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REVIEW PAPER

Antiangiogenic therapy combined with immune checkpoint blockade in renal cancer

Teele Kuusk[1] · Laurence Albiges[2] · Bernard Escudier[2] · Nikolaos Grivas[1] · John Haanen[3] · Thomas Powles[4] · Axel Bex[1]

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Abstract Antiangiogenic therapy with vascular endothelial growth factor (VEGF) inhibitors is the current first-line treatment in metastatic renal cell carcinoma (mRCC). Immunotherapy with checkpoint inhibitor has been recently added to the armamentarium of mRCC treatment. These therapies are based on treatment with antibodies that block programmed cell death-1 (PD-1), programmed cell death ligand 1 (PD-L1) pathways, demonstrating impressive response rates and improved survival in several tumour types. So far, nivolumab is the only approved anti-PD-1 monoclonal antibody after VEGF therapy in mRCC. According to preclinical and clinical studies, combination therapies with VEGF- and checkpoint inhibitors have synergistic effect achieving improved response rates. However, toxicity in some combinations is high. In this article, we present a review of the ongoing trials with these drug combinations for RCC.

✉ Axel Bex
a.bex@nki.nl

[1] Department of Urology, The Netherlands Cancer Institute, Postbus 90203, 1006 BE Amsterdam, The Netherlands

[2] Department of Medical Oncology, Gustave Roussy, 114 rue Edouard Vaillant, 94805 Villejuif, France

[3] Department of Medical Oncology, The Netherlands Cancer Institute, Postbus 90203, 1006 BE Amsterdam, The Netherlands

[4] Department of Medical Oncology, The Royal Free NHS Trust and Barts Cancer Institute, Queen Mary University of London, London, UK

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Introduction

Renal cell carcinoma (RCC) represents 5 and 3% of all malignancies in men and women, respectively [1, 2]. In Europe, the incidence and mortality are approximately 85/100.000 and 35/100.000, respectively [3]. Fifteen per cent of the patients with primary RCC are diagnosed with metastatic disease, while 30% of initially locally treated patients develop recurrent disease and systemic progression during the course of the disease [3]. Systemic therapy with vascular endothelial growth factor (VEGF) signalling axis targeting agents is the first-line treatment for metastatic RCC (mRCC) [4, 5]. In addition to established first- and second-line molecular-targeted therapies, immunotherapeutic agents are introduced into the treatment algorithm and are currently actively studied. In 2015, nivolumab was the first immune checkpoint inhibitor to be approved by the Food and Drug Administration (FDA) and European Medicine Agency (EMEA), as second-line treatment for mRCC.

Neoangiogenesis and the immune system play a central role in RCC. The earliest proof for the essential role of VEGF in RCC pathogenesis came from understanding of the genetic basis of the von Hippel-Lindau (VHL) familial syndrome [6]. Later studies showed the impact of VHL gene mutations on the upregulation of VEGF and expression of other angiogenic factors, which are of significance in RCC development and progression [7]. Early observations of spontaneous regression of metastases after radical nephrectomy suggested an importance of the immune

system in RCC. The main cause of this regression was believed to be a T- and B cell-mediated antitumour immunity [8]. However, with the exception of high-dose intravenous interleukin-2 (IL-2), treatment with cytokines such as interferon-α or subcutaneous IL-2 had only modest activity [9]. Despite a consistent rate of 5–10% of patients being in complete remission and potentially cured after high-dose IL-2, the high adverse event rate and the inability to predict responders did not favour this treatment option. After the introduction of VEGF-targeted therapy for the treatment of clear cell RCC, combinations of these drugs with cytokines have been studied [10]. Unfortunately, with the exception of bevacizumab and interferon-α, combinations were either ineffective or too toxic. The lower adverse event rate seen with PD1/PDL1 inhibitors has led to a revival in the investigation of combinations of drugs acting on VEGF and immune checkpoint inhibition in mRCC. This rationale is further supported by the observation that antiangiogenic agents have an effect on antitumour immune responses and T cell trafficking to the tumour [11, 12]. It has also been shown that checkpoint inhibition modulates tumour vessels [13]. Combining agents that act on these two major oncogenic pathways synergistically may result in better response and potential benefit from these therapies. In this article, we review the current literature and ongoing trials on combination therapies of VEGF-tyrosine kinase inhibitors (TKI), VEGF-monoclonal antibodies (mAB) and immunotherapeutic agents (checkpoint inhibitors) for RCC.

