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Review:

The evolving role of monoclonal antibodies in the treatment of patients with advanced renal cell carcinoma: a systematic review

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Abstract

Introduction: While the majority of the vascular endothelial growth factor (VEGF) and mammalian target of rapamycin (mTOR) inhibitors currently used for the therapy of metastatic renal cell carcinoma (mRCC) are small molecule agents inhibiting multiple targets, monoclonal antibodies are inhibitors of specific targets, which may decrease off-target effects while preserving on-target activity. A few monoclonal antibodies have already been approved for mRCC (bevacizumab, nivolumab), while many others may play an important role in the therapeutic scenario of mRCC.

Areas covered: This review describes emerging monoclonal antibodies for treating RCC. Currently, bevacizumab, a VEGF monoclonal antibody, is approved in combination with interferon for the therapy of metastatic RCC, while nivolumab, a Programmed Death (PD)-1 inhibitor, is approved following prior VEGF inhibitor treatment. Other PD-1 and PD-ligand (L)-1 inhibitors are undergoing clinical development.

Expert opinion: Combinations of inhibitors of the PD1/PD-L1 axis with VEGF inhibitors or cytotoxic T-lymphocyte antigen (CTLA)-4 inhibitors have shown promising efficacy in mRCC. The development of biomarkers predictive for benefit and rational tolerable combinations are both important pillars of research to improve outcomes in RCC.

1. Introduction

RCC originates from the renal tubular epithelium and accounts for approximately 85% of kidney cancers and for 1.9% of all cancers diagnosed worldwide. Clear cell (cc)-RCC is the most common histologic type. In the United States, RCC is the eighth and the sixth most frequent cancer in women and men, respectively, and its incidence has been rising by 1.6% a year since the year 2000 [1, 2]. While patients with localized[3] or locally advanced[4] kidney cancer have a possibility for cure by undergoing radical surgery, most of the patients with metastatic RCC eventually succumb to the disease, with a median overall survival of 18 to 26 months in patients treated with first-line anti- VEGF agents[5]. Multiple VEGF and mTOR inhibitors have supplanted cytokines as the cornerstone of therapy for cc-RCC. VEGF inhibitors include small molecule VEGF receptor

tyrosine kinase inhibitors (TKIs) sunitinib, pazopanib, sorafenib and axitinib, and the monoclonal antibody, bevacizumab, while mTOR inhibitors include temsirolimus and everolimus [6-17]. The median progression free survival (PFS) with first-line sunitinib, pazopanib or bevacizumab plus interferon is 8 to 11 months, while second-line axitinib or everolimus yield a median PFS of 4-5 months. High dose interleukin (IL)-2 remains an option in highly selected patients [18]. Additional survival increments have occurred in 2015 in the salvage therapy space by the emergence of cabozantinib, a MET, AXL and VEGF receptor targeting TKI, and nivolumab, an anti PD-1 targeting monoclonal antibody, for patients with progression following VEGF inhibitors [19, 20].

The importance of monoclonal antibodies for the treatment of solid malignancies has progressively increased over the past 20 years[21]. Soluble ligands, such as VEGF, membrane antigens of immune cells, such as CD-20 or CTLA4, or surface cancer proteins, such as HER2-neu and EGFR have been successfully targeted using monoclonal antibodies approved for clinical use[22-26]. The availability of monoclonal antibodies has expanded the possibilities to manufacture therapeutic agents directed against a specific molecular target, with a different and often more favorable toxicity profile vs. conventional chemotherapy or immunotherapy agents. This review describes emerging monoclonal antibodies for treating RCC. Multiple PD-1 and PD-L1 inhibitors are undergoing clinical development, and combinations of inhibitors of the PD1/PD-L1 axis with VEGF inhibitors or CTLA-4 inhibitors are being vigorously investigated. The development of biomarkers predictive for benefit and rational tolerable combinations are both important pillars of research to improve outcomes in RCC. We review available and emerging monoclonal antibodies for the therapy of RCC.

2. Search strategy

A systematic analysis of the literature was conducted on 1st January 2016 by performing a search of Medical Subject Heading (MeSH) terms on PubMed using the MESH terms “Antibodies, Monoclonal” and “Kidney Neoplasms”. Reviewed articles were written in English and provided clinical data concerning (1) safety and (2) efficacy of monoclonal antibodies employed as therapeutic agents against kidney cancer. Clinical data

from prospective, interventional phase 1-4 trials as well from both prospective and retrospective observational clinical studies were reviewed. A MESH subsequent search was conducted using the MESH terms for each of the monoclonal antibody-based therapeutic agent and "Kidney Neoplasms". Review articles and editorials were excluded from the systematic analysis but were also reviewed in order to identify additional original articles of interest. Review articles, editorials, commentaries, non-clinical studies and clinical studies not focusing on safety/efficacy of monoclonal antibody-based therapeutic agents were cited as necessary to support the clinical findings reported. Only original articles published between 1st January 2006 and 1st January 2016 were considered for inclusion in the systematic review on PUBMED. Abstracts published by the American Society of Clinical Oncology and the European Society of Medical Oncology between 1st January 2006 and 1st January 2016 were also considered, but full, peer-reviewed papers were given priority for inclusion in this review article. The number of full articles included and excluded by the systematic searches conducted using MESH terms on PUBMED is shown in figure 1. A few abstracts (< 10) have also been reviewed.

3. Clinical evidence supporting molecular targets for which approved monoclonal antibodies are available

3.1 VEGF

Bevacizumab is a monoclonal antibody that inhibits the VEGF signaling pathway, which plays a crucial role in renal cell carcinoma[27]. It is approved for the first-line treatment of mRCC in combination with interferon on the grounds of the PFS improvement achieved in two separate, similarly designed phase III trials comparing bevacizumab + interferon vs. interferon alone. In the randomized, double-blind AVOREN trial[28] enrolling 649 patients with previously untreated mRCC, interferon α plus bevacizumab was associated with longer PFS vs. interferon α plus placebo [10.2 months vs 5.4 months; HR 0.63, 95% confidence interval (CI) 0.52-0.75; p=0.0001], with the most commonly reported grade 3 or worse adverse

events including fatigue (40 [12%] patients in the bevacizumab group vs 25 [8%] in the control group) and asthenia (34 [10%] vs 20 [7%]). Consistent results were obtained in the phase III CALGB 90206 trial or bevacizumab + interferon vs. interferon alone[8]. Mature results from either of these two trials failed to show an advantage in overall survival (OS) associated with the use of bevacizumab, which has been primarily attributed to the confounding effect of subsequent treatments[26, 29, 30]. Although neither of these two trials included patients with brain metastases, bevacizumab + interferon showed activity in a case series of 4 RCC patients with brain metastases[31].

On the basis of the efficacy of bevacizumab + interferon, subsequent clinical research has focused on the feasibility of (1) using different doses of bevacizumab and interferon, (2) administering bevacizumab as a single agent, (3) using bevacizumab + interferon in settings other than the first-line setting and (4) combining bevacizumab with alternative targeted, immunotherapy and also chemotherapy agents. The contributing role of interferon to the efficacy of interferon + bevacizumab is not clear. Evidence from phase II trials support the use of bevacizumab alone[32] [33]. Single agent bevacizumab also showed activity patients receiving bevacizumab in the second- or third-line setting[34]. Activity of second line therapy based on bevacizumab and interferon was also reported anecdotally in a patient who had progressive disease after sunitinib treatment [35].

