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*Original*

PD-1 blockade therapy in renal cell carcinoma: current studies and future promises / Massari, F; Santoni, Matteo; Ciccarese, C; Santini, D; Alfieri, S; Martignoni, G; Brunelli, M; Piva, Francesco; Berardi, Rossana; Montironi, Rodolfo; Porta, C; Cascinu, Stefano; Tortora, G.. - In: CANCER TREATMENT REVIEWS. - ISSN 0305-7372. - (2015). [10.1016/j.ctrv.2014.12.013]

*Availability:*

This version is available at: 11566/206328 since: 2022-05-23T16:24:20Z

*Publisher:*

*Published*

DOI:10.1016/j.ctrv.2014.12.013

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# PD-1 blockade therapy in renal cell carcinoma: Current studies and future promises

F. Massari <sup>a</sup>, M. Santoni <sup>b,†</sup>, C. Ciccarese <sup>a</sup>, D. Santini <sup>c</sup>, S. Alfieri <sup>d</sup>, G. Martignoni <sup>e</sup>, M. Brunelli <sup>e</sup>, F. Piva <sup>f</sup>, R. Berardi <sup>b</sup>, R. Montironi <sup>g</sup>, C. Porta <sup>h</sup>, S. Cascinu <sup>b</sup>, G. Tortora <sup>a</sup>

<sup>a</sup> Medical Oncology, Azienda Ospedaliera Universitaria Integrata, University of Verona, Piazzale L.A. Scuro 10, 37124 Verona, Italy

<sup>b</sup> Clinica di Oncologia Medica, AOU “Ospedali Riuniti”, Polytechnic University of the Marche Region, via Conca 71, 60126 Ancona, Italy

<sup>c</sup> Department of Medical Oncology, University Campus Bio-Medico Roma, Oncologia Medica, Rome, Italy

<sup>d</sup> SSD Oncologia medica Tumori Testa e Collo, Fondazione IRCCS – Istituto Nazionale dei Tumori, Via Venezian, 1, 20133 Milano, Italy

<sup>e</sup> Department of Pathology and Diagnostic, Azienda Ospedaliera Universitaria Integrata, University of Verona, Piazzale L.A. Scuro 10, 37124 Verona, Italy

<sup>f</sup> Department of Specialistic Clinical and Odontostomatological Sciences, Polytechnic University of Marche, 60131 Ancona, Italy

<sup>g</sup> Section of Pathological Anatomy, Polytechnic University of the Marche Region, School of Medicine, United Hospitals, Ancona, Italy

<sup>h</sup> Medical Oncology, IRCCS San Matteo University Hospital Foundation, piazzale C. Golgi 19, 27100 Pavia, Italy

## ABSTRACT

RCC is considered an immunogenic tumor with a prominent dysfunctional immune cell infiltrate, unable to control tumor growth. Evasion of immune surveillance, a process defined immune-editing, leads to malignant progression. The striking improvement of knowledge in immunology has led to the identification of immune checkpoints (such as CTLA-4 and PD-1), whose blockage enhances the antitumor immunity. The interaction between PD-1, an inducible inhibitory receptor expressed on lymphocytes and DCs, and PD-L1 ligand, expressed by tumor cells, results in a down-regulation of the T-cell response. Therefore, the PD-1/PD-L1 axis inhibition by targeted-antibodies, increasing the T-cell proliferation and cytotoxicity, represents a promising mechanism to stimulate the anti-tumor activity of the immune system, improving the outcomes of cancer patients. Several PD-1 and PD-L1 inhibitors have been evaluated in different tumor types, showing promising results. The interesting correlation between lymphocytes PD-1 expression and RCC advanced stage, grade and prognosis, as well as the selective PD-L1 expression by RCC tumor cells and its potential association with worse clinical outcomes, have led to the development of new anti PD-1/PD-L1 agents, alone or in combination with anti-angiogenic drugs or other immunotherapeutic approaches, for the treatment of RCC. In this review we discuss the role of PD-1/PD-L1 in RCC, focusing on the biological rationale, current clinical studies and promising therapeutic perspectives to target the PD-1 pathway.