Mechanism of action of VEGF and checkpoint inhibitors in RCC

Inactivation of VHL tumour suppressor gene induces hypoxia which in turn triggers hypoxia-inducible factor (HIF)-1, causing activation of pro-angiogenic factors. VEGF upregulation results in neoangiogenesis, which facilitates the access of tumour cells to the general circulation causing systemic disease [14]. Tumour angiogenesis enhances activity of myeloid-derived suppressor cells (MDSC) and tumour-associated macrophages (TAM) suppressing innate antitumour immunity. It has been demonstrated that VEGF receptor tyrosine kinase inhibitor (VEGFR-TKI) sunitinib is suppressing angiogenic genes resulting in inhibition of angiogenesis in pretreated primary tumour tissue [15]. In preclinical models, it has been shown that antiangiogenic therapy decreased MDSC and reprogrammed immunomodulatory phenotype of TAM. The evolution of VEGFR-TKIs, namely sunitinib, pazopanib, sorafenib, axitinib, cabozantinib, lenvatinib in combination with mTOR inhibitor (everolimus) and monoclonal antibody against VEGF (bevacizumab) in combination with interferon-α, has improved mRCC prognosis by increasing progression-free survival (PFS) and impacting on overall survival (OS) [16–21]. Currently, sunitinib, pazopanib and bevacizumab with interferon-α are first-line options while nivolumab, cabozantinib, axitinib, sorafenib, everolimus alone and combination with lenvatinib are second-line treatment options in clear cell mRCC [4, 5].

Reciprocal action between the immune system and tumour development and progression has been a challenging topic in immunology. It is well known that the immune system prevents cancer development in many different pathways. However, cancer cells have also mechanisms against host immune system activity. At first, the innate and adaptive immune system both cooperate to eradicate tumour cells before clinically detectable disease [22]. After that, the adaptive system continues its attack against tumour cells, which survive. However, tumour cell types finally develop that are not recognized by the adaptive immune system. This happens through different mechanisms: tumour cells can become insensitive to immune effector mechanisms or immune checkpoint proteins may become dysregulated, typically via expression of inhibitory ligands and receptors that regulate T cell effector functions in the tumour microenvironment. This induces an immunosuppressive tumour microenvironment resulting in the escape phase, where tumour development is not prevented by the host immune system leading ultimately to clinically detectable disease [22].

Normally, microbes as well as cancer cells evoke activation of the immune system and in this process immune checkpoints are protecting the host cells from autoimmunity and self-destruction. Cancer cells are able to co-opt immune checkpoint pathways and thus avoid immune eradication. Therefore, immune checkpoint inhibitory antibodies act on tumour cells indirectly by targeting lymphocyte receptors or their ligands for reactivating and enhancing internal antitumour immunity. Checkpoint receptors are expressed on T lymphocytes (CTLA-4) and on T-, B-lymphocytes and natural killer (NK) cells such as programmed death-1 receptor (PD-1) and programmed death ligand 1 (PD-L1). Immune checkpoint blockade with monoclonal antibodies targets and blocks these inhibitory receptors, thereby inducing immune responses at different levels [23–25]. Pembrolizumab and nivolumab target the PD-1 receptor while atezolizumab, avelumab and durvalumab block its ligand (PD-L1). Ipilimumab and tremelimumab target CTLA-4 [25, 26]. Nivolumab has shown an OS benefit compared to everolimus in patients with mRCC previously treated with antiangiogenic therapy and is currently the only approved checkpoint inhibitor for the treatment of mRCC [27].