In view of the clinical efficacy of mTOR inhibitors in RCC administered as single agents [12, 16], and their different toxicity profile with respect to bevacizumab, multiple clinical trials have explored the combination of temsirolimus or everolimus with bevacizumab. In 50 untreated and 30 previously treated RCC patients, the combined use of bevacizumab and everolimus was associated with a median PFS of 9.1 and 7.1 months, respectively.[36] Conversely, accrual was terminated early in a phase II study enrolling 10 patients receiving bevacizumab+everolimus after an anti-VEGF agent because of unacceptable toxicity[37], while the RECORD-2 study randomizing 365 patients to first-line everolimus + bevacizumab or interferon + bevacizumab did not show any differences between the two arms, with similar discontinuation rates and exposure to treatment [37, 38]. Disappointing results were also associated with the combination of bevacizumab plus temsirolimus[39, 40]. The large phase III INTORACT trial conclusively proved that first-line temsirolimus plus

bevacizumab was not superior to bevacizumab + interferon[41], while the 4-arm phase II BEST trial did not show any difference between bevacizumab alone, bevacizumab plus temsirolimus, bevacizumab plus sorafenib or bevacizumab + interferon [42]

Bevacizumab has also been tested in combination with sunitinib in two phase I trials for the first line treatment. Both Sunitinib and Bevacizumab target the VEGF pathway and demonstrate activity against advanced RCC, but the results of both trials were disappointing due to prohibitive toxicities, in particular hypertension and vascular events.[43, 44] Although the combination of sunitinib+bevacizumab was also reported in a case series[45] in the second-line setting, its further development is not planned based on the toxicity concerns deriving from phase I data. Other trials have evaluated the combination of bevacizumab with agents not approved in RCC. A phase I trial[46] evaluated treatment using bortezomib plus bevacizumab on the hypothesis that bortezomib, a proteasome inhibitor agent that suppresses HIF-1α transcriptional activity, could overcome the HIF-1α resistance pathway. The toxicities were manageable and 5 of 20 evaluable patients showed SD lasting longer than 6 months, with three patients exhibiting a radiographic PR. This combination warrants further experimentation [46]. A few studies have also assessed the feasibility of combined anti-EGFR and anti-VEGF treatment, on the basis of the results obtained in preclinical models suggesting potential synergism[47]. In a series of 50 patients receiving neoadjuvant bevacizumab, alone or in combination with erlotinib, no radiological responses were reported, with 9 patients (20.9%) showing delayed wound healing 4 weeks postoperatively[48]. A phase II, randomized, double-blind, placebo-controlled trial assessed the benefit associated with the combined use of erlotinib plus bevacizumab in 104 patients receiving first-line treatment of mRCC. The most common grade 3/4 adverse events (> 5% of patients) were hypertension, rash, proteinuria, diarrhea, and hemorrhage, with one treatment-related death occurring in the bevacizumab + erlotinib arm [gastrointestinal (GI) perforation]. Although the safety profile of the combination treatment was acceptable, bevacizumab + erlotinib did not seem to provide additional clinical benefit compared with bevacizumab alone [49]. Bevacizumab has also been combined with chemotherapy agents such as capecitabine and gemcitabine[50, 51] The combination of gemcitabine, capecitabine and bevacizumab was evaluated in a phase 2 trial enrolling 29 patients with mRCC, most of

whom had been previously treated with a VEGF-TKI. Seven patients (24%) had a PR (the duration was 6 to 22 months). Median OS and PFS were 9.8 months (95% CI: 6.2, 14.9) and 5.3 months (95% CI: 3.9, 9.9), respectively. The regimen was well tolerated, and anticipated toxicities were reported. This combination appeared to have moderate activity, particularly in patients in the poor risk group and those previously exposed to TKI [52]. Bevacizumab activity was also assessed in combination with chemotherapy (gemcitabine and 5-fluorouracil) and immunotherapy (IL-2 and interferon-alfa-2a) for the treatment of 27 patients with mRCC. The highest dose tested was gemcitabine 1000 mg/m^2 and 5-fluorouracil 600 mg/m^2 with bevacizumab 10 mg/kg, IL-2 1 MIU/m^2 and interferon -α-2a 3 MIU. The most common adverse events were fever, thrombocytopenia and neutropenia. A 33% response rate, measured according to the RECIST criteria, was reported[53], which suggests that such a combination should be further explored. Concomitant inhibition of the PDGFR, VEGF and EGFR signaling pathways by the use of imatinib, bevacizumab and erlotinib has also been explored in a phase I/II trial with no encouraging data[54] Similarly, bevacizumab in combination with high or low doses of IL-2 did not show encouraging activity [55] [56]. Reviewed clinical trials on bevacizumab are summarized in table 1.

3.2. Programmed Death (PD)-1 / PD-ligand (L)-1

The PD-1 protein is a member of the CD28 family that is expressed by activated T and B cells and binds to its ligands PD-L1 (B7-H1) and PD-L2 (B7-DC) expressed on tumor and stromal cells. The immune-inhibitory effect associated with the activation of the PD-1 pathway plays an important role in mediating tumor immune escape. The PD-1/PD-L1 interaction inhibits T survival, proliferation, and effector functions such as cell killing and cytokine release[57], and it can also mediate resistance of tumor cells to the attack of cytotoxic lymphocytes[58]. Conversely, T cell responses against tumor-specific antigens are augmented by inhibition of PD-L1 (B7-H1) in in vitro models [59]. PD-L1 expression on cancer cells is common in all patients with RCC, particularly in patients with biallelic VHL inactivation and sarcomatoid features [60, 61]. Nivolumab (BMS-936568, MDX-1106) is a fully human IgG4 monoclonal antibody directed against PD-1 that showed an excellent safety profile in several phase I trials conducted in solid tumors [62] [63]. Of note, nivolumab was able to provide prolonged responses lasting > 4 years after treatment suspension[64, 65]. In the CheckMate

