• Turajlic S, Swanton C, Boshoff C. Kidney cancer: the next decade. J Exp Med. 2018;215: 2477–79. **This short review offers an insight into the upcoming therapeutic strategies in renal cell carcinoma.**
14. López JI, Pulido R, Cortés JM, Angulo JC, Lawrie CH. Potential impact of PD-L1 (SP-142) immunohistochemical heterogeneity in clear cell renal cell carcinoma immunotherapy. Pathol Res Pract. 2018;214:1110–4.
15. López JI, Pulido R, Lawrie CH, Angulo JC. Loss of PD-L1 (SP-142) expression characterizes renal vein tumor thrombus microenvironment in clear cell renal cell carcinoma. Ann Diagn Pathol. 2018;34:89–93.
16. Munari E, Zamboni G, Lunardi G, Marchionni L, Marconi M, Sommaggio M, et al. PD-L1 expression heterogeneity in non-small cell lung cancer: defining criteria for harmonization between biopsy specimens and whole sections. J Thorac Oncol. 2018;13: 1113–20.
17. Ascierto ML, McMiller TL, Berger AE, et al. The intratumoral balance between metabolic and immunologic gene expression is associated with anti-PD-1 response in patients with renal cell carcinoma. Cancer Immunol Res. 2016;4:726–33.
18. Finger LR, Pu J, Wasserman R, Vibhakar R, Louie E, Hardy RR, et al. The human PD-1 gene : complete cDNA, genomic organization, and developmentally regulated expression in B cell progenitors. Gene. 1997;197:177–87.
19. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med. 2000;192:1027–34.
20. Latchman Y, Wood CR, Chernova T, Chaudhary D, Borde M, Chernova I, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. Nature Immunol. 2001;2:261–8.
21. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. Ann Rev Immunol. 2005;23:515–48.
22. Okazaki T, Maeda A, Nishimura H, Kurosaki T, Honjo T. PD-1 immunoreceptor inhibits B-cell receptor-mediated signaling by recruiting src homology 2-domain-containing tyrosine phosphatase 2 to phosphotyrosine. Proc Natl Acad Sci U S A. 2001;98: 13866–71.
23. Sheppard KA, Fitz LJ, Lee JM, Benander C, George JA, Wooters J, et al. PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3zeta signalosome and downstream signaling to PKCtheta. FEBS Lett. 2004;574:37–41.
24. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Ann Rev Immunol. 2008;26:677–704.
25. Dong Y, Sun Q, Zhang X. PD-1 and its ligands are important immune checkpoints in cancer. Oncotarget. 2017;8:2171–86.
26. Nielsen C, Ohm-Laursen L, Barington T, Husby S, Lillevang ST. Alternative splice variants of the human PD-1 gene. Cell Immunol. 2005;235:109–16.
27. Dong H, Zhu G, Tamada K, Chen L. B7-H1, a third member of the B7 family, coestimulates T-cell proliferation and interleukin-10 secretion. Nat Med. 1999;5:1365–9.
28. Escors D, Gato-Canas M, Zuazo M, et al. The intracelular signalosome of PD-L1 in cancer cells. Signal Transduct Target Ther. 2018;3:26.

29. He XH, Liu Y, Xu LH, Zeng YY. Cloning and identification of two novel splice variants of human PD-L2. Acta Biochim Biophys Sinica. 2004;36:284–9.
30. He XH, Xu LH, Liu Y. Identification of a novel splice variant of human PD-L1 mRNA encoding an isoform-lacking Igv-like domain. Acta Pharmacol Sinica. 2005;26:462–8.
31. Ding H, Wu X, Gao W. PD-L1 is expressed by human renal tubular epithelial cells and suppresses T cell cytokine synthesis. Clin Immunol. 2005;115:184–91.
32. Chen Y, Zhang J, Li J, Zou L, Zhao T, Tang Y, et al. Expression of B7-H1 in inflammatory renal tubular epithelial cells. Nephron Exp Nephrol. 2006;102:e81–92.
