

# Checkpoint modulation - A new way to direct the immune system against renal cell carcinoma

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The introduction of targeted therapies like the tyrosine kinase (TKI) and mammalian target of rapamycin (mTOR) inhibitors has improved patients' survival in general. Nevertheless the prognosis remains limited. Therapies with a new mode of action are urgently warranted, especially those who would provoke long-term responders or long-lasting complete remissions as observed with unspecific immunotherapy with the cytokines interleukin-2 and interferon- $\alpha$ . In the recent years a deeper understanding of the underlying immunology of T cell activation led to the development of checkpoint inhibitors, which are mainly monoclonal antibodies and which enhances the presence of the co-stimulatory signals needed for T cell activation or priming. This review discusses the clinical data and ongoing studies available for the inhibition of the PD-1 (CD279) and CTLA-4 (CD152) axis in mRCC. In addition, potential future immunological targets are discussed. This approach of T-cell activation or re-activation by immunological checkpoint inhibition holds the inherent promise to directly affect the tumor cell and thereby to potentially cure a subset of patients with mRCC.

## Introduction

Metastatic disease in renal cell carcinoma (mRCC) is present in about 20 to 30% of patients at the time of initial diagnosis. Another third will develop metastatic disease later on. In general, if this tumor stage is present, the prognosis was poor and mRCC was regarded as a therapy-refractory disease. The discovery that interleukin-2 is a T cell stimulating cytokine paved the way to the first effective therapies in metastatic RCC. The cytokines IL-2 and IFN- $\alpha$  alone or in combination with 5-Fluouracil

significantly improved the survival of mRCC patients. The cytokines, applied in different dose regimes and termed unspecific immunotherapy, led to a remarkable clinical benefit in terms of disease stabilization or remission in up to 30% of patients.<sup>1,2</sup> Despite some long lasting responses or complete remissions the majority of patients had a survival benefit of only some months.<sup>1,3</sup> Therefore cytokine-based immunotherapy is currently replaced by the targeted therapy of mammalian target of rapamycin (mTOR) and tyrosine kinase inhibitors (TKI) for the treatment of mRCC patients. These agents improved median overall survival up to 30 months.<sup>4,5</sup> In addition, the multiplicity of substances allows for a second-line therapy and potential subsequent therapies.<sup>6,7</sup>

Unspecific immunotherapy did not only lead to the activation of the immune system to target the cancer cells. Adverse immune effects like increased frequencies of regulatory T cells (Tregs) and decreased frequencies of circulating myeloid and plasmacytoid dendritic cells were reported in cytokine treated mRCC patients, which may explain the limitations of this therapy.<sup>8,9</sup> These facts and a missing well-defined mode of action with a T-cell response not specifically directed against the RCC tumor cells were the major drawbacks of this unspecific stimulation of the immune system in the recent years.

Currently, in RCC a transformation from the ancient unspecific therapy with cytokines to rather specific approaches, which directly target the renal cell cancer cell and the tumor microenvironment is observed.<sup>10</sup> One of the underlying principles in specific immunotherapy is that tumors express antigens the so called tumor-associated antigens (TAAs) that are recognized by (cytolytic) T lymphocytes (CTLs) derived from the tumor-bearing patient.<sup>11</sup> The described approaches of active immunotherapy have in common that TAAs shall activate naïve T cells, which then target the tumor. Several randomized immunotherapy trials have been reported and are on its ways in the adjuvant or metastatic setting. For example, AGS-003 (Argos Therapeutic, NC, USA) is a dendritic cell based (DC) vaccine based on individual tumor mRNA combined with synthetic CD40L RNA.<sup>12</sup> Vitespen (Oncophage®; Antigenics Inc., MA, USA) is an autologous tumor derived heat shock protein Gp96 preparation.<sup>13</sup> Reniale® (Liponova, Hanover, Germany) is an autologous DC vaccine and IMA901 (Immatics, Tübingen, Germany) is a synthetic peptide vaccine.<sup>14,15</sup> The results of these trials are promising, but

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none of the vaccines has gained general market status in Europe or the US. Currently, phase III studies are ongoing for AGS-003 and IMA901 for a further evaluation.<sup>16,17</sup> Unfortunately, the immune system can be controlled and edited by local or systemic environments to prevent an effective T cell activation at checkpoints of T cell activation.