## INTRODUCTION

Since renal cell carcinoma (RCC) is considered an immunogenic tumor, a number of different immunotherapeutic approaches have been attempted in the past [1]. Indeed, the lack of significant antitumor activity observed with chemotherapy, together with the recognition of the frequent presence of several immunologic dysfunctions in RCC, even at diagnosis and in the absence of metastases [2], have rendered this tumor a privileged field for the development and clinical application of immunotherapy. Furthermore, RCC usually presents a prominent immune cell

infiltrate that consists of a number of cell types, including T cells, natural killer (NK) cells, dendritic cells (DCs) and macrophages. Despite being strongly infiltrated, immune dysfunction promotes RCC tumor growth and evasion. The tumor-induced changes in DC differentiation and the induction of anergy-associated genes in T cells can partially explain the impaired antitumor response [3]. Older immunotherapeutics (mainly cytokines) failed to impact on immune system anergy, and thus also on patients' survival. A growing body of evidence suggests that novel immune modulating agents, and especially antibodies blocking several immune checkpoints, such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed death receptor-1 (PD-1), can significantly enhance antitumor immunity [4]. While CTLA-4 inhibits T-cells activated during the priming phase of T-cell activation, PD-1 exerts its inhibitory activity during the effector phase of T-cell activation in the tumor microenvironment (Fig. 1). The main differences of the therapeutic strategies targeting PD-1/PD-L1 and CTLA-4 are summarized in Table 1. PD-1 is an immunoinhibitory receptor discovered in 1992 by Ishida et al. [5]. PD-1 belongs to the CD28/CTLA-4 family and it presents both an immunoreceptor tyrosine-based switch motif (ITSM) and an immunoreceptor tyrosine-based inhibitory motif (ITIM) able to recruit anti-SRC homology phosphatase (SHP)-1 and SHP-2 protein tyrosine phosphatases to modulate inhibitory signaling [6,7]. PD-1 is inducibly expressed on CD4+ and CD8+ T cells, NK cells, B cells and monocytes within 24 h from their immunological activation [8]. Its expression is increased by a large series of cytokines, such as IL-2, IL-7, IL-15 and IL-21. Two PD-1 ligands (PD-L1, also called B7-H1, and PD-L2, also known as B7-DC), have been described. While PD-L1 is expressed on several cells, including resting T cells, B cells, macrophages, DCs cells, vascular endothelial cells, and pancreatic islet cells, PD-L2 is expressed only on macrophages and DCs [8]. PD-L1 and PD-L2 inhibit T-cell proliferation and adhesion, as well as cytokine production. PD-L1 seems to modulate T-cell function in peripheral tissues, whereas PD-L2 regulates immune T-cell activation in lymphoid organs [9]. Interferons (IFN  $\alpha$ ,  $\beta$ ,  $\gamma$ ) and cytokines (IL-4 and IL-10) are most potent upregulators of PD-L1 expression. Many other pro-inflammatory factors, such as growth cell stem factors (G-CSF), bacterial lipopolysaccharide, and vascular endothelial growth factor (VEGF), can induce PD-L1 expression as well [4]. PD-L1 also binds CD28 (B7.1), which is expressed on T activated cells, resulting in T cell inhibition regardless of interactions between PD-L1 and PD-1 [10]. On the other hand, PD-L2/B7-DC is expressed on myeloid DCs, T activated cells and non-hematopoietic tissues (e.g. lung), especially in healthy patients. PD-L2 seems to inhibit Type 2 T-helper (Th-2) lymphocytes, but its role has not been completely clarified [10]. In this review, we analyzed the role of PD-1 and its ligands in RCC and the future perspectives of this therapeutic strategy.

## **MATERIALS AND METHODS**

All published papers were obtained from the Pubmed database, using the subsequent MeSH (Medical Subject Heading) terms: "PD-1", "PDL-1", "PD1/PDL1 pathway", "immunotherapy", "personalized therapy", "precision medicine", "target", "targeted therapy", each combined with "metastatic renal cell carcinoma". The ongoing trials were searched out on the official website [www.clinicaltrials.gov](http://www.clinicaltrials.gov), with the last search on October 2014.