33. Zhang J, Chen Y, Li J, et al. Renal tubular epithelial expression of the coinhibitory molecule B7-DC (programmed death-1 ligand). J Nephrol. 2006;19:429–38.
34. Frigola X, Inman BA, Lohse CM, Krco CJ, Cheville JC, Thompson RH, et al. Identification of a soluble form of B7-H1 that retains immunosuppressive activity and is associated with aggressive renal cell carcinoma. Clin Cancer Res. 2011;17:1915–23.
35. Yao H, Wang H, Li C, Fang JY, Xu J. Cancer cell-intrinsic PD-1 and implications in combinatorial immunotherapy. Front Immunol. 2018;9:1774.
36. Folkl A, Bienzle D. Structure and function of programmed death (PD) molecules. Vet Immunol Immunopathol. 2010;134:33–8.
37. Thompson RH, Dong H, Lohse CM, Leibovich BC, Blute ML, Cheville JC, et al. PD-1 is expressed by tumor-infiltrating immune cells and is associated with poor outcome for patients with renal cell carcinoma. Clin Cancer Res. 2007;13:1757–61.
38. MacFarlane AW 4th, Jillab M, Plimack ER, et al. PD-1 expression on peripheral blood cells increaseswith stage in renal cell carcinoma patients and is rapidly reduced after surgical tumor resection. Cancer Immunol Res. 2014;2:320–31.
39. Mazanet MM, Hughes CC. B7-H1 is expressed by human endothelial cells and suppresses T cell cytokine synthesis. J Immunol. 2002;169:3581–8.
40. Chen J, Jiang CC, Jin L, Zhang XD. Regulation of PD-L1: a novel role of pro-survival signaling in cancer. Ann Oncol. 2016;27:409–16.
41. Gibbons-Johnson RM, Dong H. Functional expression of programmed death-ligand 1 (B7-H1) by immune cells and tumor cells. Front Immunol. 2017;8:961.
42. Noman MZ, Desantis G, Janji B, Hasmim M, Karray S, Dessen P, et al. PD-L1 is a novel direct target of HIF-1α, and its blockade under hypoxia enhanced MDSC-mediated T cell activation. J Exp Med. 2014;211:781–90.
43. Tatli Dogan H, Kiran M, Bilgin B, Kiliçarslan A, Sendur MAN, Yalçin B, et al. Prognostic significance of the programmed death ligand 1 expression in clear cell renal cell carcinoma and correlation with the tumor microenvironment and hypoxia-inducible factor expression. Diagn Pathol. 2018;13:60.
44. Chemnitz JM, Parry RV, Nichols KE, June CH, Riley JL. SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation. J Immunol. 2004;173:945–54.
45. Kulpa DA, Lawani M, Cooper A, Peretz Y, Ahlers J, Sekaly RP. PD-1 coinhibitory signals: the link between pathogenesis and protection. Semin Immunol. 2013;25:219–27.
46. Patsoukis N, Brown J, Petkova V, Liu F, Li L, Boussiotis VA. Selective effects of PD-1 on Akt and Ras pathways regulate molecular components of the cell cycle and inhibit T cell proliferation. Sci Sign. 2012;5:ra46.
47. Patsoukis N, Li L, Sari D, Petkova V, Boussiotis VA. PD-1 increases PTEN phosphatase activity while decreasing PTEN protein stability by inhibiting casein kinase 2. Mol Cell Biol. 2013;33:3091–8.
48. Peng W, Chen JQ, Liu C, Malu S, Creasy C, Tetzlaff MT, et al. Loss of PTEN promotes resistance to T cell-mediated immunotherapy. Cancer Discov. 2016;6:202–16.
49. George S, Miao D, Demetri GD, Adeegbe D, Rodig SJ, Shukla S, et al. Loss of PTEN is associated with resistance to anti-PD-1 checkpoint blockade therapy in metastatic uterine leiomyosarcoma. Immunity. 2017;46:197–204.
50. Brenner W, Farber G, Herget T, Lehr HA, Hengstler JG, Thuroff JW. Loss of tumor suppressor protein PTEN during renal carcinogénesis. Int J Cancer. 2002;99:53–7.