### Immunosurveillance and immunoediting

The hypothesis of immunosurveillance and the concept of immunoediting both describe the biological – immunological approach of cancer development.<sup>18–20</sup> The original concept of the immunosurveillance hypothesis formulated by Sir Macfarlane Burnet and Lewis Thomas postulates that small accumulations of tumor cells develop within the body. These tumor cells provoke an effective cellular immune reaction, which protects from neoplastic disease and leads to the regression of the tumor with no signs of clinical existence.<sup>18,20</sup> Later on this hypothesis was re-formulated to the concept of immunoediting, which consists of 3 phases, elimination, equilibrium and tumor escape. The elimination corresponds to the immunosurveillance. In the equilibrium the immune system allows the selections and promotion of different tumor cells with the capacity to survive the immune attacks. In the escape phase the immunologically sculptured tumor leads to an uncontrolled outgrowth of tumor cells within the immunocompetent host and the tumor becomes clinically apparent.<sup>19</sup> Thereby the tumor has different mechanisms to render itself “invisible” to the host’s immune system and to silence immune cells.<sup>21</sup> These tumor-driven mechanisms of immune suppression includes the downregulation of HLA molecules and/or tumor associated antigens (TAA), which leads to a decreased immunogenicity or the induction of suppressive cytokines like IL-10 or TGF- $\beta$ .<sup>22</sup> These cytokines can lead to recruitment of regulatory T cells (T<sub>regs</sub>), myeloid derived suppressor cells (MDCS) or tumor-associated M2 macrophages which can act as immunosuppressors.<sup>21,23,24</sup>

Therefore, agents called immunomodulators or checkpoint inhibitors, which counteract tumor-induced immunosuppression are an external editing of the immune system to potentially shift tumor growth from the escape phase to the equilibrium state or toward tumor elimination.

### Checkpoints and the regulation of the immune system

Activated T cells eliminate pathogens and tumor cells, but an exaggerated activation provokes autoimmune disorders. To understand the biology of checkpoint inhibition, it is of importance to figure out the control of T cells with its activating and inhibitory co-receptors, which are major regulators of T cell response and mediate tolerance to “self.”<sup>25,26</sup>

In order to prime, e.g. to activate naïve T cells the antigen-presenting cell (DC/APC) must present the TAA in the correct major histocompatibility complex molecule (signal one) in the presence of a co-stimulus, e.g., the expression of CD80 (B7–1) and CD86 (B7–2).<sup>25</sup> A high expression of such co-stimulatory molecules is achieved by the activation of APCs. If the co-stimulatory signal is not present, the DC cells might present the antigen on to the CD8+ T cell as a tolerogenic signal, which

is also mediated through PD-1 (CD279) and CTLA-4 (CD152) (Fig. 1).<sup>27,28</sup> This phenomenon termed peripheral tolerance is influenced by the antigen-specific tolerogenic role of DCs.<sup>22,27,28</sup>

### CD152 – CTLA-4

The discovery that the B7-family members CD80/86 act as ligands to CD28 provided the molecular basis for the additional co-stimulatory signal needed for T cell activation.<sup>29</sup> This view of T cell co-stimulation had to be adopted with the discovery of cytotoxic T-lymphocyte antigen-4 (CTLA-4, CD152), which inhibits CD80/86, but binds with a much higher affinity than CD28.<sup>30</sup> Antibody binding experiments demonstrated that CTLA-4 ligation blocks CD28-dependent T cell activation.<sup>31</sup> Here, crosslinking of anti-CTLA-4 antibodies inhibited IL-2 production. In this context, it is thought that CTLA-4 prevents an exaggerated T cell activation if the co-stimulatory activation of CD80/86 to CD28 is provided.<sup>32</sup> For example, CTLA-4 deficient mice develop massive lymphoproliferation and fatal multi-organ tissue destruction as an expression of autoimmunity.<sup>33</sup> On the other hand, in preclinical models CTLA-4 blockade augments anti-tumor immunity and prolonged survival in a mice colon carcinoma model.<sup>34</sup>