025 trial[66], 821 cc-RCC patients who had been treated with one or two lines of antiangiogenic VEGF targeting therapy were randomized to receive intravenous nivolumab (3 mg/ kg every 2 weeks) or oral everolimus (10-mg daily) . Nivolumab vs. everolimus was able to yield an OS of 25.0 months (95% CI, 21.8 to not estimable) vs. 19.6 months (95% CI, 17.6 to 23.1). The hazard ratio for death was 0.73 (98.5% CI, 0.57 to 0.93; P = 0.002), thus meeting the pre-specified criterion for superiority (P≤0.0148). Similarly to other immunotherapy agents such as sipuleucel-T in prostate cancer [67, 68], nivolumab yielded an advantage in OS, but it did not yield a statistical advantage in PFS. Although a median PFS of approximately 4.5 months was reported both in the nivolumab and everolimus groups, in the sub-group of patients who had not progressed at 6 months, the median progression-free survival significantly favored nivolumab (15.6 months vs. 11.7). The CheckMate 025 trial also allowed therapy beyond RECIST progression which makes the optimal duration of treatment an unanswered question [68]. Differently from sipuleucel-T, nivolumab was associated with a better response rate with respect to the comparator arm (nivolumab vs. everolimus, 25% vs. 5%; P<0.001). Nivolumab showed an excellent safety profile, with severe fatigue and anemia, the most frequently reported serious adverse events associated with the use of nivolumab, being reported only in 2% of patients. A greater advantage of nivolumab vs. everolimus was reported in patients at poor vs. intermediate-good prognosis, with the HR for death of patients at poor prognosis being 0.47, and the HR for death of patients at good and intermediate prognosis being 0.89 and 0.73, respectively. Such a heterogeneity did not prove to be statistically significant, and these differences may simply be due to the higher number of events of death in patients at poor prognosis. Unfortunately, nivolumab efficacy was not related to PD-1 ligand expression levels in the CheckMate 025 trial, although such an evaluation did not take into account the heterogeneous expression of PD-1 ligand in primary vs. secondary lesions[69], so the value of PD-1 ligand expression in metastases is unknown. Furthermore, PD-1 ligand expression levels did not appear to change before and after treatment in another prospective trial involving 91 mRCC patients undergoing re-biopsy after receiving different doses of nivolumab[70]. In this regard, it must be noted that Conversely, early clinical deterioration captured by quality of life assessment at 8 weeks may be tightly associated with decreased nivolumab efficacy, with a hazard ratio for survival of 0.99

vs. 0.68 and 0.62 of nivolumab vs. everolimus, respectively, in patients with worse vs. stable and improved scores on the Functional Assessment of Cancer Therapy–Kidney Symptom Index–Disease Related Symptoms (FKSI-DRS) after 8 weeks[71]. In the latter trial[70], the biological activity of nivolumab was demonstrated by a median percent change in CD3+, CD4+,CD8+ tumor-associated lymphocytes of 69%, 180% , and 117%, respectively. The results of an exploratory analysis of the outcomes associated with nivolumab treatment beyond progression have been presented at ASCO 2016. With a similar proportion of patients receiving (38%) and not receiving (36%) post-progression nivolumab in the nivolumab-treated arm, treatment beyond progression was associated with a median overall survival of 28.1 months vs 15.0 months (P<0.001). These results appear intringuing, although optimal duration of nivolumab therapy requires to be defined in adequately designed randomized-controlled trials[72]. Reviewed clinical trials on nivolumab are summarized in table 2.

Pembrolizumab, another humanized monoclonal antibody that prevents the binding of PD-1 to PD-L1/2, showed an encouraging safety and activity profile when administered in combination with anti-VEGF TKI axitinib in a phase Ib trial enrolling 11 mRCC patients. Diarrhea, headache, hypothyroidism, arthralgia and fatigue were the most common adverse events reported, while partial responses or disease stabilization were reported in 10 patients[73]. Axitinib has also been combined with another fully human IgG1 anti-PD-L1 monoclonal antibody, avelumab[74], and such a combination is being currently tested vs. sunitinib in the JAVELIN Renal 101 phase III trial (NCT02684006). Bevacizumab has also proven to be safe when administered with anti PD-L1 monoclonal antibody atezolizumab [75], while combinations of sunitinib or pazopanib, and PD-1 inhibitors have displayed increased GI and hepatic toxicities [76] . Furthermore, combinations of nivolumab plus ipilimumab (NCT02231749) and atezolizumab plus bevacizumab (NCT02420821) are being compared with sunitinib as first-line therapy in phase III trials.

4.0. Clinical evidence supporting molecular targets for which monoclonal antibodies are under development

4.1. VEGF and PlGF

Aflibercept is a recombinant fusion protein consisting of human VEGF receptor extracellular domains fused to the F_c portion of human IgG_1 that binds to all isoforms of VEGF as well as placental growth factor (PlGF)..A phase II trial evaluated aflibercept for mRCC previously exposed to a VEGF TKI [77]. Of 94 patients enrolled, 59 and 35 received 4 mg/kg and 1 mg/kg doses respectively. At the 4mg/kg dose, 61% of patients were progression free at 8 weeks and was considered to merit further study.

4.2. VEGF receptor (R)-2

Ramucirumab is a recombinant IgG1 monoclonal antibody that binds VEGFR-2 and blocks the interaction between VEGFR-2 and VEGF, thus inhibiting VEGF-related endothelial proliferation and migration.[78] In a phase 2 study, single agent ramucirumab was administered to 39 patients with mRCC after failure of a previous VEGF TKI. The objective response rate was 5.1% (95% CI, 0.6%-17.3%). The 12-week disease control rate was 64.1% (95% CI, 47.2%-78.8%), while the median PFS was 7.1 months (95% CI, 4.1-9.7 months) and the median OS was 24.8 months (95% CI, 18.9-32.6 months). The most frequent drug-related adverse events were headache, fatigue, hypertension, epistaxis, acute renal failure, hemoptysis, hypertension, infusion reaction and proteinuria. Cerebral ischemia, myocardial infarction, cardiorespiratory arrest, hypertensive crisis, proteinuria and hemoptysis were among the most serious adverse events. The encouraging activity of ramucirumab must be weighed against its toxicity profile when considering further development to treat RCC [79].

4.3. Endoglin

Endoglin is a HIF-1- induced membrane receptor that is highly expressed on proliferating endothelial cells and mediates resistance to VEGF pathway inhibitors[80]. TRC105 is an anti-endoglin IgG1 monoclonal antibody that enhances the activity of VEGF inhibitors in preclinical models[81]. A phase 1b dose escalation study assessed safety, pharmacokinetics, and anti-tumor activity of TRC105 in combination with

bevacizumab. Thirty-eight patients with solid tumors were enrolled in this trial. TRC105 and bevacizumab were well tolerated at their recommended single agent doses (10 mg/kg). Grade 3 suspected adverse reactions included anemia (the dose limiting toxicity of TRC105 established as a single agent), headache (the most common adverse event), and fatigue. Fifteen patients who had previously progressed on bevacizumab or VEGF receptor tyrosine kinase inhibitor (VEGFR TKI) treatment experienced reductions in tumor volume, including two PRs by RECIST, and six remained without progression for longer periods than during their prior VEGF inhibitor therapy.TRC105 was well tolerated with bevacizumab and clinical activity was observed in a VEGF inhibitor refractory population. [81] An ongoing randomized phase II trial is evaluating the addition of TRC105 to second or third line axitinib following previous VEGF TKIs, based on feasibility and preliminary evidence of activity of this combination[82].