Rationale for using combination of antiangiogenic agents and immunotherapy

Earlier studies have shown that anti angiogenic therapy can elicit or enhance antitumour immunity, whereas reciprocally the immune system can induce angiogenesis [23, 28, 29]. Therefore, there is a bidirectional link and synergy between antiangiogenic agents and immunotherapy [28] (Fig. 1). Antiangiogenic agents are capable to reverse immunosuppression by decreasing immunosuppressive cells (MDSCs, regulatory T cells), immunosuppressive cytokines (IL-10, TGF β) and inhibitory molecules on T cells (PD-1) [28]. Moreover, VEGF receptor inhibitors drive tumour cells to activate immune checkpoints and therefore a combination of VEGF- and checkpoint inhibitors makes sense [23, 29]. Combination of anti-VEGF therapy with immunotherapy, though not checkpoint inhibitors, has demonstrated improved PFS in mRCC already in 2007 in two trials of bevacizumab in combination with interferon-α leading to approval as a first-line therapeutic option in mRCC [10]. In addition, recent research on intratumoral immune components such as tumour infiltrating lymphocytes (TIL) or MDSCs in tumour tissue of sunitinib pretreated primary RCC have demonstrated potential synergism for TKI with anti-PD-(L)1 therapy [30]. Pretreatment with sunitinib improved TIL expansion by reduction in intratumoral content of MDSC. Furthermore, the function of tumour infiltrating T lymphocytes may be inhibited in an immunosuppressive tumour microenvironment by T regulatory cells and expression of PD-L1. It has been shown that patients treated with antiangiogenic therapy have increased Treg and PD-L1 expression in their primary tumour tissue and this is associated with poor survival. Thus, combination therapy may be effective for patients with mRCC [31]. Recently published translational and clinical data on the combination of bevacizumab with atezolizumab (anti-PD-L1) in 10 patients demonstrated that combination therapy improves antigen-specific T cell migration thus enhancing antitumour activity. Durable partial responses (PR) and stable disease (SD) were observed in eight patients. This durable clinical benefit may be due to an addition of dissimilar response kinetics, since VEGFR-TKIs produce fast but non-durable response, but PD-1 inhibitors are slow to act but the response is long-lasting and thorough [32] (Table 1).

Combination therapy trials in advanced and metastatic RCC

Several trials have been performed or are ongoing to assess different combinations of antiangiogenic agents with checkpoint inhibitors in RCC. A phase I study (Checkmate-016, NCT01472081) in mRCC compared combination therapy of nivolumab, an anti-PD-1 inhibitor, with sunitinib, pazopanib or ipilimumab [33] (Tables 2, 3). Starting dose for nivolumab was 2 mg/kg (maximum 5 mg/kg) intravenously every 3 weeks until progressive disease (PD), toxicity or other reason for discontinuation, while standard dose for sunitinib and pazopanib was 50 mg and 800 mg, respectively. Primary outcome measures of the study were safety and tolerability of the different combinations while secondary outcome were the objective response rate (ORR) and the duration of response. In the sunitinib and nivolumab arm, no dose-limiting toxicities (DLT) were seen and the arm with higher dose (5 mg/kg) of nivolumab was expanded (up to 33 patients). The nivolumab (2 mg/kg)–pazopanib combination arm (20 patients) was closed due to early DLT. Moreover, adverse event rate was high with both combinations. A 82 and 70% rate of grade 3–4 toxicity were seen in the nivolumab–sunitinib and nivolumab–pazopanib arm, respectively. The most common grade 3–4 adverse events for the nivolumab–sunitinib and nivolumab–pazopanib combination were liver enzymes rise, hypertension, hyponatremia and lymphocytopenia. Regarding the effectiveness, nivolumab–sunitinib and nivolumab–pazopanib combinations showed

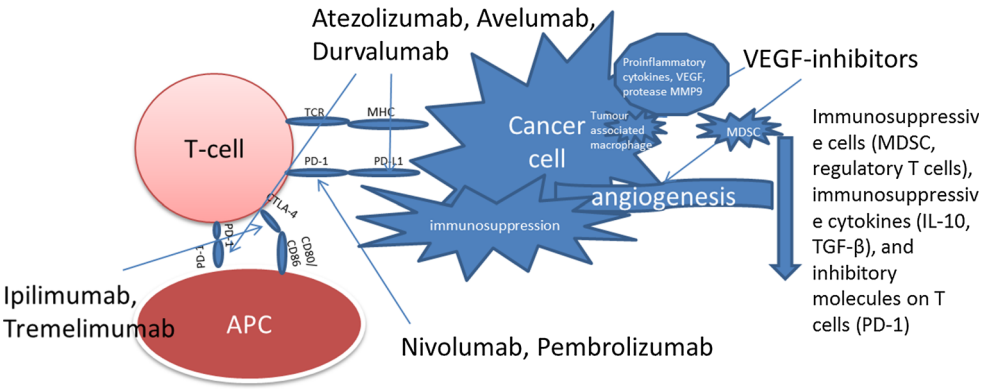

Fig. 1 Synergistic effect of VEGFR- and checkpoint inhibitors

Table 1 Phase III studies with immune checkpoint inhibitors in combination with VEGF-targeted therapy for patients with treatment-naïve mRCC