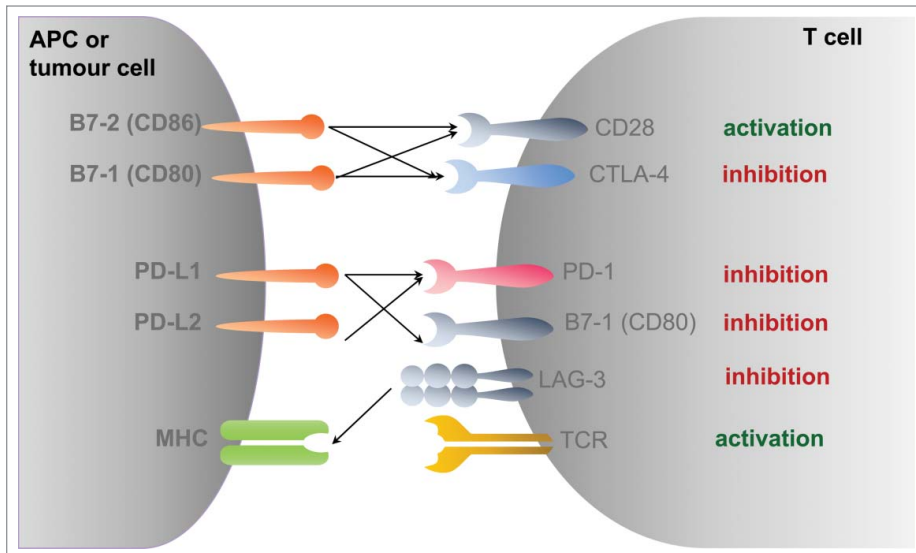
In the clinic, ipilimumab (Yervoy, Bristol-Myers Squibb, New York City, NY, USA) and tremelimumab (Pfizer, New York City, NY, USA) are 2 humanized antibodies, which binds to CTLA-4 and thereby re-activate T cell effector function (Table 1). So far, only ipilimumab is approved for the treatment of metastatic melanoma.<sup>35</sup> Data from 2 large phase III trials are available for Ipilimumab. In the first trial patients with unresectable stage III or IV melanoma who had progressed after initial first-line therapy were randomized in a 3:1:1 fashion to receive ipilimumab (3 mg/kg b.w.) plus the glycoprotein 100 (gp100) peptide vaccine vs. ipilimumab alone vs. gp100 peptide vaccine alone. Overall survival was improved to 10.0 months vs. 6.4 months in the ipilimumab + gp100 combination arm compared to gp100 alone. Patients who received ipilimumab alone demonstrated an overall survival of 10.1 months.<sup>35</sup> In the second trial ipilimumab (10 mg/kg) was administered to previously untreated patients with metastatic melanoma in combination with dacarbazine (850 mg/m<sup>2</sup>) compared to dacarbazine vs. placebo. In the ipilimumab plus dacarbazine group overall survival was significantly improved by 2.1 months with higher survival rates at 2 and 3 (20.8% vs. 12.2%) years follow-up.<sup>36</sup>

The second CTLA-4 monoclonal antibody tremelimumab failed to demonstrate a significant prolongation in survival in a large phase III study of metastatic melanoma patients.<sup>37</sup>