51. Shin Lee J, Seok Kim H, Bok Kim Y, Cheol Lee M, Soo PC. Expression of PTEN in renal cell carcinoma and its relation to tumor behavior and growth. J Surg Oncol. 2003;84:166–72.
52. Hager M, Haufe H, Kemmerling R, Mikuz G, Kolbitsch C, Moser PL. PTEN expression in renal cell carcinoma and oncocytoma and prognosis. Pathology. 2007;39:482–5.
53. Dong P, Xiong Y, Yue J, Hanley SJB, Watari H. Tumor-intrinsic PD-L1 signaling in cancer initiation, development and treatment: beyond immune evasion. Front Oncol. 2018;8:385.
54. Gupta HB, Clark CA, Yuan B, Sareddy G, Pandeswara S, Padron AS, et al. Tumor cell-intrinsic PD-L1 promotes tumor-initiating cell generation and functions in melanoma and ovarian cancer. Sign Transduct Target Ther. 2016;1:16030.
55. Clark CA, Gupta HB, Sareddy G, Pandeswara S, Lao S, Yuan B, et al. Tumor-intrinsic PD-L1 signals regulate cell growth, pathogenesis, and autophagy in ovarian cancer and melanoma. Cancer Res. 2016;76:6964–74.
56. Qiu XY, Hu DX, Chen WQ, et al. PD-L1 confers glioblastoma multiforme malignancy via Ras binding and Ras/Erk/EMT activation. Biochim Biophys Acta Mol basis Dis. 1864;2018:1754–69.
57. Wang Y, Wang H, Zhao Q, Xia Y, Hu X, Guo J. PD-L1 induces epithelial-to-mesenchymal transition via activating SREBP-1c in renal cell carcinoma. Med Oncol. 2015;32:212.
58. Verbiest A, Couchy G, Job S, Zucman-Rossi J, Caruana L, Lerut E, et al. Molecular subtypes of clear cell renal cell carcinoma are associated with outcome during pazopanib therapy in the metastatic setting. Clin Genitourin Cancer. 2018;16:e605–12.
59. Iacovelli R, Nole F, Verri E, et al. Prognostic role of PD-L1 expression in renal cell carcinoma. A systematic review and meta-analysis. Targ Oncol. 2016;11:143–8.
60. Wang Z, Peng S, Xie H, Guo L, Cai Q, Shang Z, et al. Prognostic and clinicopathological significance of PD-L1 in patients with renal cell carcinoma: a meta-analysis based on 1863 individuals. Clin Exp Med. 2018;18:165–75.
61. Abbas M, Steffens S, Bellut M, Eggers H, Großhennig A, Becker JU, et al. Intratumoral expression of programmed death ligand 1 (PD-L1) in patients with clear cell renal cell carcinoma (ccRCC). Med Oncol. 2016;33:80.
62. Ueda K, Suekane S, Kurose H, Chikui K, Nakiri M, Nishihara K, et al. Prognostic value of PD-1 and PD-L1 expression in patients with metastatic clear cell renal cell carcinoma. Urol Oncol. 2018;36:499.e9–499.e16. https://doi.org/10.1016/j.urolonc.2018.07.003.
63. Thompson RH, Kuntz SM, Leibovitz BC, et al. Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up. Cancer Res. 2006;66:3381–5.
64.• Choueiri TK, Motzer RJ. Systemic therapy for metastatic renal-cell carcinoma. N Eng J Med. 2017;376:354–66. **Exhaustive review of the spectrum of systemic therapies available for patients with advanced renal cancer.**
65. Flynn M, Pickering L, Larkin J, Turajlic S. Immune-checkpoint inhibitors in melanoma and kidney cancer: from sequencing to rational selection. Ther Adv Med Oncol. 2018;10:1–16.

66. Liang J, Liu Z, Zou Z, Tang Y, Zhou C, Yang J, et al. The correlation between the immune and epithelial-mesenchymal transition signatures suggests potential therapeutic targets and prognosis prediction approaches in kidney cancer. Sci Rep. 2018;8:6570.