## **EXPRESSION AND ROLE OF PD-1 AND ITS LIGANDS IN RCC**

Several studies have investigated the biological and prognostic role of the expression of PD-1 and its ligands (especially PDL-1) in RCC. Generally, activated T-cells, B-cells, NK and DCs, monocytes cells express PD-1 in order to restrict auto-immunity during inflammatory states such as infections. However tumors express the PD-L1 ligand to take advantage of this mechanism, down-regulating the T-cell response. PD-L1 is not expressed on normal kidney tissues, but is expressed in a significant proportion of both primary and metastatic RCC specimens [11–14]. Thompson and his colleagues evaluated the immunohistochemical expression of PD-1 and PD-L1 in a large series of nephrectomy

specimens. They showed that PD-1 was expressed in the 56% of patients whose tumor contained mononuclear cell infiltrates, while it was not expressed on RCC tumor cells. In addition, PD-1 expression was correlated with advanced tumor stage, the presence of necrosis or sarcomatoid differentiation and poorer worse 5-years cancer-specific survival [11,15]. Furthermore, Frigola et al. developed an ELISA to quantify soluble PD-L1 in a series of 172 RCC patients. They found higher preoperative soluble PD-L1 levels in patients with higher tumor stage, grade and poorer survival [16]. PD-1 and PD-L1 are both expressed on CD4 + CD25 + regulatory T cells (Tregs), but whether they influence function of this regulatory T cells is not completely clear [8]. Tregs infiltration promotes the pro-angiogenic phenotype of RCC and is associated with stage and grade of RCC [17]. Interestingly, the percentage of tumor infiltrating Tregs is reduced by both sunitinib and sorafenib treatment [18,19], while it is improved by everolimus [20]. Kang et al. found a significant correlation between the presence of tumor-infiltrating PD1-positive lymphocytes or FoxP3-positive Tregs and poorer relapse-free survival (RFS), distant metastatic relapse (DMR) and OS of patients with clear cell RCC [21]. In addition, Chen et al. showed that PD-1 modulates extrathymic Treg differentiation into peripherally induced Treg (pTreg) cells. Otherwise, PD-1 seems to be dispensable for thymic Treg-cell (tTreg) development and suppressive function [22]. These data suggest that anti-PD-1/PD-L1 agents enhance T-cell response by influencing the activity of conventional T cells as well as by suppressing the differentiation of pTreg cells. Furthermore, a high frequency of NK cells among the lymphocytic infiltrate seems to predict a better prognosis in patients with RCC [23]. Both sorafenib and sunitinib have been shown to enhance tumor cell sensitivity to NK cell killing. This event was correlated with the up-regulation of the ligands for the NK activating receptor NKG2D on RCC cells [24]. McFarlane et al. showed that substantial levels of PD-1 are expressed also on cytolytic CD56dim NK cells of patients with RCC. PD-1+ CD56dim NK cells had an activated effector phenotype, characterized by higher levels of perforin and granzyme B [7]. Based on these data, the role of NK cells in the tumor response induced by anti-PD-1 antibodies should be further investigated. Recently, Choueiri et al. presented at the 2014 symposium dedicated to genitourinary tumors of the American Society of Clinical Oncology (ASCO) a sub-analysis from the COMPARZ study comparing sunitinib and pazopanib as first-line therapy for mRCC patients. In this trial, increased PD-L1 tumor expression, as well as tumor CD8+ T cell count, were associated with shorter OS in patients treated with pazopanib or sunitinib, supporting the necessity of prospective trials to investigate and validate PD-L1 as a potential biomarker in patients who receive TKI therapy [25].

## **THERAPEUTIC PERSPECTIVES**

As mentioned above, the PD-1/PD-L1 pathway blockade by targeted- antibodies (against PD-1 or PD-L1) is able to avoid the T cell inhibition. Preclinical and early clinical studies demonstrated that molecules targeting PD-1/PD-L1, such as monoclonal antibodies, increase proliferation and cytolytic activity of T cells resulting in durable objective responses in patients with advanced RCC, melanoma and non-small lung cancer [26–28]. Moreover, PD-L1 expression correlates with worse clinical outcomes, increased risk of progression and cancer specific death [11] (Table 2, [27,29–31]. Therefore, the inhibition of T-cell activation by the PD-1 immune checkpoint is not only a prognostic factor but also a promising target for novel immunotherapeutic approaches. Here we summarize the preliminary results and illustrate the ongoing trials on the use of anti PD-1/PD-L1 agents, used alone or in combination with anti-angiogenic or other immunotherapeutic approaches, for the treatment of RCC.

## **PD-1 BLOCKING ANTIBODIES AND FUSION PROTEINS**

To date several PD-1 and PD-L1 inhibitors have been evaluated in different types of malignancies including, nivolumab, pembrolizumab and pidilizumab (Table 3).