### CTLA-4 – Ipilimumab phase II study

In RCC the results of one phase II trial are available.<sup>38</sup> In the first cohort 21 metastatic RCC patients were treated with ipilimumab at a loading dose of 3 mg/kg and then with 1 mg/kg every 3 weeks (n = 21). All patients had previous IL-2 treatment (Table 2). Three (14%) of these patients had a grade III toxicity of enteritis presenting with diarrhea. One of the 21 patients, the

one with diarrhea, had a partial response. In the second cohort 40 patients received 3 mg/kg of ipilimumab every 3 weeks. 26 patients had a previous IL-2 therapy.<sup>38</sup> 43% of patients had a severe immune-mediated toxicity (14 grade III, 2 grade IV and 1 grade V), with enteritis (n = 13) and hypophysitis (n = 1). One patient with adrenal metastasis showed primary adrenal insufficiency and one patient had aseptic meningitis. Of the patients with enteritis 3 had a colonic perforation and one underwent colectomy for bleeding.<sup>38</sup> Among the 40 patients there were 5 partial responses at 7, 8, 12, 17 and 21 months' duration. The association between autoimmune toxicity and objective tumor regression was significant; there was no patients responding to therapy who had no autoimmune event.<sup>38</sup>



**Figure 1.** Stimulatory pathways of T cell activation or inhibition.

#### CTLA-4 – Tremelimumab in combination with sunitinib phase I study

In a phase I study 28 patients, who had received  $\leq 1$  previous systemic treatment, received tremelimumab (6 mg/kg, 10 mg/kg, or 15 mg/kg) intravenously once every 12 weeks and oral sunitinib (50 mg daily for 4 weeks then 2 weeks off or 37.5 mg daily as a continuous dose) in a 3+3 dose escalation scheme.<sup>39</sup> The primary objective was drug safety. Dose limiting toxicities were observed in 2 of 5 patients receiving 50 mg sunitinib and tremelimumab 6 mg/kg, in 3 of 6 patients receiving tremelimumab 15 mg/kg plus continuously sunitinib at 37.5 mg and in 3 of 7 patients in the expansion cohort of tremelimumab 10 mg/kg plus sunitinib 37.5 mg daily. There was one sudden death in the tremelimumab 10 mg/kg plus sunitinib group. The onset of rapid acute renal failure was the most common DLT. Due to these results a further investigation of tremelimumab doses greater than 6 mg/kg plus sunitinib 37.5 mg daily was not recommended.<sup>39</sup>

#### PD-1/PD-L1 axis

Programmed death 1 (PD-1) is a key immune checkpoint receptor expressed by activated T cells, where it terminates immune responses upon antigen stimulation to avoid massive immune destruction. The two ligands PD-L1 (B7-H1) and PD-L2 (B7-DC) expressed in peripheral and tumor tissue bind to the PD-1 receptor on the T cell surface.<sup>40</sup> Here, PD-L1 seems to be selectively expressed by tumor cells and cells in the tumor micro-environment in response to inflammatory stimuli, where it can inhibit cytokine production and the cytolytic activity of PD-1 positive, tumor-infiltrating CD4+ and CD8+ T cells.<sup>40-42</sup> PD-1 directed antibodies inhibit the interaction between PD-1 receptor and its ligands, while anti-PD-L1 antibodies block interactions between PD-L1 and both PD-1 and CD80.<sup>43,44</sup>

The interesting point in the disruption of the PD-1/PD-L1 axis is whether there will be a long-lasting anti-tumor response and a tumor control after the cessation of the blockade. This would reflect a persistent anti-tumor immunity with the

**Table 1.** Overview of available checkpoint modulators for cancer immunotherapy

Target pathway	Substance	Company	Reference
CTLA-4	Ipilimumab	BMS	35,36
	Tremelimumab	Pfizer	37,38
PD-1	Nivolumab (BMS-936558)	BMS	44, 47, 48
	Pembrolizumab (MK-3475)	Merck	53
	AMP-224	Amplimmune	54
	Pidilizumab (CT-011)	CureTech	55
PD-L1	MEDI4736	MedImmune/AstraZeneca	56
	MPDL3280A	Roche	57
	MSB0010718C	MerckSerono	58
	BMS-936559	BMS	43
LAG-3	IMP321	Immutep	59