Study	N	Therapy	Endpoint	Subtype
KEYNOTE-426 NCT02853331 [41]	840	Pembrolizumab 200 mg iv every 3 weeks + axitinib 5 mg po twice daily vs sunitinib 50 mg po once daily on schedule 4/2	PFS central review OS	Clear cell component with or without sarcomatoid features
JAVELIN Renal 101 NCT02684006 [42]	583	Avelumab 10 mg/kg iv every two weeks + axitinib, 5 mg po twice daily vs sunitinib 50 mg po on schedule 4/2	PFS	Clear cell component
IMmotion 151 NCT02420821 [45]	900	Atezolizumab 1200 mg iv on days 1 and 22 of each 42-day + bevacizumab 15 mg/kg iv on days 1 and 22 of each 42-day cycle vs sunitinib 50 mg po on schedule 4/2	PFS investigator reviewed OS in participants with detectable PD-L1	Clear cell histology and/or a component of sarcomatoid carcinoma
NCT02811861 [37]	735	Lenvatinib 18 mg po + everolimus 5 mg po or lenvatinib 20 mg po + pembrolizumab 200 mg iv every 3 weeks vs sunitinib 50 mg po on a schedule 4/2	PFS	Clear cell component

ORR of 52 and 45%, respectively. The response was seen 6 weeks after treatment initiation in 41 and 56% in combinations of nivolumab with sunitinib and pazopanib, respectively, and demonstrated long-lasting effects up to 13 and 17 months in the sunitinib–nivolumab and pazopanib–nivolumab combinations, respectively. Median progression-free survival (PFS) was 48.9 and 31.4 months for sunitinib and pazopanib combinations, respectively. This study showed higher response rates for combination therapy compared to monotherapy, although toxicity was higher.

A recently launched phase I/II will be investigating the combination of nivolumab with tivozanib, a VEGFR-TKI in advanced RCC (TiNivo trial).

At least five trials investigate pembrolizumab, another anti-PD-1 inhibitor, in combinations with monoclonal antibodies against VEGF- or antiangiogenic VEGFR-TKI. Pembrolizumab (MK-3475) has been studied with the combination of bevacizumab in a phase Ib study [34]. Sixteen patients with mRCC who had at least one systemic therapy failure were enroled. Pembrolizumab (200 mg every 3 weeks) was given in combination with bevacizumab (either at 10 mg/kg or 15 mg/kg every 3 weeks). No grade 3–4 AEs were recorded. Seventy-one per cent of 14 patients who were evaluable for response demonstrated PR, 29% had PD. To conclude, pembrolizumab and bevacizumab at maximum dose were safe and recommended to continue in a phase II study (NCT02348008) BTCRC-GU14-003.

The other phase I study of a monoclonal antibody against VEGF, aflibercept, in combination with pembrolizumab enrols patients with solid tumours and mRCC who have been previously treated with VEGFR-TKIs [35] (NCT02298959). They receive pembrolizumab and ziv-aflibercept intravenously on day 1 and cycles are repeated every 2 weeks. Results are pending.

Further combinations of pembrolizumab with VEGFR-TKI were investigated in phase Ib/II studies. One such trial enroled eight RCC patients among other patients with solid tumours who had progressed after first-line therapy [36] to receive pembrolizumab (200 mg) intravenously once every 3 weeks and a daily oral dose of lenvatinib (24 mg or 20 mg)(NCT02501096). Grade 3 adverse events with 24 mg of lenvatinib were arthralgia and fatigue; however, no DLTs were reported in the arm combining pembrolizumab and lenvatinib 20 mg. ORR for this combination was 69%. Half of the mRCC patients showed PR and the other half SD. The maximum daily tolerated dose of lenvatinib in the combination was confirmed as 20 mg and a phase III study testing the combination against sunitinib, a first-line standard, is ongoing [37] (Table 1).

Interestingly, other VEGFR-TKI combinations with pembrolizumab may not be necessarily comparable regarding their toxicity profile. Another phase I/II study combined pembrolizumab with pazopanib, 600 or 800 mg [38] (Keynote-018, NCT02014636). Sixty-five per cent of patients developed grade 3 hepatotoxic AEs and toxicity appeared recurrently after re-initiation of treatment. The investigators concluded that liver function deterioration was related to pazopanib. ORR was 60 and 20% for pazopanib 800 mg and 600 mg, respectively. One patient in the pembrolizumab–pazopanib 800 mg arm showed complete response (CR).