### **Nivolumab (BMS-936558)**

Nivolumab is a fully human monoclonal antibody and represents the most studied PD-1 inhibitor. In a Phase I clinical trial, 39 patients with advanced solid tumors were treated with escalating doses of nivolumab [26]. The agent seemed to be well tolerated, and no maximum tolerated dose was identified as no dose-limiting toxicities were observed. In this phase I study have been reported one complete response and two partial responses, and one of the patients who developed a partial response lasting over 16 months had metastatic RCC. A favorable safety profile and preliminary evidence of clinical activity led to the design of a multi-dose trial of nivolumab in 296 patients, confirming antitumor efficacy in melanoma, RCC and NSCLC [26]. In this study, cumulative response rates were 18% for patients with non-small-cell lung cancer (NSCLC), 28% for patients with melanoma, and 27% (9 of 33 patients) for patients with RCC. None of the PD1- tumors did respond, suggesting PD1 as a biomarker of resistance to nivolumab, although it is now clear that PD-L1 tumors can also respond to this agent (Table 2). Recently, the results of a randomized, dose-ranging phase II trial of nivolumab in 168 pretreated mRCC patients were presented. At a dose of 0.3 mg/kg, nivolumab reported a median duration of response of 15.7 months and a median OS of 18.2 months. More than 50% of responders with all doses had objective responses lasting more than 12–20 months. The rate of serious adverse events (AEs) was lower than 17% [32]. Furthermore, Choueiri and his group showed that response to nivolumab were more frequent in patients with higher PD-L1 tumor expression, but were also seen in patients with PD-L1 RCC [33]. At present, a phase III study is comparing nivolumab to everolimus as a second-line therapy for patients with metastatic RCC (NCT01668784).

### **Pembrolizumab (MK-3475) and pidilizumab (CT-011)**

Pembrolizumab (MK-3475) is a humanized IgG4 PD-1-blocking antibody that was investigated in a phase I trial enrolling patients with advanced solid tumors including RCC. The most common AEs were fatigue, pruritus and dyspnea. Antitumor activity was observed [34]. Nowadays, pembrolizumab is under study in the neoadjuvant setting (NCT02212730) and in combination with INCB024360, an orally available inhibitor of indoleamine 2,3-dioxygenase (IDO1), in patients with advanced solid tumors including RCC (NCT02178722). Concerning Pidilizumab (CT-011), it is a humanized monoclonal antibody targeting PD-1 under evaluation in several hematologic diseases. Its use in combination with vaccines will be described below.

### **AMP-224**

AMP-224 is the first recombinant B7-DC-Fc fusion protein tested in cancer patients. AMP-224 blocks the interaction between PD-1 and its ligands PD-L1 and PD-L2, likely by the depletion of PD-1 high expressing T-cells representing exhausted effector cells [35]. The preliminary results from the phase I study showed a high rate of Infusion reactions (69%), without showing drug-related inflammatory AEs commonly reported with PD-1 blocking antibodies. AMP-224 showed antitumor activity in patients with advanced solid tumors [35], but the final results are awaited yet.

### **PD-L1 BLOCKING ANTIBODIES**

PD-L1 inhibitors, BMS-936559 (MDX- 1105) and MPDL3280A, have been tested in advanced RCC patients (Table 3).

### **BMS-936559**

BMS-936559 is a fully human IgG4, anti-PD-L1 that inhibits the binding of the PD-L1 ligand to both PD-1 and CD80. In a recent Phase I study, this molecule was investigated in a group of patients with

different solid tumors, including previously treated metastatic RCC. A total of 207 patients including those with NSCLC (n = 75), melanoma (n = 55), RCC (n = 17), and ovarian cancer (n = 17) were treated with escalating doses of BMS-936559 (0.3, 1, 3, and 10 mg/kg). Objective response rates of 6–17% were observed depending on cancer type across all doses. In the small RCC cohort (n = 17), 82% were previously treated with antiangiogenic therapy and 41% received immunotherapy. Two patients had an objective response (12%; 95% CI: 2–36), which lasted for 4 and 17 months. An additional seven patients had stable disease for more than 24 weeks. [28]. As a whole, these data suggest the potential activity also of this agent against RCC.