**Table 2.** Published clinical trials of checkpoint modulators for mRCC as discussed in the review

Target pathway	Trial Design	Trial Drug	Setting all mRCC	Number	Dosing	Results	Reference
CTLA-4	Phase II	Ipilimumab	after previous IL-2	first cohort n = 21 pts.	loading dose of 3 mg/kg 1 mg/kg every 3 weeks 3 mg/kg every 3 weeks	1 partial response	<sup>38</sup> NCT00057889
			after previous IL-2 or no previous treatment	second cohort n = 40 pts.		5 partial responses at months 7, 8, 12, 17 and 21	
PD-1	Phase I 3+3 dose escalation study	Tremelimumab	≤1 previous systemic treatment	n = 28 pts.	6 mg/kg, 10 mg/kg, or 15 mg/kg once every 12 weeks plus sunitinib 50 mg (4/2) or 37.5 mg continuously	Investigation of tremelimumab doses > 6 mg/kg plus sunitinib 37.5 mg daily were not recommended	<sup>39</sup> NCT00372853
			>1 previous systemic treatment	n = 33 with mRCC out of 296 pts. with solid cancers	1 mg/kg or 10 mg/kg every 2 weeks until progression (maximum up to 24 months)	objective responses in 4 of 17 patients (24%) objective responses in 5 of 16 (31%) at a dose of 10.0 mg/kg	<sup>44</sup> NCT00730639
PD-L1	Phase I dose escalation study	BMS-936559	>1 previous systemic treatment	n = 17 with mRCC out of 207 pts. with solid cancers	10 mg/kg every 2 weeks in 6 weeks cycles with up to 16 cycles	objective response in 2 of 17 patients (12%, all at the 10 mg/kg dose), responses lasting 4 and 17 months. stable disease in 7 (41%) lasting at least 24 weeks.	<sup>43</sup> NCT00729664
MHC class II	Phase I dose-escalation trial	IMP321	most pts. were pretreated with sunitinib or sorafenib	n = 21 pts.	s.c. biweekly for 6 injections with 3 to 6 pts. at 0.05, 0.25, 1, 2.5, 6.25, and 30 mg per injection	no objective tumor response observed stable disease at 3 months in 7 of 8 evaluable patients at doses > 6 mg and in 3 of 11 pts. at doses > 6 mg	<sup>59</sup> NCT00351949



generation of an effective immunologic memory for an ongoing tumor control. Several antibodies have been used in early clinical trials that either target PD-1 or PD-L1.<sup>45</sup> Subsequently, the results of antibodies administered in RCC patients are discussed, mainly based on the published trial results and results of clinical trial, which were presented at the annual meeting of the American society of clinical oncology (ASCO) in 2014.

### PD-1 antibody

BMS-936558 also named nivolumab is a fully humanized anti-PD-1 antibody (Bristol-Myers Squibb, New York City, New York) that has shown anti-tumor activity in 296 patients with solid cancers.<sup>44</sup> Nivolumab was given in a starting dose of 1 mg/kg and then expanded to 3 and 10 mg/kg in cohorts of 3 to 6 patients, among these 33 had mRCC at doses of 1 and 10 mg/kg. The objective response rates were 4 of 17 patients (24%) treated with a dose of 1.0 mg/kg and 5 of 16 (31%) treated with a dose of 10.0 mg/kg. Five out of 8 patients, who started treatment one year before analysis, had a response longer than one year. Stable disease, defined as stability longer than 24 weeks, was observed in an additional 9 (27%) patients. Of all 296 patients treated 14% had a grade 3 or 4 drug-related adverse event (AE) with diarrhea (11%), rash (12%), pruritus (9%), pneumonitis (3%) and increased levels of alanine (4%) or aspartate aminotransferase (3%) or thyroid-stimulating hormone (3%). Endocrine disorders of hypophysitis (2%) and hyperthyroidism (1%) were rare.