Table 2 Phase I/II studies with immune checkpoint inhibitors in combination with VEGF-targeted therapy with response rate

Trial	Study name, setting	Study name, combination (dose)	Nr of patients (evaluable for response/all)	ORR nr (%)	CR nr (%)	PR nr (%)	SD nr (%)	PD nr (%)	mPFS months
Phase I, NCT01472081[a]	Checkmate 016, mRCC, first line [33]	Nivolumab 5 mg/kg q3w + sunitinib 50 mg	33	17 (52%)	1 (3%)	16 (48%)	10 (30%)	1 (3%)	48.9
Phase I, NCT01472081[a]	Checkmate 016, mRCC, first line [33]	Nivolumab2 mg/kg q3w + pazopanib 800 mg	20	9 (45%)	0	9 (45%)	7 (35%)	4 (20%)	31.4
Phase I, NCT02496208[a]	mRCC, second line [49]	Nivolumab 3 mg/kg q2w + cabozantinib 40 mg	38/40 (3 mRCC)	12 (32%)	1 (5%)	11 (29%)1mRCC	20 (53%)	NA	NA
Phase I, NCT00372853	mRCC, first or second line [46]	Tremelimumab 6–15 mg/kg q12w + sunitinib 37.5 or 50 mg	21/28	16 (76%)	NA	9 (43%)	7 (33%)	NA	NA
Phase Ib, NCT02348008, BTCRG-GU14-003	mRCC, second line [34]	Pembrolizumab 200 mg q3w + bevacizumab 10 or 15 mg/kg	14/16	NA	NA	10 (71%)	NA	4 (29%)	NA
Phase Ib/II, NCT02501096	mRCC, second line [36]	Pembrolizumab 200 mg q3w + lenvatinib 24 or 20 mg	13 (8 mRCC)	9 (69%)	0	7 (54%)	6 (46%)	0	NA
Phase I/II, NCT02014636	Keynote-018, mRCC, first line [38]	Pembrolizumab 2 mg/kg q2w + pazopanib 600 or 800 mg	20	60% for 800 mg, 20% for 600 mg	1 (5%)	NA	NA	NA	NA
Phase Ib, NCT02133742	mRCC, first line [40]	Pembrolizumab 2 mg/kg q3w + axitinib 5 mg	52	37 (71%)	3 (6%)	34 (65%)	10 (19%)	2 (3.8%)	15.1
Phase Ib, NCT02493751	JAVELIN Renal 100, mRCC, first line [40]	Avelumab 10 mg/kg q2w + axitinib 3 or 5 mg twice a day	6	6 (100%)	0	6 (100%)	0	NA	NA
Phase II, NCT01984242[a]	Immotion 150, first line [44]	Atezolizumab 1200 mg q3w + bevacizumab 15 mg/kg	305 (101 in combination arm)	32%	7%	NA	NA	NA	11.7

[a] Response rate only for VEGFR-TKI or VEGF-monoclonal antibody and checkpoint inhibitor combination arm

Table 3 Phase I/II studies with immune checkpoint inhibitors in combination with VEGF-targeted therapy and grade 3–4 adverse events, either reported as number or percentage, or both if available

	Checkmate 016 (nivolumab + sunitinib) [33]	Checkmate 016 (nivolumab + pazopanib) [33]	Nivolumab + cabozantinib [49]	Tremelimumab + sunitinib [46]	Pembrolizumab + bevacizumab [34]
Nr of patients	33	20	24 (3 mRCC)	28	16
Grade 3–4 AEs	27 (82%)	14 (70%)	7 (29%)	17 (61%)	0%
Fatigue	9%	15%	2 (8%)	1	
Nausea, vomiting					
Artralgia					
Hypertension	18%	10%			
Hand-foot syndrome					
Mucositis				1	
Pneumonitis					
Aseptic meningitis			1/40		
colitis			1/40		
Elevated ALT	18%	20%			
Elevated AST	9%	20%			
Elevated ALT/AST					
Elevation of lipase			3 (13%)		
Hypercalcemia					
Hyperuricemia					
Hypophosphatemia			4 (17%)		
Hyponatriemia	15%		4 (17%)		
Lymphocytopenia	15%				
Neutropenia					
Proteinuria					
Diarrhoea	9%	20%	2 (8%)		
Renal insufficiency				2	
Respiratory insufficiency					
Dyspnoea				1	
Headache					
Tumour pain					
Postoperative wound infection					
Death				1	
Weight loss					