4.4. Carbonic Anhydrase IX

The carbonic anhydrase IX (CAIX, G250) is a heat-sensitive transmembrane cell-surface antigen expressed on more than 85% of RCCs, whose expression is related to overexpression of hypoxia-related molecules, such as HIF-1α, and correlates with responsiveness to IL-2 therapy. The role of the RCC immunotherapy base on the use of anti-CAIX monoclonal antibody (cG250 or girentuximab) was studied also in addition to other established therapy for kidney cancer. A phase I clinical trial was conducted to evaluate safety, pharmacokinetic, biodistribution, tumor response rates of cG250 monoclonal antibody in monotherapy. Repeated intravenous doses of up to 50 mg/m^2 of cG250 were safe. No grade 3 or 4 toxicities and no dose limiting toxicities (DLT) occurred. cG250 has a long half-life and targets cc-RCC effectively (cG250 tumour localization was evaluated by gamma camera imaging). One patient showed a CR, 9 patients had SD, and 3 had PD[83]. Unfortunately, an international multicenter phase III trial (ARISER) of 864 patients could not demonstrate an improvement in outcomes using adjuvant girentuximab following surgery for localized high risk disease[84]. However, in a hypothesis-generating analysis, patients with a high tumor CAIX score appeared to derive a benefit with improved disease-free survival. Two clinical trials evaluated the treatment based on the monoclonal antibody plus IL-2. Because the suggested working mechanism of anti-G250 antibody is by antibody dependent cell-mediated cytotoxicity (ADCC) and the number of ADCC effector cells

can be increased by a low dose IL-2 pulsing schedule, a multicenter study investigate whether the association of these two molecules could improve RCC patients clinical outcomes. Thirty-five patients with cc-RCC were enrolled, clinical benefits were achieved in 8 of 35 patients (23%), 3 PR and 5 SD. Mean survival was 22 months. In general treatment was well tolerated with little toxicity[85]. The second study evaluated monoclonal antibody cG250 (which recognizes the CAIX antigen and induces ADCC) with low dose subcutaneous IL-2 in patients with advanced RCC. The primary endpoints of the trial were immunological effects and toxicity: an increased percentage of circulating CD3-/CD16+CD56+ NK cells was observed, some patients showed enhanced ADCC or lymphokine-activated killer cell activity; weekly cG250 with daily low-dose subcutaneous IL-2 is well tolerated with no adverse events attributable to cG250. No antitumor responses were observed in this trial [86]. However, a phase I/II trial combined the monoclonal antibody cG250 and interferon-α-2a to treat 31 patients with clear cell progressive mRCC. Two patients showed PR and 14 patients SD, 1 patient had complete remission lasting at least 17 months, 9 patients had SD of 24 weeks or longer. The median OS observed was 30 months and the 2-year survival was 57%. Patients receiving extended treatment showed a significantly longer 2-year survival rate than discontinued patients (79 vs. 30%; P=0.0083). In general, treatment was well tolerated with little toxicity[87].

Two separate studies were conducted to evaluate toxicity and efficacy of the radioimmunotherapy (RIT) based on the infusions of lutetium 177-girentuximab, an anti-CAIX monoclonal antibody, in patients with mRCC. In the phase 1 trial the maximum tolerated dose (MTD) was determined to be 2405 MBq/m^2 (higher doses were associated with dose-limiting myelotoxicity). Seventeen of 23 [74%] patients demonstrated SD 3 months after the treatment, and one patient showed a PR that lasted 9 months. Mean growth of target tumor lesions was reduced from 40.4% (95% CI, ± 17.0) to 5.5% (95% CI, ± 5.3; p<0.001) at 3 months after the treatment. The phase 2 radioimmunotherapy trial included 14 patients with progressive cc-mRCC, who received ^{177}Lu-girentuximab intravenously. The treatment was generally well tolerated but resulted in grade 3-4 myelotoxicity in most patients. The therapy resulted in disease stabilization in 9 of 14 patients, but myelotoxicity (prolonged thrombocytopenia in particular) prevented retreatment in some patients. [88, 89].

In conclusion, RIT with ^{177}Lu-gentuximab may stabilize previously progressive cc-mRCC. Furthermore with the ^{111}In-gentuximab imaging the radiation absorbed doses to normal tissues and tumor lesions during RIT with ^{177}Lu-gentuximab can be estimated in order to predict hematologic toxicity after treatment with ^{177}Lu-gentuximab [90].

4.5. Hepatocyte Growth Factor – MET axis

Accumulating evidence suggests that the Hepatocyte Growth Factor (HGF)- c-Met pathway is implicated in proliferation of RCC tumor cells. In fact, c-Met is frequently expressed and constitutively phosphorylated in RCC, and patients with high serum levels of HGF/SF have a poor prognosis[91].[92, 93] AMG 102 is a fully human monoclonal antibody that targets HGF/scatter factor (SF), thus preventing its binding to c-Met. In a phase 2 study by Schoffski et al, 61 patients with mRCC were administered AMG-102 (40 at 10 mg/kg; 21 at 20 mg/kg), with 92% of them having received previous anti-VEGF therapy. The median PFS was 3.7 (1.8-7.6) months at 10 mg/kg and 2.0 (1.8-3.7) months at 20 mg/kg, while the median OS (95% CI) was 14.9 (9.4 to not evaluable) months at 10 mg/kg and 17.6 (7.1 to not evaluable) months at 20 mg/kg. The most common adverse events, reported in ≥10% of patients, were oedema (45.9%), fatigue (37.7%) and nausea (27.9%). Grade 3-4 oedema was the most common grade 3-4 event, occurring in 9.8% of patients [94]. Since only one patient had a confirmed PR, the potential usefulness of AMG-102 is unclear, and further studies are warranted.

4.6. 5T4

The oncofoetal trophoblast antigen 5T4 is a transmembrane glycoprotein expressed on a variety of solid cancers, such as ovarian, gastric, non-small cell lung, colorectal and RCC, while 5T4 is poorly expressed in healthy adult tissues, which makes it an ideal target for antibody-based therapy. Naptumomab estafenatox is an immunotoxin consisting of a mutated variant of the superantigen staphylococcal enterotoxin A (SEA/E-

120), which works as a tumor-targeted superantigen (TTS), linked to a fragment antigen binding (Fab) moiety of a monoclonal antibody recognizing the tumor-associated antigen 5T4. Targeting of superantigens towards tumors induces a local recruitment of patients' own cytotoxic T cells, which kill tumor cells directly and through accumulation of inflammatory cytokines. The use of Naptumomab estafenatox for RCC appears promising because of the high expression of 5T4, with >95% of tumors being positive for this antigen. Thirty-nine patients were enrolled in the MONO study, a phase I study conducted of naptumomab estafenatox (ABR-217620) in monotherapy. Patients with pancreatic cancer, non-small-cell lung cancer and RCC received ABR-217620 in escalating doses to determine the MTD, which was 15 µg/kg (RCC). DLTs were fever, hypotension, acute liver toxicity, and vascular leak syndrome. Fourteen patients (36%) had SD by RECIST on day 56 [95]. In a phase 2/3 trial conducted in UK, RCC patients were randomized to receive naptumomab estafenatox (Nap)+interferon (IFN)-α or IFN-α, addition of Nap to IFN-α might prolong OS (HR=0.59, p=0.020) and PFS (HR = 0.62, p = 0.016) in a subgroup of patients with low IL-6, a biomarker for immune responsiveness, and normal levels of anti-SEA/E-120 antibodies, a biomarker for drug exposure [96]. In another phase 2 study, 43 RCC patients were treated with different doses of ABR-214936, a recombinant fusion protein of a murine Fab recognising the antigen 5T4 and a modified form of SEA. Treatment was associated with well tolerated nausea and moderate fever. Median time to progression (TTP) was 4.0 months and median survival was 19.7 months, with a 2-year survival of 42% [97].