Based on these results a large phase III trial of 822 patients with metastatic or advanced RCC was initiated (CheckMate 025).<sup>46</sup> RCC patients with 1 or 2 prior anti-angiogenic therapies were randomized to receive either nivolumab (3 mg/kg every 2 weeks) vs. everolimus (10 mg p.o daily). Primary study aim is overall survival. The study has terminated recruitment and is currently under follow-up. Final data collection for primary outcome measure is announced for February 2016.<sup>46</sup>

In advanced treatment-refractory melanoma (n = 107, 62% had at least 2 prior therapies) nivolumab treated patients demonstrated an overall survival of 16.8 months with one and 2 y survival rates of 62% and 43%. Nivolumab doses ranged between 0.1 and 10 mg/kg, including a dose expansion cohort.<sup>47</sup> In a phase III study (CheckMate 066) previously untreated metastatic melanoma patients without a BRAF mutation were randomized 1:1 to nivolumab (3 mg/kg every 2 weeks) vs. dacarbazine (1000 mg/m<sup>2</sup> every 3 weeks). Progression-free survival was significantly prolonged in the nivolumab group (5.1 vs. 2.1 months) and the overall survival rate at one year was 72.9% in the nivolumab arm compared to 42.1% in the dacarbazine arm, while median OS was not reached in the nivolumab arm so far.<sup>48</sup>

These improvements obtained with nivolumab in the treatment of melanoma patients underline the expectations, which are anticipated from the ongoing RCC trials.

### PD-1 antibody – dose escalation

In the primary study of nivolumab in solid cancer no dose–toxicity relationship was observed with 0.3 to 10 mg/kg.<sup>44</sup> At ASCO 2014 a dose ranging phase II study of nivolumab was

presented, which assessed the primary aim of a dose response-relationships of nivolumab in pretreated mRCC patients (at least 1 prior antiangiogenic therapy; n = 168).<sup>49</sup> Patients were randomized 1:1:1 to 0.3, 2 and 10 mg/kg nivolumab i.v. every three weeks with treatment until progression or intolerable toxicity. Patients received a median of 6 (0.3 mg/kg) to 8 (2 and 10 mg/kg) doses of nivolumab. Reasons for drug discontinuation were predominately progressive disease in 81%, 74% and 69% of patients and drug-related toxicity in 2%, 9% and 7% of patients in the 0.3, 2 and 10 mg/kg arms. Treatment related adverse events of any grade III or IV were 5% (0.3 mg/kg), 17% (2 mg/kg) and 13% (10 mg/kg) in the respective group. Objective response rate was not different between the groups (20–22%) and most patients responding had the response within their first 3 months. Interestingly, complete responses were rare with a rate of 0–2%. Progression-free survival was similar in all groups with 2.7 months, 4.0 months and 4.2 months for the 0.3, 2 and 10 mg/kg groups (p = 0.9). In addition, median overall survival ranged between 18.2 months (0.3 mg/kg), 25.5 months (2 mg/kg) and 24.7 months (10 mg/kg), which is quite a promising result for second and third-line treated patients.

### PD-1 antibody in combination with VEGF-TKI

Data of a phase II study of nivolumab in combination with the TKI sunitinib or pazopanib in mRCC patients was presented at this years ASCO annual meeting as an abstract.<sup>50</sup> Nivolumab dose (every 3 weeks) was escalated from 2 mg/kg to 5 mg/kg, sunitinib (S + N; n = 33) and pazopanib (P + N, n = 20) given in the recommended dosage in previously treated patients with the primary aim to assess overall tolerability and safety. In the sunitinib arm nivolumab was escalated from 2 mg/kg (n = 7) to 5 mg/kg (n = 7) and then expanded in another 19 patients who were treatment naive. In the pazopanib arm 10 patients were treated with 2 mg/kg and no further escalation as the maximum tolerated dose was reached. Severe adverse events grade III or IV occurred in 82% of the S+N arm and in 70% of the P+N2 arm, including TKI class related AEs like hypertension, diarrhea, liver enzyme elevations and fatigue. Immune associated AE of pneumonitis and endocrinopathy were rare, but renal and hepatic AE were higher than anticipated for VEGF-TKI or nivolumab monotherapy. Treatment was discontinued due to AE in 12 (36,4%, S+N arm) and 5 (25%, P+N2 arm) patients. No treatment related death occurred. Efficacy was higher in the combinations than anticipated for the single agents. At the first assessment at 6 weeks 41.2% (7/17) and 55.6% (5/9) responded, 30% (n = 10) and 35% (n = 7) had a stable disease in the S+N and the P+N2 arm. Ongoing responders were observed in 58.8% (10/17, S+N) and 33.3% (3/9, P+N) of patients. Interestingly, time to response was relatively short with most patients responding within 6 to 12 weeks and 5 patients responded following the discontinuation of the therapy.<sup>50</sup>