Table 3 continued

	Pembrolizumab + lenvatinib [36]	Pembrolizumab + axitinib [39]	JAVELIN Renal 100 (avelumab + axitinib) [40]	Keynote-018 pembrolizumab + pazopanib [38]	Atezolizumab + bevacizumab [43]
Nr of patients	13 (8 mRCC)	52	6	20	12
Grade 3–4 AEs	9 (69%)	28 (53%)	5 (83%)	13 (65%)	7 (58%)
Fatigue	1	6%			
Nausea, vomiting					
Artralgia	1				
Hypertension	1	17%	33%		3
Hand-foot syndrome			17%		
Mucositis			17%		
Pneumonitis					
Aseptic meningitis					
colitis					
Elevated ALT		6%			
Elevated AST					
Elevated ALT/AST	1			13 (65%)	
Elevation of lipase			17%		
Hypercalcemia					1
Hyperuricemia					
Hypophosphatemia					
Hyponatriemia	1				
Lymphocytopenia					
Neutropenia					
Proteinuria			17%		
Diarrhoea	1	10%			
Renal insufficiency					
Respiratory insufficiency					1
Dyspnoea					
Headache		8%			
Tumour pain					1
Postoperative wound infection					1
Death					
Weight loss		6%			

Finally, a phase Ib study investigated pembrolizumab in combination with axitinib in 52 treatment-naïve patients. The trial determined that the safe dose of axitinib was 5 mg twice daily and 2 mg/kg every 3 weeks for pembrolizumab [39]. Severe grade 3–4 adverse events included hypertension, diarrhoea and headache. Seventy-one per cent of the patients obtained objective response, with 3 CR 34 PR and 10 had SD.

Similar combinations were studied with monoclonal antibodies against PD-L1. For example, axitinib was further investigated in a phase Ib study which evaluated the safety, pharmacokinetics and pharmacodynamics of axitinib (3 or 5 mg twice a day) in combination with the anti-PD-L1 inhibitor avelumab (10 mg/kg every 2 weeks) in first-line advanced RCC [40]. Grade 3–4 adverse events occurred in 5/6 patients, hypertension being the most common one. No discontinuation due to treatment-related toxicity was observed. Confirmed PR was observed in six patients. The dose combination with avelumab and axitinib regarded as safe was 10 mg/kg and 5 mg, respectively. Both pembrolizumab and avelumab combinations with axitinib were considered encouraging and are currently being tested in phase 3 trials against the standard sunitinib in untreated mRCC [41, 42] (Table 1).

Like the anti-PD-1 inhibitor pembrolizumab, atezolizumab, another monoclonal antibody against PD-L1, has been studied in combination with bevacizumab in phase I and II studies [43, 44]. In a phase I study, atezolizumab (20 mg/kg every 3 weeks) was administered with bevacizumab (15 mg/kg every 3 weeks) in 12 patients. Atezolizumab-related grade 3 adverse events occurred in 3% of the patients; however, grade 3–4 AEs accounted for 58%. ORR was observed in 40%, one patient had a CR and almost half of the patients experienced SD. These results suggested a safety and efficacy of the combination in mRCC which led to a randomized phase II study. In the phase II study, atezolizumab was administered either as monotherapy (103 patients) or in combination with bevacizumab (101 patients) versus sunitinib (101 patients) in patients with previously untreated locally advanced or metastatic RCC (IMmotion150, NCT01984242). This trial provides the first randomized data of VEGFR-TKI versus single-agent PD-L1 inhibitor in first line. The results were encouraging especially in patients with higher expression of PD-L1 on immune cells and were presented at the 2017 American Society of Clinical Oncology Genito-urinary (ASCO GU) symposium recently. ORR ranged from 32, 25, 29% in the atezolizumab–bevacizumab combination, the atezolizumab-mono and sunitinib arm, respectively. Interestingly, complete response rate was the highest in the atezolizumab arm (11%) followed by the combination (7%) and sunitinib arm (5%), respectively. Diverse response rates were seen in patients with higher expression of PD-L1 favouring the combination or atezolizumab only arms. As with previous combinations, AE rate was high: 64, 41 and 69% of patients developed grade 3–4 AEs in the combination, atezolizumab only and sunitinib arm, respectively. Atezolizumab only arm side effects were similar to side effects reported for nivolumab. There were one treatment-related AE leading to death in the combination arm and two in the sunitinib arm. Promising results from the phase I/II studies have led this combination to a phase III study in which patients with treatment-naïve mRCC were randomized to atezolizumab with or without bevacizumab versus sunitinib monotherapy (IMmotion 151, NCT02420821) [45] (Table 1). Atezolizumab and bevacizumab combination will also be further studied in phase 1 trials with entinostat, histone benzamide deacetylase inhibitor (NCT03024437) and obinutuzumab, anti CD20 monoclonal antibody (NCT03063762). Both trials have been registered early this year.

