



Cytokine therapy for renal cell cancer: the evolving role of immunomodulation

Renal cell carcinoma (RCC) is a chemo-resistant malignancy. For many years the only treatment for advanced disease was immunotherapy; the cytokines IFN- α and IL-2 were widely used but offered limited objective response rates of 10–20% and only modest survival benefits in the majority of cases. The last decade has witnessed a marked increase in the availability of novel targeted therapies demonstrating anti-tumor activity and, more importantly, providing a meaningful impact on overall survival. However, to date, immunotherapy, in the form of high-dose IL-2, remains the only treatment modality able to induce durable complete remissions and/or cure in metastatic RCC, albeit in a small minority of patients. A priority for immunotherapy research should be to investigate strategies that may augment the effectiveness of immunomodulation and enable a greater proportion of patients with advanced RCC to benefit from this treatment. A number of approaches are discussed; combinations with molecularly targeted agents may potentiate response and survival, immune checkpoint inhibitors such as CTLA-4 and PD1 antibodies are showing encouraging activity in early clinical trials and vaccines are of renewed interest. Taking these and other novel immunomodulatory agents forward will require the use of immune-related response criteria in clinical trial design and, most importantly, the ability to identify reliable predictors of benefit so that appropriate patients may be selected for immunotherapeutic treatments.

KEYWORDS: biomarkers ■ CTLA-4 ■ IL-2 ■ immunomodulation ■ immunotherapy ■ PD-1 ■ renal cell cancer

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Renal cell carcinoma (RCC) is the most common tumor of the kidney. Surgery remains the primary curative treatment modality; however, a quarter of patients present with advanced disease while a third of those who undergo nephrectomy for localized disease eventually relapse. RCC is highly resistant to conventional chemotherapy [1] and for a long time the only therapeutic options for advanced RCC were cytokine therapies such as IFN- α and IL-2. However, the outlook for metastatic RCC has improved significantly in the last 10 years with the introduction of inhibitors of the VEGF receptor (VEGFR) and the mammalian target of rapamycin (mTOR). Here, we discuss the role of immunomodulation within the modern day context of targeted therapies, including an up to date review of the effectiveness of cytokine therapy and a discussion of IL-2 response predictors. The recent success of immune-based therapies in other tumor types has fuelled a renewed interest in immunomodulation in RCC. Therapeutic strategies are under investigation to harness and augment host innate anti-tumor immunity or inhibit negative immune regulators. The development of novel immunotherapies will require parallel biological studies, a careful evaluation of potentially serious immune-related side effects and modified response assessment criteria.

Immunobiology of advanced renal cell cancer

The immunoresponsiveness of RCC was first suggested by the observation of spontaneous remissions. Objective regression rates of 7% (including complete remissions) have been reported with placebo or active surveillance [2,3]. In addition, the increased incidence of renal cancer among patients receiving chronic post-transplant immunosuppressive therapy provided further evidence implicating immune-related mechanisms in renal carcinogenesis [4]. Circulating activated T cells and intratumoral T-cell infiltrates have been detected in patients with cancer and found to correlate with survival, suggesting that the immune system is capable of recognizing and eliminating tumor cells [5].

Disappearance of histologically proven metastatic disease after debulking nephrectomies has been described [6,7] generating the hypothesis that resection of the primary tumor may remove a reservoir of tumor-secreted immunosuppressive factors and restore host immune reactivity to residual cancer cells. For instance, immune inhibitory molecules, such as B7-H1, are expressed on the surface of T cells within the tumor and serum of patients with metastatic RCC and shown to be associated with

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poor prognosis [8,9]. Tumoral secretion of IL-10 or VEGF, both reported to attenuate dendritic cell activation and differentiation, may provide another means of evading innate host immunity [10]. Finally, Foxp3⁺/CD25⁺ regulatory T cells (Tregs) are critical negative regulators contributing to immune tolerance. RCC primary tumors frequently demonstrate dense Treg infiltrates and the presence of Tregs is associated with aggressive histology and poor survival post-nephrectomy [11]. These and other tumor-associated negative immune regulators may suppress both local and systemic innate immunity. On this basis, a number of therapeutic strategies have been explored in an effort to upregulate anti-tumor immunity [12].

Cytokine-based therapies:

IFN- α & IL-2

■ IFN- α

Interferons are endogenous cytokines produced in response to viral infections and antigens or induced by other cytokines such as TNF or interleukins. The anti-tumor effects of exogenous IFN- α are poorly understood but are likely to involve a combination of immunomodulation via upregulation of cytotoxic T lymphocytes (CTLs), natural killer (NK) cells and macrophages, as well as antiproliferative and anti-angiogenic effects [13].