### PD-1 antibody in combination with ipilimumab

Another phase I study by Hammers et al. was presented as an abstract at the ASCO meeting 2014.<sup>51</sup> Patients were treated with

combinations of nivolumab and ipilimumab 4 times in 3 weeks intervals followed by continuous treatment of nivolumab 3 mg/kg every 3 weeks. Combination therapy was either nivolumab 3 mg/kg plus ipilimumab 1 mg/kg (N3 + I1, n = 21) vs. nivolumab 1 mg/kg plus ipilimumab 3 mg/kg (N1+I3, n = 23). About 80% of patients had received a previous systemic treatment. Treatment related AE led to the discontinuation of therapy in 2 (9.5%) and 6 (26.1%) of N3+I1 and N1+I3 treated patients. Treatment related adverse events grade III and IV were higher in the N1+I3 arm (60.9% vs. 28.6%) with gastrointestinal disorders and hepatic dysfunction. According to the authors grade III or IV AE were manageable with established guidelines. Responses were higher in the combination than reported for each monosubstance of nivolumab and ipilimumab in RCC. Progression-free survival was 64% and 64% in the N3+I1 and N1+I3 arm. Median duration of response was 31.1 weeks (4.1 to 32.1+) in the N3+I1 arm and not reached in the N1+I3 arm (12.1+ to 35.1+). Objective response was observed in 9 of 21 (43%) and 10 of 23 (43%) and stable disease in 5 (24%) and 8 (35%) patients for N3+I1 and N1+I3. Most responses occurred within 6 to 12 weeks and 2 of the N3+I3 and 4 of the N1+I3 responders exhibited ongoing responses.<sup>50</sup>

The encouraging results of this phase I study led to the initiation of a phase III study in mRCC patients as a first-line treatment with the combination of nivolumab 3 mg/kg with ipilimumab 1 mg/kg intravenously every 3 weeks for 4 doses and then nivolumab 3 mg/kg solutions intravenously every 2 weeks compared to sunitinib monotherapy (CheckMate 214). Approximately 1070 patients are intended to be enrolled.<sup>52</sup>

### PD-L1 antibody

The second antibody that targets the PD-1 receptor-ligand axis is BMS-936559 a fully humanized PD-L1 specific antibody (Bristol-Myers Squibb, New York City, New York).<sup>43</sup> PD-L1 antibody has been evaluated in 207 patients with advanced solid tumors, among 17 patients with mRCC treated at a dosage of 10 mg/kg in 6 weeks cycles on days 1, 15, and 29 of each cycle. Two of the 17 patients showed an objective response with duration of 4 and 17 months; 7 patients had stable disease at the 24-week follow-up. Most events were low grade, with treatment-related grade 3 or 4 events in 19 of 207 (9%). The most common drug-related adverse events were fatigue, infusion reactions, diarrhea, arthralgia, rash, nausea, pruritus, and headache. Potential immune-related adverse events were observed in 81 of 207 patients (39%); the included hypothyroidism, hepatitis, and one case each of sarcoidosis, endophthalmitis, diabetes mellitus, and myasthenia gravis.<sup>43</sup>