4.7. Cytotoxic T-Lymphocyte Antigen (CTLA)-4

CTLA4 is a receptor that inhibits the function and proliferation of the T cells. This inhibitory receptor has a key role in peripheral tolerance of T cells for both normal and tumor-associated antigens. The potential role of antibodies against CTLA4 in patients with mRCC was evaluated in two different trials. A phase 1 study evaluated the combination of sunitinib (50 mg daily for 4 weeks then 2 weeks off or 37.5 mg daily as a continuous dose) and tremelimumab (an antibody against CTLA4; 6 mg/kg, 10 mg/kg, or 15 mg/kg). The patients enrolled experienced DLT and one of them suffered a sudden death. Overall, rapid-onset renal failure was the most common DLT. Nine of 21 patients who were evaluable for response achieved PRs (43%; 95% CI, 22%-66%). Due to these results further investigations of tremelimumab plus sunitinib is not recommended [98]. The second study was a phase II study of ipilimumab conducted in patients with mRCC

(previously treated with IL-2 or not). Major adverse events were enteritis and endocrine deficiencies of presumed autoimmune origin, 33% of patients experienced a grade 3 or 4 immune-mediated toxicity. One of 21 patients receiving the lower dose had a PR, 5 of 40 patients at the higher dose had PRs (95% CI for cohort response rate 4% to 27%) and responses were seen also in patients who had previously not responded to IL-2. Of note, a strong association between autoimmune events and tumor regression was reported (response rate was 30% with adverse events, 0% without adverse events) [25]. In one phase II trial, generally pre-treated patients with mRCC were randomized to receive nivolumab 3 mg/kg + ipilimumab 1 mg/kg (N3I1) or nivolumab 1 mg/kg + ipilimumab 3 mg/kg (N1I3) IV Q3W for 4 doses then nivolumab 3 mg/kg IV Q2W until progression/toxicity[85]. Grade 3–4 related adverse events occurred in 19 patients and objective response rate was 29% with N3 + I1 and 39% with N1 + I3. Thus, nivolumab + ipilimumab showed acceptable safety and encouraging durable antitumor activity. A phase III trial is comparing first-line sunitinib versus the N3I1 regimen.

4.8. TGF-β

Transforming growth factor-β (TGFβ) is a pleiotropic cytokine that is involved in the differentiation, motility and adhesion of cancerous cells, as well as in angiogenesis and formation of extracellular matrix. Furthermore, it suppresses host antitumor immunity. GC1008 (Fresolimumab) is a human anti-TGFβ monoclonal antibody that blocks all the three isoforms of this cytokine. In the phase I study conducted by Morris et al., the safety and activity of GC1008 was evaluated in patients with previously treated advanced malignant melanoma and RCC. No DLT was observed, and the maximum dose, 15 mg/kg, was determined to be safe. Acute infusion reaction, neutropenia, thrombocytopenia, fatigue, diarrhea, vomiting and grade 2 transaminases or bilirubin elevation were observed but skin toxicity (consisting in eruptive keratoacanthomas, hyperkeratosis, cutaneous squamous cell carcinoma and basal cell carcinoma) was the most common drug-related adverse events. The median PFS for all 29 patients was 11.1 weeks (range, 4.1–44.4 weeks). The median TTP/PFS for the PR and SD patients was 24 weeks (range, 16.4–44.4 weeks). Further studies of single agent and combination treatments are required[99].

4.9. Interleukin (IL)-6

The multifunctional cytokine IL-6 is elevated in numerous infectious, inflammatory, autoimmune diseases, and cancer. It induces tumor growth, angiogenesis, invasion and metastasis. High levels of IL-6 correlate with metastatic progression, poorer prognosis and response to IL-2 therapy of renal cancer. As a result, IL-6 may serve as a potential therapeutic target. Furthermore, C-reactive protein (CRP), whose synthesis is stimulated by IL-6, may serve as a pharmacodynamic (PD) marker of IL-6 activity. Siltuximab is a chimeric monoclonal antibody that specifically binds IL-6. A phase I/II study was conducted to evaluate the safety, efficacy, pharmacokinetics (PK) and PD of Siltuximab in 68 patients with mRCC. Although only one patient showed a PR lasting approximately 8 months, approximately 50% of patients had SD that was sustained for a minimum of 11 weeks. **[100]** Siltuximab appeared to be safe in a three-part phase I/II study in patients with progressive mRCC, with no MTD or immune response observed. The adverse events reported were fatigue, chest pain, back pain, dyspnoea, hypertension, transaminases increased, syncope, pain, musculoskeletal pain, and hypercalcaemia. In part 2, SD (≥11weeks) or better was achieved by 11 out of 17 (65%) 3 mg/kg treated patients (one PR ~8 months, 10 SD) and 10 out of 20 (50%) 6 mg/kg treated patients (10 SD). In part 3 65% of patients achieved SD. On the basis of these results Siltuximab stabilised disease in >50% of progressive mRCC patients. Given the favourable safety profile of siltuximab and poor correlation of tumour shrinkage with clinical benefit demonstrated for other non-cytotoxic therapies, further evaluation of dose-escalation strategies and/or combination therapy may be considered for patients with RCC. [101]

4.10. TNF-α

Tumor necrosis factor α (TNF-α) is an inflammatory cytokine with antitumor activity at high dose. Nevertheless, it is secreted by many tumor cells, RCC included, having a role in tumor growth (as autocrine growth factor) and metastasis at pathologic levels. Indeed TNF-α is associated with resistance, poor prognosis and cachexia in cancer patients. Infliximab is a chimeric human-murine monoclonal antibody antihuman TNF-α, it inhibits the bond between TNF-α and its receptors and activates complement-

mediated cell death. In two sequential phase II trials Infliximab was administered at standard and high dose in patients with immunotherapy-resistant or refractory RCC. At standard doses (5 mg/kg of infliximab), 16% of patients showed PR and another 16% of patients achieved SD Median duration of response was 7.7 months. At high doses (10 mg/kg of infliximab), 61% of patients obtained SD, with a median duration of response of 6.2 months. Higher doses were associated with grade 1-2 toxicities including mucositis, headache, myalgia, anemia, flushing, fatigue, peripheral edema and infection. Higher baseline serum levels of TNF-α were associated with PD and poor prognosis[102] A phase I/II trial was conducted administering infliximab in combination with sorafenib in patients with advanced RCC. Median PFS and OS were 6 (95% CI, 4.8-7.2) and 14 months (95% CI, 10-19), respectively. The most common toxicities observed were diarrhea, alopecia, lymphopenia, hand-foot syndrome and rash. Grade 3 adverse events were rash, fatigue, hand-foot syndrome and infection. Since no evidence of improved efficacy of the combination vs. sorafenib alone in RCC was obtained, the further development of the combination of infliximab with sorafenib in advanced RCC was not recommended. [103]