A Cochrane analysis of four randomized trials, involving over 600 patients demonstrated an overall survival (OS) advantage of 3.8 months (11.4 vs 7.6 months, $p < 0.001$) for IFN- α compared with noncytokine treatment (medroxyprogesterone or vinblastine alone) [14]. Side effects included flu-like symptoms, depression, abnormal liver function tests and weight loss. An updated review, in 2010, of eight randomized trials involving over 1300 patients comparing IFN alone ($n = 5$) [15–19] or in combination with agents unlikely to have significant activity in RCC (with vinblastine, $n = 2$; with IFN- γ , $n = 1$) [20–22] demonstrated a sevenfold higher response rate compared with control and a significant improvement in mortality [23]. However, activity remains modest with objective response rates ranging from 5 to 20% and median OS from 9 to 15 months in the IFN- α groups (TABLE 1).

Despite preclinical evidence of synergy, the addition of chemotherapy does not improve survival compared with IFN- α alone [24–26]. In contrast, debulking nephrectomy potentiates benefit from IFN- α . Two trials have established that cytoreductive surgery prolongs median survival

compared with IFN- α alone in selected patients with good performance status [27,28]. Although IFN- α monotherapy was widely used in Europe, it did not have US FDA approval for the treatment of RCC in the USA. Today, given the low response rate, potential toxicity and demonstrated efficacy of the VEGFR inhibitor, sunitinib and the mTOR inhibitor, temsirolimus over IFN- α alone in Phase III randomized trials, IFN- α is unlikely to have a significant role as a single agent in the management of advanced RCC [29,30].

IFN- α has been ascribed anti-angiogenic properties and two large trials have shown that the combination of IFN- α with the VEGF monoclonal antibody, bevacizumab significantly improved response rate and progression-free survival (PFS) compared with IFN- α alone, although neither trial demonstrated an impact on OS [31,32]. Unfortunately, neither study included a third bevacizumab alone arm making it difficult to assess the true added benefit of IFN- α over bevacizumab alone which is known to have single-agent activity in Phase II trials [33].

■ IL-2

High-dose intravenous bolus IL-2 was first approved in 1992 on the basis of an objective response rate of 15% in a series of Phase II trials [34]. Of the responses, a third were complete regressions and follow-up long-term survival data showed that median duration of response for all responders was 54 months (range: 3–131 months) and that the median duration of response for complete responders was not yet reached but was greater than 80 months [35]. These results have been confirmed by other groups showing complete response rates of 7% associated with a median survival of more than 120 months [36]. However treatment with high-dose IL-2 is associated with severe toxicity. The most severe and potentially life-threatening side effects are attributable to IL-2-induced capillary leak with resulting peripheral and pulmonary oedema, hypotension and renal failure frequently requiring invasive monitoring in intensive care. Other side effects include fevers, rash, cardiac arrhythmias, metabolic acidosis and neurotoxicity. Although treatment-associated mortality was 4% in early trials, supportive measures such as careful fluid balance and blood pressure monitoring, use of vasopressors or intravenous bicarbonate, as needed, have been shown to allow the safe delivery of high-dose IL-2 and more recent trials have not experienced significant treatment-associated deaths [37].

Table 1. Prospective randomized studies of IFN- α .

Randomization	Number of patients	Response rate [†]	Median survival (months) [†]	Ref.
Studies evaluating single agent IFN-α				
IFN- α vs MPA	145	4.5 vs 2.5%	15.2 vs 14.9 NS	[15]
IFN- α vs MPA	335	14 vs 1% p = 0.001	9 vs 6.8 p = 0.013	[16]
IFN- α vs MPA	60	6 vs 3%	NR	[19]
IFN- α vs FAMP	32	12 vs 12%	8.8 vs 9.9	[18]
IFN- α vs IFN- γ vs IFN- α + IFN- γ	89	4.7 vs 4.7 vs 4.2%	NR	[17]
Studies evaluating IFN-α with VBL or IFN-γ				
IFN- α + IFN- γ vs IFN- γ	88	10 vs 0%	10.9 vs 7.0	[20]
IFN- α + VBL vs VBL	160	16.5 vs 2.5% p = 0.0025	15.5 vs 8.7 p = 0.005	[21]
IFN- α + VBL vs MPA	89	20 vs 0% p = 0.001	NR	[22]

[†]Significance values included when reported.
FAMP: 5-fluorouracil + doxorubicin + cisplatin + mitomycin; MPA: Medroxyprogesterone acetate; MRC: Medical Research Council; NR: Not reported; NS: Not significant; VBL: Vinblastine.