### Future perspective

Immunotherapy of metastatic RCC has evolved from the rather unspecific approaches of the cytokine area to specific immunotherapy. Currently, one of the main foci in specific immunotherapy is the use of checkpoint inhibitors. Here, targeting the PD-1/PD-L1 axis is the most heavily investigated area and is regarded as the lead axis. Among the discussed antibodies, for which clinical results are available, several other PD-1

antibodies like pembrolizumab (MK-3475, Merck Sharp & Dohme Corp., Whitehouse Station, NJ, USA.<sup>53</sup>), AMP-224 (Amplimmune Inc., Gaithersburg, MD, USA<sup>54</sup>), Pidilizumab (CT-011, Curetech, Yavne, Israel<sup>55</sup>) and PD-L1 antibodies like MEDI4736 (MedImmune/AstraZeneca, London, U.K.<sup>56</sup>), MPDL3280A (Genentech/Roche, Basel, Switzerland<sup>57</sup>), and MSB0010718C (MerckSerono, Darmstadt, Germany<sup>58</sup>) are in different stages of preclinical and clinical development in phase I/II studies of various cancer entities (Table 1).<sup>45</sup>

Checkpoint inhibition with nivolumab and ipilimumab activates or inhibits co-stimulatory signals in T cell activation. This new mode of action demonstrated a prolonged overall survival for melanoma patients and it is anticipated that this will also be achieved in mRCC patients. Nevertheless this “manipulation” of the immune system also leads to new probably class specific immune related adverse events which might be dose limiting. As observed so far, these immune related AE lead to autoimmune reactions like hypothyroidism, hepatic dysfunction or hepatitis, sarcoidosis, endophthalmitis, gastrointestinal dysfunction like enteritis or myasthenia gravis. Especially the combination of ipilimumab and nivolumab in the dose of I3 + N1 seemed to be dose limiting.<sup>50</sup>

Immunological checkpoints can inhibit or activate T cells. These leads to the speculation if immunomodulation should target co-stimulatory molecules, which inhibit or the activate T cells. Based on the clinical results available from the 2 lead substances nivolumab and ipilimumab, especially in melanoma, it seems to be more beneficial to target the inhibitory axis, in terms of efficacy and reduced side effects.

Checkpoint inhibition is not limited to the CTLA-4 and the PD-1/PD-L1 axis. Different monoclonal antibodies, which activate or inhibit T cell function, are under development. Among the target molecules antibodies against the tumor necrosis family receptors (TNFR) with OX40 (CD134), 4-1BB (CD137), GITR (glucocorticoid-induced TNFR-related protein/ tumor necrosis factor receptor superfamily member 18) and CD40 are available and warrant further evaluation in RCC.<sup>45</sup> The future will show if any antibody directed checkpoint inhibition would become a paradigm changing therapy in mRCC.

Not only co-stimulatory signals are the target of T cell activation therapies, but also the direct agonize of the MHC class II receptor. IMP321 is a LAG-3 fusion protein, which agonizes MHC class II-driven dendritic cell activation. IMP321 was evaluated in a dose escalation phase I study in mRCC patients.<sup>59</sup> In this study IMP321 induced both sustained CD8 T-cell activation and an increase in the percentage of long-lived effector-memory CD8 T cells. The safety of IMP321 was excellent, as no clinical significant local or systemic treatment-related adverse events were recorded; 10% of patients experienced grade I local reactions. In the 21 patients treated no objective response occurred, but in patients with higher doses PFS was significantly better compared to lower doses.<sup>59</sup> These interesting results warrant further investigation in combination studies, not only with standard anti-angiogenic therapy.

Although checkpoint inhibitors are attributed to the treatment modality of specific immunotherapy it has to be stressed

that only the mode of action is specific, e.g. the target receptor is well defined. On the other hand, the provoked effector functions by the respective checkpoint modulation are not well defined. It is not known against which respective tumor-associated antigens the T cells will be activated and in which strength the anti-tumor immune response is provoked. For the future, these facts warrant that checkpoint modulators are not only be used as a stand-alone drug, but rather in combination with a vaccination specific approach in which the immune response is direct against specific TAA and the strength of the response can be measured.

## Disclosure of Potential Conflicts of Interest

AS is subinvestigator, JB and PJG are principal investigator of the nivolumab CheckMate25 clinical trial. PJG is investigator of the nivolumab Checkmate214 study. JB, AS and PJG have received honoraria for advisory role from BMS.

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