4.11. CD-70

CD70 is a member of the TNF ligand family that is expressed on activated antigen presenting cells such as B lymphocytes and dendritic cells and binds to the CD27 receptor expressed on T lymphocytes. The CD70 - CD27 interaction mediates co-stimulation of NK, B, and T cells and induces differentiation of naive CD4 T lymphocytes into IFN-γ producing T helper 1 (Th1) cells [104]. The attractiveness of the CD70-CD27 pathway for therapeutic purposes in RCC is based on its role in increasing the regulatory T cell (Treg) / effector T cell (Teff)ratio, thus allowing the tumor to escape immune response[105]. The experimental agent SGN-75 is constituted by anti-CD70 IgG1 monoclonal antibody SGN-70 that is chemically bound to the cytotoxic compound named monomethyl auristatin F. SGN-75 was evaluated in a phase I dose-escalation study in patients with CD70-positive relapsed/refractory non-Hodgkin lymphoma or mRCC. Fifty-eight patients were enrolled (39 RCC, 19 NHL). Administration every 3 weeks was better tolerated than weekly dosing and the MTD in RCC patients was 3 mg/kg. The most common adverse events generally manageable, included

corneal epitheliopathy and dry eye (57% of patients), fatigue (40% of patients), nausea (30% of patients) and thrombocytopenia (26% of patients). The antitumor activity was encouraging and included 1 complete response (CR), 2 PRs, while 20 patients showed SD, warranting further testing of this agent. Proof of the biological activity of SGN-75 was obtained by the observation of a substantial depletion of CD70-positive peripheral blood lymphocytes after SGN-75 treatment [106].

5.0. Conclusions

Bevacizumab has been the most extensively investigated monoclonal antibody in RCC, although its use according to its label indication is far less frequent in kidney cancer with respect to oral TKIs such as sunitinib. This may be due, among other reasons, to the intravenous vs. oral route of administration of bevacizumab vs. sunitinib and the approved use of bevacizumab in combination with interferon. Furthermore, in a meta-analysis of 11 randomized-controlled trials, a longer PFS was associated with sunitinib vs. bevacizumab + IFN-α use (HR = 0.79, 95% CrI: 0.64 - 0.96), and temsirolimus + bevacizumab (HR = 0.74, 95% CrI: 0.56 - 0.96), while no difference in PFS between sunitinib and either axitinib, pazopanib or tivozanib was reported [107]. Although toxicities associated with bevacizumab make it feasible to combine it with multiple targeted, immunotherapy and chemotherapy agents, the evidence suggesting some advantage of the use of single agent bevacizumab vs. bevacizumab-containing regimens is scarce. In this regard, the results obtained in terms of PFS and OS with gemcitabine-capecitabine-bevacizumab in patients with sarcomatoid features [50] are promising and are consistent with those obtained in other clinical trials evaluating the combined use of chemotherapy + targeted agents in RCC patients with aggressive features[108]. While combination of bevacizumab with mTOR inhibitors is not more effective vs. the use of bevacizumab alone, combination of bevacizumab plus oral TKIs such as sunitinib is prohibitively toxic. Combination of bevacizumab with novel immunotherapy agents, such as nivolumab and other PD-1/PD-L1 inhibitors, may be feasible on the grounds of different mechanisms of action and non-overlapping toxicity profiles, although the advantages of such a combination treatment must be weighed against its increased

financial burden. The immunotherapy agents reviewed here target either surface white blood cells molecules with inhibitory activity (PD-1, CTLA-4) or cytokines mediating cancer proliferation or exerting immunosuppressive activity. Additional trials are required in order to assess the best combination/sequence of use of these and other novel emerging monoclonal antibodies directed against key molecular targets involved in immune response and cancer growth.

6.0. Expert opinion

The use of monoclonal antibodies holds great promise for RCC and may be accompanied by excellent therapeutic index. The findings obtained with the monoclonal antibody nivolumab in the CheckMate 025 trial are unprecedented in the treatment scenario of RCC. Unlike everolimus and axitinib, which failed to show any advantage in OS after failure of TKI treatment, nivolumab was able to yield a significant survival advantage vs. everolimus, with a hazard ratio for death of 0.73 (98.5% CI, 0.57 to 0.93; P = 0.002). The results obtained in the CheckMate trial established a paradigm change for two main reasons. First, the intrinsic immunogenicity of RCC has been successfully exploited for therapeutic purposes via the use of novel antibody-based immunotherapy agents, so nivolumab paved the way for experimentation and testing of the wealth of immunotherapy agents directed against PD-1 and other additional immunological targets[109]. Second, unlike targeted agents, whose clinical activity was primarily based on improvements in PFS, nivolumab did not yield any advantage in PFS, although its use was associated with increased radiological responses (25% vs. 5%; P<0.001). The lack of an association between PFS and OS is similar to that associated with another immunotherapy agent, that is Sipuleucel-T, in prostate cancer [68] and underlines the need for additional surrogate end points, both clinical and biological, other than PFS. In contrast, cabozantinib extended PFS and OS following VEGF inhibitors, although the toxicity profile needs to be considered when selecting patients for this agent. Although PD-1 and PD-L1 are the most promising therapeutic targets for RCC, we have shown that monoclonal antibodies can also target key cytokines (TNF □, IL-6, endoglins and TGF-□) and surface immune molecules (CTLA-4), although the results obtained with such agents are still preliminary.

The aforementioned data with monoclonal antibodies needs to be viewed in the context of emerging agents and combinations belonging to other classes. For example, the combination of lenvatinib plus everolimus was associated with a significantly longer median PFS of 12.9 months compared to 5.6 months with everolimus in a randomized phase II trial, with [110, 111]. Finally, given the explosion in the number of approved agents and combinations being developed, it is imperative to co-develop precision medicine to select the right patient for the right drug or combination. Presently, nivolumab is the most extensively studied immunotherapy drug in renal cell carcinoma, but patent rights do not prevent other pharmaceutical companies from developing drugs against established therapeutic targets such as PD-L1 and PD-1, which has led to sharp increase of the number of monoclonal antibody-based immunotherapy drugs being investigated in clinical trials. Differently from the multiple bevacizumab-based treatment combinations which we showed failed to provide any additional benefit, combined VEGF and PD-1/PD-L1 inhibition may be the key to improving mRCC outcomes in the next 5 years, on the basis of the non overlapping side effects and biological targets of the therapeutic agents involved. The increasing costs associated with the availability of multiple agents may prevent access to novel effective agents to most patients. On the other hand, a virtuous loop may be created by the abundance of multiple monoclonal antibodies directed against the same/similar targets (e.g. pembrolizumab, nivolumab, atezolizumab, avelimumab are directed against PD-1/ PD-L1), with market competition of similarly effective agents possibly leading to decreased therapy costs.