In an effort to improve the toxicity profile, a number of alternative regimens using intermediate doses of intravenous IL-2 [38,39] or subcutaneous IL-2 in combination with IFN- α have been tested and have failed to show the same rate of durable complete responses (TABLE 2) [15,39–43]. A biochemotherapy combination of IFN- α , subcutaneous IL-2 and 5-fluorouracil (Atzpodien schedule) showed encouraging activity in two early trials with response rates as high as 39% [42,44]. However, the large RE04 trial that randomized 1000 patients with advanced RCC to first-line treatment with the Atzpodien schedule or IFN- α alone, failed to show any improvement in OS for the biochemotherapy regimen (TABLE 2) [26]. In summary, the benefit of IL-2 appears to be limited to a small subset of patients who achieve durable complete responses with high-dose intravenous treatment. However, the significant toxicity, cost and limited efficacy of IL-2 has narrowed its use to selected young, fit patients treated in a few specialized centers.

Patient selection for high-dose IL-2 immunotherapy

Given the low response rate and significant toxicity of currently available immunotherapies, should anyone be considered for IL-2? While newer targeted therapies have demonstrated significant activity in metastatic RCC, their role to date has only been established in the palliative setting. Despite low response rates, there is compelling evidence that immunotherapy using

high-dose IL-2 may result in prolonged remissions, and even cures, in a minority of patients (7–10%). It is unclear why only a small number of patients achieve durable remissions with IL-2 and whether this subset of patients can be reliably identified and selected for high-dose IL-2 with curative intent. High-dose IL-2 is only suited to young, fit patients with intact organ function and a performance status of 0, likely to tolerate the potentially reversible but significant treatment-associated morbidity. In addition, a number of candidate clinical, biochemical and tumor-related factors have been investigated.

Clinical predictors

One of the best-described models used to predict benefit from immunotherapy is the modified Memorial Sloan Kettering Cancer Centre (MSKCC) prognostic criteria; patients who have undergone debulking nephrectomy and present with a normal performance status, lactate dehydrogenase, calcium and hemoglobin, as well as a time to treatment of more than 1 year have the best outcome with immunotherapy [45]. Other clinical and serum predictors have been incorporated into predictive models that may be of value, such as number and sites of metastatic disease, elevated C-reactive protein and high neutrophil count [46,47].

Tumor-related predictors

A number of tumor-related predictors have also been proposed. There is compelling evidence that response to immunotherapy is limited to clear

Table 2. Selected randomized trials of IL-2 in advanced renal cell cancer.

Treatment	Number of patients	Response rate	Complete response rate	OS difference	Ref.
High dose iv. IL-2					
HD IL-2 [†] vs low dose iv. IL-2 + IFN- α	99	17 vs 11%	5.6 vs 0%	NS	[43]
HD IL-2 [‡] vs low dose iv. IL-2 vs SQ IL-2	397	21 vs 11 vs 10% p < 0.05 for HD IL-2	7 vs 4 vs 2%	NS	[41]
HD IL-2 [§] vs SQ IL-2 + IFN- α	186	23 vs 10% p = 0.018	8.4 vs 3.2%	p = 0.001 if liver or bone mets and p = 0.04 if primary <i>in situ</i>	[40]
Intermediate dose iv. IL-2					
ID IL-2 [†] vs IFN- α vs ID IL-2 + IFN- α	425	6.5 vs 7.5 vs 18.6% p = 0.01 for ID IL-2 + IFN	1.4 vs 0 vs 0.7%	NS	[39]
ID IL-2 [†] + IFN- α vs SQ IL-2 + IFN- α	155	17.9 vs 21.3%	3.8 vs 1.3%	NS	[38]
Biochemotherapy combinations using subcutaneous IL-2					
SQ IL-2 + IFN- α + 5FU vs TAM	78	39 vs 0%	17 vs 0%	NR	[44]
SQ IL-2 + IFN- α + 5FU (Arm A) vs Arm A + cis-RA (Arm B) vs IFN- α + VB (Arm C)	341	31 vs 26 vs 20%	5 vs 8 vs 6%	p = 0.04 for A vs C and p = 0.02 for B vs C	[42]
SQ IL-2 + IFN- α + 5FU vs IFN- α	1006	23 vs 16% p = 0.045	2.3 vs 2.3%	NS	[26]
[†] IL-2 1.33 mg/m ² intravenous every 8 h, days 1–5 and 15–19. [‡] IL-2 720,000 U/kg intravenous every 8 h. [§] IL-2 600,000 U/kg intravenous every 8 h days 1–5 and 15–19 (equivalent to 1.33 mg/m ²). ^{††} 18 MIU/m ² /day days 1–5. cis-RA: 13-cis-retinoic acid; HD: High dose; ID: Intermediate dose; IFN: Interferon; iv.: Intravenous; mets: Metastases; NR: Not reported; NS: Not significant; OS: Overall survival; SQ: Subcutaneous; TAM: Tamoxifen.					