### **MPDL3280A and MEDI4736**

MPDL3280A is another human, monoclonal antibody that targets PD-L1, blocking PD-L1 from binding the PD-1 receptor; it showed similar efficacy to nivolumab in patients with melanoma and patients with lung cancer, and is now being studied in other cancers, including renal [29]. In this phase I study reported by Cho et al., it was tested in 53 RCC patients for safety and efficacy. The majority of patients (83%) had received prior systemic therapy; 38% received immunotherapy, 57% received tyrosine kinase inhibitors, and 36% received antiangiogenic therapy. In this study were enrolled patients with different RCC types: clear cell (87%), papillary (7%) and sarcomatoid (4%) histology. The objective response rate was 13% for the RCC cohort, 13% for the clear cell histology group and as high as 17% for the non-clear cell histology group. Stable disease of 24 weeks or longer was observed in 32% and the 24-weeks PFS was 53% in the total RCC cohort. It is important to underline that, although PD-L1-positive tumors were more likely to respond, was detected some response to treatment with MPDL3280A even in patients with PD-L1- tumors. Furthermore, MEDI4736, an anti-PD-L1 antibody, has shown antitumor activity and an acceptable safety profile in patients with advanced solid tumors [36].

### **Combination with VEGF/VEGFR targeting agents**

Angiogenesis and immunosuppression work hand-in-hand in the RCC microenvironment. Beyond their antiangiogenic activity, several studies have revealed that TKIs, such as sorafenib, sunitinib, pazopanib and axitinib, may exert immunomodulatory effects depending on their different selectivity for c-kit and fms-related tyrosine kinase 3 (FLT3) receptors expressed on hematopoietic stem cells and precursor cells [37]. The immunological effects of TKIs on the immune system are complex and, at a certain extent, still ill-defined. As far as the first-generation multikinase inhibitors Sunitinib and Sorafenib, presently available data seem to suggest that Sorafenib may exert immune suppressive effects, while the effects of Sunitinib are not so clear, being immune stimulatory in the vast majority – but not all – the studies reported [38]. Furthermore, sorafenib has been shown to induce autophagy and suppress activation of tumor associated macrophages (TAMs) [39], which significantly correlate with tumor microvessel density and VEGF levels in RCC [40]. Moreover, sunitinib may enhance the immune anticancer response by decreasing the number and effectiveness of suppressor cell (Tregs and myeloid-derived stem cells, MDSCs), which are markedly involved in the pro-angiogenic phenotype of RCC [17,41]. Based on these findings, the combination of PD-1/PD-L1 blocking agents with antiangiogenic drugs has been evaluated in preclinical and clinical studies. At 2014 ASCO Annual Meeting, Dr. Amin presented the results of a phase I study of nivolumab in combination with sunitinib or pazopanib as first line therapy (NCT01472081). This combination showed a manageable safety profile, with a PFS rate at 24 weeks of 78% in the arm treated with nivolumab and sunitinib and 55% for the arm receiving nivolumab plus pazopanib [42]. Presently, a phase II study is comparing nivolumab in combination with ipilimumab vs nivolumab plus bevacizumab as neoadjuvant therapy in mRCC patients eligible for cytoreductive nephrectomy. Furthermore, pembrolizumab is under studying in combination with pazopanib (NCT02014636) or

axitinib (NCT02133742) in treatment naïve subjects with advanced RCC. As for anti-PD-L1 antibodies, a phase II trial is investigating MPDL3280A alone or in combination with bevacizumab vs. sunitinib as first-line therapy in patients with mRCC (NCT01984242). In addition, two phase I studies are evaluating MPDL3280A alone (NCT01375842) or in combination with MEK inhibitor cobimetinib in patients with advanced solid tumors including mRCC (NCT01988896).

### **Combination with other immunotherapies**

An emerging concept in cancer immunology is that inhibitory ligands, such as PD-L1/2, are induced in response to immune attack, leading to adaptive resistance by tumors [43,44]. This potential mechanism suggests that PD-1/PD-L1/2 blocking agents might synergize with other agents that enhance endogenous antitumor immunity [4,45]. Here we summarize the most promising agents for such combined strategies.