Compared to the anti-PD-1 or PD-L1 combinations with VEGF-targeted therapy, only one study investigated anti angiogenic therapy in combination with a monoclonal antibody against CTLA-4. A phase I study examining the combination of tremelimumab, a CTLA-4 inhibitor, with sunitinib enroled 28 mRCC who had received none or only one previous systemic treatment [46]. The patients were treated with tremelimumab (6–15 mg/kg intravenously) once every 12 weeks and sunitinib (50 mg daily on 4 on 2 off weeks schedule or 37.5 mg continuously). Two of five patients in 50 mg sunitinib plus tremelimumab (6 mg/kg) arm experienced DLTs, resulting in closure of the sunitinib 50 mg dose arm. Half of the patients on sunitinib 37.5 and tremelimumab (15 mg/kg) developed DLTs. One patient receiving tremelimumab (10 mg/kg) plus daily sunitinib (37.5 mg) died. Finally, the tremelimumab (10 mg/kg) plus daily sunitinib (37.5 mg) combination was expanded with seven patients and three of those experienced DLTs. The most common DLT was acute renal failure. Finally, ORR was 76%. However, due to high toxicity of renal failure, tremelimumab doses higher than 6 mg/kg combined with sunitinib (37.5 mg) were not recommended and not further studied.

In comparison with the multitude of studies performed or ongoing for clear cell RCC, only two studies are currently enroling patients with non-clear cell subtypes. Specifically, a phase II trial with atezolizumab and bevacizumab combination is accruing patients with advanced or metastatic non-clear cell RCC. Both drugs will be administered intravenously every 3 weeks. Results are awaited [47] (NCT02724878). Another combination trial is a phase Ib trial of durvalumab, a PD-L1 inhibitor, in combination with either savolitinib, a selective c-MET-TKI, or tremelimumab which enrols a papillary RCC cohort in VEGFR-

TKI refractory mRCC patients (CALYPSO, NCT02819596) [48].

The only triple combination trial is a phase I study in pretreated metastatic genitourinary cancer patients which compared combination therapy of nivolumab with cabozantinib and a triple combination of cabozantinib, nivolumab and ipilimumab. Forty patients with genitourinary cancers, among whom three patients with mRCC, were enroled. Preliminary results were presented at the 2017 American Society of Clinical Oncology Genito-urinary (ASCO GU) symposium (NCT02496208). Grade 3–4 AEs were hypophosphatemia, hyponatremia, elevated lipase. The combination of nivolumab and cabozantinib was well tolerated and did not cause grade 4–5 toxicities, immune-related AEs or DLTs. There were no additive toxicities also in a triple arm. Nivolumab 3 mg/kg every 2 weeks with cabozantinib 40 mg daily and nivolumab 3 mg/kg, cabozantinib 40 mg and ipilimumab 1 mg/kg was recommended to proceed to a phase II study. ORR in 38 evaluable patients for this combination was 32%, and one of the 3 mRCC patient had a PR [49].

In conclusion, given the many potential immune checkpoint inhibitor combinations with VEGF-targeted therapy that have been or are currently investigated in early phase I/II trials it may not come as a surprise that no less than 4 such combination trials are currently being investigated in treatment-naïve mRCC patients challenging sunitinib, a first-line standard of care, in randomized controlled phase III settings [50] (Table 1). From these studies, the IMmotion 151 trial has finished accrual and data may be presented as early as autumn 2017.