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Article highlights Box

- Monoclonal antibodies hold a great potential for the treatment of metastatic renal cell carcinoma, with two agents of this pharmacologic class currently approved (anti-VEGF agent bevacizumab and anti-PD-1 agent nivolumab)

- Bevacizumab has shown to prolong progression-free survival in the first line setting of treatment of metastatic renal cell carcinoma, while nivolumab has shown to prolong overall survival in the second-line setting after failure of one anti-VEGF treatment. Bevacizumab has been approved in combination with interferon, although the contributing role of interferon to the therapeutic activity of the combination is uncertain.
- A number of studies have been conducted to explore the feasibility to combine bevacizumab with agents other than interferon, but none of these have shown promising results
- Although the PD-1 /PD-L1 axis is the most promising target for the development of novel agents, a large number of prospective trials are being conducted to explore the use of monoclonal antibodies directed against multiple alternative targets, such as MET, endoglin, 5T4, CTLA-4, CD70, and others
- Promising results may derive from the combined inhibition of the VEGF and PD1/PD-L1 axis in the next 5 years, although the advantages of such as combination must be weighed against its increased toxicities and financial costs.

Table 1. Clinical trials reviewed

	Experimental agent and mechanism of action	Study design	Experimental combination	PFS	OS	Grade 3-4 toxicities
Escudier et al. [22, 24]	Bevacizumab - angiogenesis inhibitor by blocking VEGF-A; Interferon α - plays an antiproliferative role activating MAP kinase signaling, increasing p53 activity and modulating functions of the immune system	Multicentre, randomized, double-blind, phase 3 trial	Interferon α-2a and bevacizumab versus interferon α-2a and placebo	10.2 mo with interferon α-2a and bevacizumab; 5.4 mo with interferon α-2a and placebo	23.3 mo with bevacizumab and interferon α-2a; 21.3 mo with interferon α-2a and placebo	Fatigue (12%), asthenia (10%), proteinuria (8%), hypertension (6%) with interferon α-2a and bevacizumab; fatigue (8%), asthenia (7%) with interferon α-2a and placebo
Rini et al. [8, 23]	Bevacizumab (see above); Interferon α (see above)	Prospective, randomized, multicenter phase 3 trial	Bevacizumab plus IFN-α versus IFN-α monotherapy	8.5 mo (7.5-9.7) with bevacizumab plus IFN-α; 5.2 (3.1-5.6) with IFN-α	18.3 mo (16.5 to 22.5) with bevacizumab plus IFN-α; 17.4 mo (14.4 to 20.0) with IFN-α	Fatigue (37%), anorexia (17%), proteinuria (15%), hypertension (11%), neutropenia (9%), nausea (7%), dyspnea (6%) with bevacizumab plus IFN-α; fatigue (30%), neutropenia (9%), anorexia (8%), nausea (5%) with IFN-α
Melichar et al. [27]	Bevacizumab (see above); Interferon α (see above)	Prospective, multicentre, open-label, single-arm, multinational, phase 2 trial	Bevacizumab with low-dose IFN	15.3 mo (11.7-18.0)	30.7 mo (25.7-not reached)	Fatigue or asthaenia (9.6%)
Hainsworth et al. [28]	Bevacizumab (see above)	Phase 2 trial	Bevacizumab	7.8 mo in previously untreated patients; 3.7		Proteinuria

				mo in patients previously treated with VEGFR-targeted agents		
Turnbull et al. [29]	Bevacizumab (see above)	Retrospective analysis	Bevacizumab	4.4 mo (2.8-9.6)	19.4 mo (9.9-not reached)	Fatigue (29%), dehydration (24%), failure to thrive (10%), constipation (10%), muscle weakness (10%)
Jonasch et al. [43]	Bevacizumab (see above); Erlotinib - tyrosine kinase inhibitor acting on EGFR	Prospective, single-arm, phase 2 trial	Bevacizumab plus erlotinib or bevacizumab alone	11 mo (5.5-15.6)	25.4 mo (11.4-not estimable)	Diarrhea (6%) with bevacizumab plus erlotinib; hypertension (5%), anemia (5%) with bevacizumab only
Hainsworth et al. [31]	Bevacizumab (see above); Everolimus - inhibits mTORC1 protein complex, thus prevents tumor cells growth and proliferation	Multicenter, community-based, nonrandomized, phase 2 trial	Bevacizumab and everolimus	8.1 mo (6.3-10.8)	18.5 mo	Proteinuria (26%), mucositis/stomatitis (15%), fatigue (12%), diarrhea (9%), anemia (5%), hyperlipidemia (5%), constipation (5%)
Harshman et al. [32]	Bevacizumab (see above); Everolimus (see above)	Investigator-initiated, phase 2 trial	Bevacizumab and everolimus	5.1 mo	21 mo	
Ravaud et al. [33]	Everolimus (see above); Bevacizumab (see above); Interferon α (see above)	Open-label, randomized, phase 2 trial	Everolimus plus bevacizumab (EVE/BEV) versus interferon α-2a plus bevacizumab (IFN/BEV)	9.3 mo with EVE/BEV; 10 mo with IFN/BEV	27.1 mo	Proteinuria (24.4%), stomatitis (10.6%), anemia (10.6%) with EVE/BEV; fatigue (17.1%), asthenia (14.4%), proteniuria (10.5%) with IFN/BEV
Merchan et al. [34]	Bevacizumab (see above); Temsirolimus - mTOR inhibitor,	Phase 2 trial	Bevacizumab and temsirolimus	5.9 mo (4.0-7.8)	20.6 mo (11.5-23.7)	Hypertriglyceridemia (14%), oral mucositis (10%), fatigue (10%), anemia

	induces cell cycle arrest in th G1 phase, blocks tumor angiogenesis by reducing synthesis of VEGF, interferes with growth, survival and proliferation of tumor cells					(8%), proteinuria (8%)
Negrier t al. [35]	Bevacizumab (see above); Temsirolimus (see above); Interferon α (see above); Sunitinib – multi-targeted receptor tyrosine kinase, it binds PDGF-Rs, VEGF-Rs, c-KIT, RET, G-CSF-R, FLT-3	Multicentre, open-label, randomized, phase 2 trial	Bevacizumab and temsirolimus or sunitinib or bevacizumab and interferon α	8.2 mo (7.0-9.6) with bevacizumab and temsirolimus; 8.2 mo (5.5-11.7) with sunitinib; 16.8 mo (6.0-26.0) with interferon α and bevacizumab		
Rini et al. [36]	Bevacizumab (see above); Temsirolimus (see above); Interferon α (see above)	Radomized, open-label, multicenter, phase 3 trial	Bevacizumab and temsirolimus versus bevacizumab and interferon α	9.1 mo (8.1-10.2) with temsirolimus and bevacizumab;9.3 mo (9.0-11.2) with bevacizumab and interferon α	25.8 mo (21.1-30.7) with temsirolimus and bevacizumab; 25.5 mo (22.4-30.8) with bevacizumab and interferon α	Proteinuria (16%), hypertension (11%), anemia (9%), mucosal inflammation (8%), hypertriglyceridemia (7%), stomatitis (7%), hypercholesterolemia (6%), asthenia (6%), hyperglycemia (6%), fatigue (5%) with temsirolimus and bevacizumab; proteinuria (13%), fatigue (11%), hypertension (10%), asthenia (10%), anemia (8%), neutropenia