### **Immune priming**

The antitumor immune response can be enhanced by agents that prime the immune system. Single or multiple tumor antigens presenting vaccines have been evaluated in RCC [46]. This list includes autologous tumor-cell vaccines, DC-based vaccines and peptide-based vaccines. Autologous tumor-lysate vaccines have been examined in a phase III trial of patients with organ-confined RCC. The difference in terms of OS rate between patients treated after nephrectomy with autologous tumor-lysate vaccines vs placebo was not significant for both 5-years (68.9% vs 62.1%) and 10-years (80.6% vs 79.2%) follow-up. Otherwise, the difference was significant for patients with pT3 tumors ( $p = 0.001$ ) and after 10 years in the overall study population (53.6% vs 36.2%,  $p = 0.022$ ) [47]. Furthermore, Flörcken et al. presented the results of a phase I/2 study of 8 patients with mRCC treated with DC-based vaccine pulsed with autologous tumor lysate and IL-2. One patient had a minimal clinical response and two experienced stable disease, without registering serious AEs [48]. AGS-003 is a DC-based vaccine. AGS-003 was evaluated in a phase II study of newly diagnosed patients with mRCC, showing a PFS of 11.9 months without reporting additive toxicity [49]. A pilot study is evaluating the cluster of CD40L RNA-transfected AGS-003 administered before surgery in patients with local advanced mRCC (NCT02170389). Concerning peptide-based vaccines, human leukocyte antigen (HLA) class I- and II-binding peptides, oncofetal antigen 5T4, and tumor-derived heat shock protein-peptide complex (HSPPC-96) represent the main peptide targets. In this regard, a phase III trial has shown no benefits in terms of RFS in high-risk patients with non-mRCC treated with autologous tumor-derived HSPPC-96 vaccine [50], although a slight benefit was reported in patients with stage I or II disease. In the same view, no benefits were reported with MVA-5T4 vaccine (containing 5T4) vs placebo in combination with sunitinib, IL-2 or IFN- $\alpha$  for the treatment of patients with mRCC [51]. On the other hand, IMA901 (containing 9 HLA class I binding proteins and 1 HLA class II-binding protein) with or without low-dose cyclophosphamide 300 mg/m<sup>2</sup> showed 83% of 18- months OS rate in the group previously treated with cytokines who received cyclophosphamide [52]. The combination of antibodies targeting PD-1/PD-L1 with vaccines may represent a potentially effective therapeutic strategy in mRCC patients. Presently this combination is under study in patients with advanced melanoma (NCT01176474), acute myelogenous leukemia (AML) (NCT01096602) or multiple myeloma (NCT01067287). As for RCC, a phase II study is evaluating the efficacy and safety of anti-PD-1 antibody pidilizumab alone or in conjunction with DC/RCC fusion cell vaccine in mRCC patients (NCT01441765).

### **T-cell modulation**

CTLA-4, also known as CD152, is an inhibitory receptor expressed by T cells. The engagement of CTLA-4 with the binding to CD80 and CD86 promotes T-cell proliferation and function [53]. A phase

II trial of the anti-CTLA-4 antibody ipilimumab was led in patients with mRCC. Partial responses were observed in the 12.5% of enrolled patients, with a duration ranged between 7 and 21 months. Immune-related serious AEs were reported in the 43% of patients [54]. At 2014 ASCO Annual Meeting, Dr. Hammers presented the results of a phase I study of nivolumab plus ipilimumab at different doses (nivolumab 3 mg/kg + ipilimumab 1 mg/kg or nivolumab 1 mg/kg + ipilimumab 3 mg/kg) in patients with mRCC (NCT01472081). This combination showed a manageable safety profile in the two arms, the objective response rate ranged from 29% (arm N3 + I1) to 39% (N1 + I3 arm) and the duration of response from 4.1 to 22.1 weeks in the N3 + I1 and 6.1 to 18.3 weeks in the N1 + I3 arm, with almost all patients (14/15) with ongoing responses at time of analysis [55]. Presently, a phase III study is comparing the combination of nivolumab and ipilimumab vs. sunitinib in previously untreated mRCC patients (NCT02231749). In addition, a phase I/II trial is studying pembrolizumab in combination with ipilimumab or IFN- $\alpha$  in patients with advanced melanoma or RCC (NCT02089685). Furthermore, a phase I trial is studying anti-PD-L1 MPDL3280A in combination with ipilimumab or IFN- $\alpha$  in patients with advanced solid tumors including mRCC (NCT02174172). Moreover, another phase I study is evaluating anti-PD-L1 MEDI4736 in combination with anti-CTLA-4 Tremelimumab in patients with advanced solid tumors including RCC (NCT01975831). Denileukin diftitox (DD) is a fusion protein of diphtheria toxin and human IL-2 and depletes cells expressing the CD25 component of the IL-2 receptor [56]. In 2010, a phase I study of 15 mRCC patients showed that DD administered before high-dose IL-2 or between IL-2 courses was associated with 33% of partial responses, with 2 complete responses and no serious AEs related to DD addition [57]. The soluble lymphocyte-activation gene-3 (LAG-3) is an agonist of major histocompatibility complex II-driven DC activation that enhances expansion of tumor-specific, cytotoxic T lymphocytes (CTLs) in vitro [58]. IMP321, a soluble LAG-3 fusion protein, was investigated in an escalating-dose phase I study of 21 patients with mRCC. IMP321 was well tolerated and 7/8 patients treated with high dose IMP321 had stable disease at 3 months, while only in 3/11 patients treated with low dose IMP321 the disease was stable at 3 months [59]. Currently, a phase I study is evaluating anti-LAG-3 BMS-986016 with or without nivolumab in patients with advanced solid tumors including RCC (NCT01968109).