Combinations in neoadjuvant or presurgical setting in mRCC

Neoadjuvant or presurgical studies are a unique opportunity to obtain sequential tumour tissue and to identify predictors of response or resistance to immune checkpoint inhibition and combination with VEGF-targeted therapy. Preclinical and early clinical research suggest that there is significantly greater therapeutic efficacy of neoadjuvant immunotherapies in eradicating early occult metastases than with an adjuvant approach, following primary tumour resection [51]. This has resulted in several neoadjuvant and presurgical phase I/II studies in localized and metastatic RCC with single-agent nivolumab and pembrolizumab which are currently ongoing [52–54]. In addition, a phase III trial schedules patients for perioperative nivolumab before nephrectomy for $\geq$T2 or T_{any} N + RCC and plans to enrol 766 patients (PROSPER EA8143). There is one pilot randomized study evaluating presurgical nivolumab monotherapy, nivolumab combination with bevacizumab and nivolumab combined with ipilimumab in patients with primary mRCC and the tumour in place [55]. This is currently the only study investigating a combination of checkpoint inhibition and antiangiogenic therapy prior to removal of the primary tumour. One arm receives nivolumab (3 mg/kg intravenously every 2 weeks for a total of 6 weeks). The second arm receives nivolumab (3 mg/kg every 2 weeks) with bevacizumab (10 mg/kg intravenosuly every 2 weeks for 6 weeks) and the third arm receives nivolumab (3 mg/kg every 3 weeks) with ipilimumab (1 mg/kg intravenously every 3 weeks for 6 weeks). In all arms, cytoreductive nephrectomy is planned after the end of drug treatment.

Combination treatment in adjuvant setting

Based on the assumption that immune checkpoint inhibition may be more effective in eliminating circulating tumour cells and micrometastases than VEGFR-targeted therapy, several randomized controlled phase 3 trials are planned to test adjuvant atezolizumab, nivolumab and pembrolizumab as single agents in patients with non-metastatic RCC and high risk of recurrence [56–59]. However, at present no combinations of immune checkpoint inhibition and VEGF-targeted therapy are being tested in the adjuvant setting. This is in part owing to conflicting results being reported with adjuvant VEGFR-TKI therapy in localized high-risk RCC [60]. In two RCTs, sunitinib did not prolong OS while it had a significant but limited benefit on disease free survival in one of the studies. Unfortunately, a threefold adverse event rate influencing some aspects of quality of life resulted in an unfavourable harms-benefits ratio for adjuvant VEGFR-TKI therapy. Although further trials evaluating VEGFR-TKIs in adjuvant setting are ongoing, it is unlikely that they will be practice changing after assessment of their contribution to value-based health care. Until data from ongoing phase III trials in the metastatic setting report significant improvement in OS, it is likely that the current adverse event profile of combined immune checkpoint inhibition and VEGF-targeted therapy prohibits their long-term administration in adjuvant studies.

Conclusions and outlook

Long-lasting remission, manageable toxicity and a synergistic effect with VEGF-targeted therapy make immune checkpoint inhibitors attractive candidates for combination therapy with antiangiogenic compounds. Clinical trials with novel checkpoint inhibitors are initiated and first results from phase I/II trials of checkpoint inhibitors with

VEGFR-TKI and VEGF-monoclonal antibodies are promising. No less than 4 phase III trials are ongoing to investigate immune checkpoint inhibitors in combination with VEGF-targeted therapy in patients with treatment-naïve mRCC. These trials are designed to identify patient subgroups for appropriate treatment selection but further studies will be needed to establish markers of resistance to therapy, dosing, optimal timing, and sequencing. In parallel, neoadjuvant and presurgical studies are ongoing and will investigate whether these combinations are effective in this setting, which, in turn, may provide a rationale for adjuvant studies in patients with non-metastatic RCC with high risk of recurrence. In addition, novel checkpoint inhibitors that ought to be less toxic are investigated actively.

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Conflict of interest AB took part in advisory boards of Pfizer, Novartis, Ipsen, Eisai and Roche. He is the PI of the EORTC SURTIME trial sponsored in part by a Grant from Pfizer to the EORTC. LA has received consulting and advisory fees from BMS, Pfizer, Novartis, Sanofi, Amgen, Bristol-Myers Squibb, Bayer, and Cerulean; and research funding from Pfizer and Novartis. BE has received fees for serving on advisory boards from Pfizer, Novartis, Bristol-Myers Squibb, Exelixis, and Roche and lecture fees from Pfizer and Novartis. JH has consulted or has an advisory role for MSD Oncology, Pfizer, and Bristol-Myers Squibb, as well as gained research funding by MSD and Bristol-Myers Squibb. TP is a company consultant for Novartis, Pfizer, GSK, has received company speaker honoraria from Novartis, Pfizer, GSK, Genentech, performed trial participation for GSK, Pfizer, BMS, Genentech, Genetech, and received grants/research support from GSK, Pfizer, and Novartis. The other authors have no conflict of interest to declare.

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