67. Beckermann KE, Douglas B, Johnson DB, Sosman JA. PD-1/PD-L1 blockade in renal cell cancer. Expert Rev Clin Immunol. 2017;13:77–84.
68. Nassar A, Radhakrishan A, Cabrero IA, Cotsonis GA, Cohen C. Intratumoral heterogeneity of immunohistochemical marker expression in breast carcinoma. A tissue microarray-based study. Appl Immunohistochem Mol Morphol. 2010;18:433–41.
69. Jilaveanu LB, Shuch B, Zito CR, Parisi F, Barr M, Kluger Y, et al. PD-L1 expression in clear cell renal cell carcinoma: an analysis of nephrectomy and sites of metastases. J Cancer. 2014;5:166–72.
70. Ellsworth RE, Blackburn HL, Shriver CD, Soon-Shiong P, Ellsworth DL. Molecular heterogeneity in breast cancer: state of the science and implications for patient care. Semin Cell Dev Biol. 2017;64:65–72.
71.• López JI, Cortés JM. A divide and conquer strategy in tumor sampling enhances detection of intratumor heterogeneity in pathology routine: a modeling approach in clear cell renal cell carcinoma. F1000Res. 2016;5:385. **This paper describes an in silico approach to a new sampling method that improves intratumor heterogeneity detection in large tumors.**
72. López JI, Cortés JM. Multi-site tumor sampling (MSTS): a new tumor selection method to enhance intratumor heterogeneity detection. Hum Pathol. 2017;64:1–6.
73. Ming D, Yang W. A divide and conquer strategy to improve diffusion sampling in generalized ensemble simulators. J Chem Phys. 2008;128:094106.
74. Kristensen VN. Divide and conquer: the genetic basis of molecular subclassification of breast cancer. EMBO Mol Med. 2011;3:183–5.
75. Horita H, Law A, Hong S, Middleton K. Identifying regulatory posttranscriptional modifications of PD-L1: a focus on monoubiquitination. Neoplasia. 2017;19:346–53.
76. Shin SJ, Jeon YK, Kim PJ, Cho YM, Koh J, Chung DH, et al. Clinicopathologic analysis of PD-L1 and PD-L2 expression in renal cell carcinoma: association with oncogenic proteins status. Ann Surg Oncol. 2016;23:694–702.
77. Hendry S, Bryne DJ, Wright GM, et al. Comparison of four PD-L1 immunohistochemical assays in lung cancer. J Thorac Oncol. 2017;13:367–76.
78. Adam J, Le Stang N, Rouquette I, et al. Multicenter harmonization study for PD-L1 IHC testing in non-small-cell lung cancer. Ann Oncol. 2018;29:953–8.
79. Tsao MS, Kerr KM, Kockx M, Beasley MB, Borczuk AC, Botling J, et al. PD-L1 immunohistochemistry comparability study in real-life clinical samples: results of blueprint phase 2 project. J Thorac Oncol. 2018;13:1302–11.
80. Munari E, Rossi G, Zamboni G, Lunardi G, Marconi M, Sommaggio M, et al. PD-L1 assays 22C3 and SP263 are not interchangeable in non-small cell lung cancer when considering clinically relevant cutoffs. An interclon evaluation by differently trained pathologists. Am J Surg Pathol. 2018;42:1384–9.
81. Brunnström H, Johansonn A, Westbom-Fremer S, et al. PD-L1 immunohistochemistry in clinical diagnosis of lung cancer: inter-pathologist variability is higher than assay variability. Mod Pathol. 2017;30:1411–21.
82. Wang C, Hahn E, Slodkowska E, Eskander A, Enepekides D, Higgins K, et al. Reproducibility of PD-L1 immunohistochemistry interpretation across various types of genitourinary and head/neck carcinomas, antibody clones, and tissue types. Hum Pathol. 2018;82:131–9. https://doi.org/10.1016/j.humpath.2018.07.024.
83. Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV, Necchi A, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single arm. Multicenter, phase 2 trial. Lancet. 2016;387:1909–20.