#### **Adoptive cell transfer (ACT) and allogeneic stem cell transplantation (ASCT)**

ACT therapy is based on T cells modified to target proteins expressed by RCC, such as MAGE-A3/12, DR4 and TRAIL. However, ACT have not demonstrated OS benefits in a series of phase I/II studies in RCC patients [60–63]. Similarly, few studies of tumorinfiltrating lymphocytes (TILs) have showed poor objective response rates [64–69]. ASCT represents an alternative method of manipulating innate immunity. However, this strategy remains investigational due to its toxicity profile, the availability of multiple targeted agents, together with the necessity of receiving an immunosuppressive therapy to prevent graft-versus-host disease and the lack of available donors [70].

#### **Granulocyte–macrophage colony-stimulating factor (GM–CSF)**

Preclinical studies in melanoma and colon cancer tumor models have revealed synergy between PD-1 blocking and the treatment with granulocyte–macrophage colony-stimulating factor (GM–CSF) [71]. Thus, this combined strategy could be evaluated in patients with mRCC.

#### **4–1BB**

4–1BB is a type 2 transmembrane glycoprotein that belongs to the TNF superfamily, expressed on activated T lymphocytes. Signaling via 4–1BB enhances cell proliferation, induces cytokine production, and prevents activation-induced cell death in T cells [72]. Presently, a phase I study of



PF-05082566, a 4-1BB agonist monoclonal antibody in combination with pembrolizumab is in course in patients with solid tumors (NCT02179918).

## **DISCUSSION**

Cancer immune surveillance is considered to be a fundamental host protection process to maintain cellular homeostasis and to inhibit carcinogenesis. Tumors evolve mechanisms to escape from immune-mediated rejection, a process called “immune editing”, which exerts a selective pressure on the tumor microenvironment, leading to malignant progression. Despite the fact that in few RCC patients, cytokines (i.e. a very ‘primitive’ form of immunotherapy) yielded long-lasting response and survival, in the last decade, VEGFR-TKI and mTOR inhibitors have been developed, suddenly overshadowing immunotherapy as the standard of care for RCC. However, the promising results obtained by novel, and more sophisticated immune modulation approaches (in particular the blockade of the PD-1 pathway, which seems to be characterized by good tolerability and durable clinical benefit), have shed new light to this approach. Complementary PD-1 pathway blockade and targeted therapies or other immunotherapeutic agents has the potential to reduce the tumor-induced immunosuppression, likely improving the outcome of mRCC patients. Nevertheless, a series of open questions remain. Firstly, what is the most effective setting for the use of these agents? A growing body of evidence suggests that starting PD-1 blocking therapy before nephrectomy, when PD-1 expression on immune cells and PD-L1 expression on RCC cells are more pronounced might be more effective [7]. Moreover, the presence of PD-1 on effector memory T cells suggests that these cells could be able to mount an effective secondary immune response against tumor recurrence [7]. Secondly, do patients continue to have tumor control after cessation of blockade of the pathway between PD-1 and PD-L1? Such tumor control could reflect the development of an effective immunologic memory to control tumor growth. However, only a longer follow-up will confirm this hypothesis. Thirdly, the efficacy and safety of PD-1/PD-L1 targeting antibodies in specific RCC subpopulations, such as elderly patients, patients with poor-risk features or non-clear cell histologies should be further investigated. In addition, predictive reliable biomarkers of response to these agents and adequate clinical/radiological criteria to evaluate RCC response to immunotherapy are urgently needed in order to identify patients who will benefit the most. In conclusion, the blockade of PD-1 pathway has the potential to be a game-changer in the therapeutic scenario of RCC. However, further efforts are needed to extend the promises realized by these agents in other tumors to RCC patients.