						(8%) with bevacizumab and interferon α
Flaherty et al. [37]	Bevacizumab (see above); Temsirolimus (see above); Sorafenib - inhibitor of tyrosine protein kinases, such as VEGF-R, PDGF-R, Raf family kinases	Randomized phase 2 trial	Bevacizumab monotherapy or bevacizumab plus temsirolimus or bevacizumab plus sorafenib or temsirolimus plus sorafenib	7.5 mo (5.8-10.8) with bevacizumab monotherapy; 7.6 mo (6.7-9.2) with bevacizumab plus temsirolimus; 9.2 mo (7.5-11.4) with bevacizumab plus sorafenib; 7.4 mo (5.6-7.9) with temsirolimus plus sorafenib		
Falchook et al. [41]	Bevacizumab (see above); Bortezomib - proteasome inhibitor	Phase 1 trial	Bevacizumab plus bortezomib			Thrombocytopenia (12%)
Bukowski et al. [44]	Erlotinib (see above); Bevacizumab (see above)	Randomized, double-blind, placebo-controlled, phase 2 trial	Erlotinib plus bevacizumab versus bevacizumab alone	9.9 mo with bevacizumab plus erlotinib; 8.5 mo with bevacizumab alone		Hypertension (31%), rash (16%), diarrhea (7.8%), proteinuria (7.8%), hemorrhage (5.9%) with erlotinib plus bevacizumab; hypertension (26%), proteinuria (5.7%) with bevacizumab alone
Gordon et al. [56]	Bevacizumab (see above); TRC105 - inhibits endoglin, which has a role in angiogenesis	Open-label, multicenter, nonrandomized, dose-finding, phase 1 trial	Bevacizumab and TRC105			Anemia (20.8%), headache (10.4%), fatigue (5.2%)
Dandamudi et al.	Bevacizumab (see above);	Phase 2 trial	Bevacizumab and	11,2 mo (5.7-11.7)		

[50]	IL-2 - has a crucial role in modulating important functions of immune system, such as tolerance and immunity, through its effects on T cells		high-dose IL-2			
Garcia et al. [51]	Bevacizumab (see above); IL-2 (see above)	Prospective, phase 2 trial	Bevacizumab and low-dose IL-2	9.6 mo (4.1-16.9)		Fatigue (42%), neutropenia (12%)
Feldman et al. [38]	Bevacizumab (see above); Sunitinib (see above)	Single-center, investigator-initiated, phase 1 trial	Bevacizumab plus sunitinib	11 mo (6.0-not reached)		Hypertension (60%), proteinuria (36%), elevated lipase (28%), thrombocytopenia (24%), hand-foot-skin reaction (16%), fatigue (12%), hyponatremia (12%), hemorrhage (12%), reversible posterior leukoencephalopathy (8%), elevated amylase (8%), rash (8%), microangiopathic hemolytic anemia (8%), hyperuricemia (8%), abdominal pain (8%), lymphopenia (8%)
Bruce et al. [39]	Bevacizumab (see above); Sunitinib (see above)	Multicenter, investigator-initiated, phase 1 trial	Bevacizumab and sunitinib			Hypertension (16.7%), leukopenia (16.7%), diarrhea (16.7%), anemia (16.7%)
Medioni et al. [40]	Bevacizumab (see above); Sunitinib (see above)	Case report	Bevacizumab and sunitinib	8.5 mo	15.1 mo	Hypertension (28.6%)

Jonasch et al. [45]	Bevacizumab (see above); Capecitabine - chemotherapeutic agent, it is converted to 5-fluorouracil, which inhibits the synthesis of thymidine monophosphate required for *de novo* synthesis of DNA; Gemcitabine - nucleoside anolog, blocks the DNA replication and inactivates the enzyme ribonucleotide reductase, which cannot produce the deoxyribonucleotides for DNA replication and repair, cell apoptosis is induced	Retrospective study	Bevacizumab, capecitabine and gemcitabine	5.9 mo	10.4 mo	
Chung et al. [47]	Bevacizumab (see above); Capecitabine (see above); Gemcitabine (see above)	Open-label, single arm, phase 2 trial	Bevacizumab, capecitabine and gemcitabine	5.3 mo (3.9-9.9)	9.8 mo (6.2-14.9)	Fatigue (21%), hand foot syndrome (7%), dyspnea (7%), emesis (7%), nausea (7%)
Buti et al. [48]	Bevacizumab (see above); IL-2 (see above); Interferon α (see above); Gemcitabine (see above); 5-fluorouracil - pyrimidine analog, thymidylate	Multicenter, dose-finding, phase 1 trial	Bevacizumab, IL-2, interferon α-2a, gemcitabine and 5-fluorouracil	TTP 6.4 mo (3.3-9.5)	22.6 mo (9.6-35.6)	Neutropenia (63%), thrombocytopenia (34%), fever (26%), pain and arthromyalgia (22%), asthenia (15%), leukopenia (11%), proteinuria (8%), hypertension (7%), hypertransaminasemia (7%)

	synthase inhibitor, thus blocks the synthesis of the thymidine, a nucleoside required for DNA replication					
Hainsworth et al. [49]	Bevacizumab (see above); Erlotinib (see above); Imatinib - tyrosine kinase inhibitor, it blocks the activity of several enzymes, such as ABL, KIT, PDGF-R	Multicenter, nonrandomized, phase 2 trial	Bevacizumab, erlotinib and imatinib	8.9 mo (6.6-10.9)	17.2 mo (12.9-21.0)	Diarrhea (50%), rash (27%), fatigue (24%), nausea/vomiting (19%), proteinuria (12%), hypertension (7%), arthrealgia (5%)

Table 1. Reviewed clinical studies on bevacizumab in renal cell carcinoma

	Mechanismo f action	Study design	Experimente d combination	PFS	OS	Grade 3-4 toxicities (incidence)
Topalian et al. [75]	Nivolumab - inhibits PD-1, whose immune-inhibitory effect mediates tumor immune escape, the PD-1/PD-L1 interaction blocks Tcells functions and induces resistance of tumor cells to cytotoxic lymphocytes	Phase 1, open-label, multicenter, multidose, dose-escalation study	Nivolumab	56 % (39-73) at 24 wk		Fatigue (5%); Dyspnea (8%)
Brahmer et al. [76]	Nivolumab (see above)	Multi-institutional, open-label, phase 1, dose-escalation study	Nivolumab			No
Lipson et al. [77]	Nivolumab (see above)	Phase 1 study	Nivolumab			
Brahmer et al. [105]	MDX-1105 – anti-PD-L1 monoclonal antibody prevents the binding of PD-L1 to its receptor PD-1, which enhances the T cells-mediated immune response to tumor cells	Phase 1, open-label, dose-escalation, multidose study	MDX-1105	53% (29-77) at 24 wk		Fatigue (8%); Dyspnea (5%)

Motzer et al. [79]	Nivolumab (see above); Everolimus (see above)	Randomized, open-label, phase 3 study	Nivolumab versus Everolimus	4.6 mo (3.7-5.4) with Nivolumab; 4.4 mo (3.7-5.5) with Everolimus	25 mo (21.8 to not estimable) with Nivolumab; 19.6 mo (17.6-23.1) with Everolimus	Anemia (8%) with Everolimus
McDermott et al. [78]	Nivolumab (see above)	Phase 1 study	Nivolumab		22.4 mo	

Table 2. Reviewed clinical studies on nivolumab in renal cell carcinoma

Figure 1