84.•• Ross K, Jones RJ. Immune checkpoint inhibitors in renal cell carcinoma. Clin Sci. 2017;131:2627–42. **Excellent review of the current status of immune checkpoint blockade in renal cell carcinoma.**
85.• Özdemir BC, Siefker-Radtke AO, Campbell MT, Subudhi SK. Current and future applications of novel immunotherapies in urological oncology: a critical review of the literature. Eur Urol Focus. 2017, https://doi.org/10.1016/j.euf.2017.10.001. **A review on the clinical data for immune checkpoint inhibition alone or in combination with other therapies for urologic malignancies.**
86. 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;33:1430–7.
87.•• Motzer RJ, Escudier B, McDermott DF, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. N Engl J Med. 2015;373:1803–1813. **This phase 3 randomized study demonstrated that patients with advanced renal cell carcinoma experienced longer survival with nivolumab treatment than with everolimus treatment after prior antiangiogenic treatment.**
88. George S, Motzer RJ, Hammers HJ, Redman BG, Kuzel TM, Tykodi SS, et al. Safety and efficacy of nivolumab in patients with metastatic renal cell carcinoma treated beyond progression: a subgroup analysis of a randomized clinical trial. JAMA Oncol. 2016;2:1179–86.
89.• Bedke J, Stühler V, Stenzl A, Brehmer B. Immunotherapy for kidney cancer: status quo and the future. Curr Opin Urol. 2018;28:8–14. **Essential review on the immunotherapy for renal cell cancer, including both checkpoint inhibitors and vaccination strategies.**
90. Bedke J, Gauler T, Grünwald V, Hegele A, Herrmann E, Hinz S, et al. Systemic therapy in metastatic renal cell carcinoma. World J Urol. 2017;35:179–88.
91. Atkins MB, Philips GK. Emerging monoclonal antibodies for the treatment of renal cell carcinoma (RCC). Expert Opin Emerg Drugs. 2016;21:243–54.
92. Motzer RJ, Tannir NM, McDermott DF, Arén Frontera O, Melichar B, Choueiri TK, et al. Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma. N Engl J Med. 2018;378:1277–90.
93. Bedke J, Stenzl A. IMA901: a peptide vaccine in renal cell carcinoma. Expert Opin Investig Drugs. 2013;22:1329–36.
94. Rini BI, Stenzl A, Zdrojowy R, Kogan M, Shkolnik M, Oudard S, et al. IMA901, a multipeptide cancer vaccine, plus sunitinib versus sunitinib alone, as first-line therapy for advanced or metastatic renal cell carcinoma (IMPRINT): a multicenter open-label, randomized, controlled phase 3 trial. Lancet Oncol. 2016;17:1599–611.
95. Maleki Vareki S, Garrigós C, Duran I. Biomarkers of response to PD-1/PD-L1 inhibition. Crit Rev Oncol Hematol. 2017;116:116–24.
96. Nishijima TF, Shachar SS, Nyrop KA, Muss HB. Safety and tolerability of PD-1/PD-L1 inhibitors compared with chemotherapy in patients with advanced cancer: a meta-analysis. Oncologist. 2017;22:470–9.
97. Brahmer JR, Lacchetti C, Schneider BJ, Atkins MB, Brassil KJ, Caterino JM, et al. Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: American Society of Clinical Oncology Clinical Practice Guideline. J Clin Oncol. 2018;36:1714–68.
98.•• Shen X, Zhao B. Efficacy of PD-1 or PD-L1 inhibitors and PD-L1 expression status in cancer: meta-analysis. BMJ. 2018;362:k3529.

Meta-analysis that proves PD-L1 expression status is insufficient to determine patients that should be offered PD-1 or PD-L1 blockade therapy.

99.• Gibney GT, Weiner LM, Atkins MB. Predictive biomarkers for checkpoint inhibitor-based immunotherapy. Lancet Oncol. 2016;17:e542–51. **Extensive review dealing with the pitfalls and possibilities to define accurate predictive biomarkers for checkpoint inhibition.**

100. Baxi S, Yang A, Gennarelli RL, et al. Immune-related adverse events for anti-PD-1 and anti-PD-L1 drugs: systematic review and meta-analysis. BMJ. 2018;360:k793.