## **CONFLICTS OF INTEREST**

The authors declare to have no conflicts of interest.

## **ACKNOWLEDGMENTS**

Supported by a grant of the Italian Association for Cancer Research (AIRC-IG 11930, AIRC 5per mille 12214).

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## TABLES

Table 1. Differences between CTLA-4 and PD-1 pathways.

CTLA-4 pathway	PD-1 pathway
Expressed only on T-lymphocytes	Expressed on T, B-lymphocytes, NK cells
Ligands: B7.1 (CD80), B7.2 (CD86)	Ligands: PD-L1 (B7-H1), PD-L2 (B7-DC)
Ligands expressed only on APC cells	Ligands expressed on APC and tumor cells
CTLA-4 blockade increases CD4+ and CD8+ T cells proliferation	PD-1/PD-L1 blockade especially increases CD8+ T cells proliferation (>CD4+ T cells)

Table 2. Response rate according to PD-L1 positivity in cancer patients treated with PD-1/PD- L1 targeting antibodies.

Agent(s)	Tumor type	Number of patients	RR (%) PD-L1 pos	RR (%) PD-L1 neg
Nivolumab [26]	Multiple solid tumors	42	36	0
MPDL3280A [28]	Kidney cancer	47	20	10
Nivolumab/ Ipilimumab [29]	Melanoma	27	40	47
Nivolumab [30]	Melanoma	34	44	17

Table 3

Current trials focused on PD-1/PD-L1 blocking agents ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)). CTLA-4 = cytotoxic T-lymphocyte-associated protein 4; DC = dendritic cell; IDO1 = indoleamine 2,3-dioxygenase 1; IFN- $\alpha$  = interferon- $\alpha$ ; LAG3 = lymphocyte-activation gene 3; RCC = metastatic renal cell carcinoma; PD-1 = programmed death-1; PD-L1 = programmed death ligand-1.

Agents	Target	Trial ID number	Phase	Description
Nivolumab	PD-1	NCT01472081	I	In combination with ipilimumab at different doses in previously treated mRCC patients
		NCT01844505	III	vs. everolimus in mRCC patients who have received prior anti-angiogenic therapy
		NCT01358721	I	Characterization of peripheral immune cells, soluble factors, tumor immune infiltrates and markers in mRCC patients treated with nivolumab
		NCT02231749	III	In combination with ipilimumab vs. sunitinib in previously untreated mRCC patients
		NCT02210117	II	In combination with ipilimumab vs nivolumab plus bevacizumab as neoadjuvant therapy in mRCC patients eligible for cytoreductive nephrectomy
		NCT01968109	I	Anti-LAG-3 BMS-986016 With or without nivolumab in patients with advanced solid tumors including RCC
Pembrolizumab	PD-1	NCT02212730	I	Alone as neoadjuvant therapy in participants undergoing RCC tumor resection
		NCT02178722	I/II	in combination with the inhibitor of IDO1 INCB024360 in patients with advanced solid tumors including RCC
		NCT02014636	I/II	In combination with pazopanib in treatment naïve subjects with advanced RCC
		NCT02133742	I	In combination with axitinib in previously untreated mRCC patients
		NCT02089685	I/II	In combination with ipilimumab or IFN- $\alpha$ in patients with advanced melanoma or RCC
		NCT02179918	I	In combination with PF-05082566, a 4–1BB agonist monoclonal antibody in patients with solid tumors
Pidilizumab	PD-1	NCT01441765	II	Alone or in conjunction with DC/RCC fusion cell vaccine in mRCC patients
MPDL3280A	PD-L1	NCT01375842	I	Patients with advanced solid tumors including RCC
		NCT01984242	II	In combination with bevacizumab vs. sunitinib as first-line therapy in patients with mRCC
		NCT01988896	I	In combination with MEK inhibitor cobimetinib in patients with advanced solid tumors including RCC
		NCT02174172	I	In combination with ipilimumab or IFN- $\alpha$ in patients with advanced solid tumors including RCC
MEDI4736	PD-L1	NCT02118337	I	In combination with anti-PD-1 MEDI0680 in patients with advanced solid tumors including RCC
		NCT01975831	I	In combination with anti-CTLA-4 Tremelimumab in patients with advanced solid tumors including RCC

Fig. 1. Blocking PD-1/PD-1L in RCC tumor microenvironment.