cell RCC [48–50]. In addition, certain histological features, such as predominant alveolar morphology and absence of papillary or granular features, have been associated with benefit from immunotherapy [50]. Carbonic anhydrase IX (CAIX) is transcriptionally regulated by hypoxia-inducible factor, which in turn is increased by the loss of the tumor suppressor gene, Von Hippel-Lindau, in the majority of clear cell RCC. High CAIX was shown to be associated with long-term benefit from IL-2 [51,52]. On this basis, Atkins *et al.* proposed a model combining histological features with CAIX expression to select patients for up-front high-dose IL-2 immunotherapy (TABLE 3) [52]. In a retrospective analysis, this model identified a good risk group that contained 96% of IL-2 responders and only 46% of non-responders. The recently reported SELECT trial was specifically designed to investigate whether the therapeutic index of high-dose IL-2 could be improved by using this model incorporating histological features and CAIX levels to select likely responders [53]. The response rate of 28% among 120 patients was higher than historical controls and may be largely attributed to the high proportion of patients with clear cell histology (>95%) and previous cytoreductive surgery (>95%). However, SELECT failed to confirm

the value of tumor-based predictive features or CAIX levels in selecting patients most likely to benefit from high-dose IL-2. The investigators suggested that host factors, such as cellular immunity, may be more important than tumor factors at predicting response to IL-2.

Immunological predictors

IL-2 requires the activation of the host immune system for tumor lysis, thus generating interest in patient-related immunologic predictive factors. Low levels of CD57⁺ NK cells, the presence of neutrophils, as well as high levels of Tregs (negative modulators of the immune response) within the tumor have been associated with poor outcome with immunotherapy [54]. Pretreatment dendritic cell phenotype or maturation has been identified as a potential marker of benefit from high-dose IL-2 [55]. Whether these immune factors provide an indirect measure of innate anti-tumor immune response capacity is unknown. Increased levels of circulating TNF- α and IL-1 with IL-2 treatment has also been associated with tumor response [56]. In summary, no immune parameters associated with benefit from IL-2 have been reliably identified to allow the selection of a subpopulation for high-dose immunotherapy.

Combining molecularly targeted therapies with immunomodulation

Another potential strategy to enhance the effectiveness of immunotherapy may involve combinations with new targeted therapies that may have both anti-tumor effects in their own right, as well as the potential to augment host immunity. Many of the molecules and signaling pathways targeted by VEGFR and mTOR inhibitors are also important in immune cell function. Not surprisingly, there is increasing evidence that many of these inhibitors can have a substantial impact on immune function, either stimulating or down-regulating the immune response [57,58]. There is an increasing interest in combining immunotherapy with targeted therapies that may potentiate anticancer immune reactivity and result in biological synergy [59]. VEGF has been shown to inhibit the maturation of dendritic cells (DCs) [10] or T cells [60] and has thus been ascribed immunosuppressive properties. Inhibition of VEGF results in activation of DCs *in vitro* and a Phase I trial showed that VEGF-Trap promoted DC maturation [61,62]. The possibility that bevacizumab may improve host immune function and increase the rate of durable responses with IL-2 is being investigated in a trial of high-dose IL-2 plus bevacizumab. In addition, the vascular effects of bevacizumab may reduce the toxicity associated with high-dose IL-2. As previously mentioned, the addition of bevacizumab to IFN- α has been shown to increase response rate and PFS [32]. In contrast, a randomized trial of IFN- α , the mTOR inhibitor, temsirolimus or the combination of both, failed to demonstrate a benefit of the combination over IFN- α alone [29]. This finding is thought to be attributable to the fact that additive toxicities in the combination arm resulted in reduced dose intensity of temsirolimus.

Myeloid-derived suppressor cells and Tregs promote immune tolerance and attenuate CTL function. Recent studies have suggested that sunitinib can reduce levels of myeloid-derived suppressor cells and Tregs, thereby potentially augmenting the effectiveness of immunotherapy [63,64]. By contrast, the multi-targeted inhibitor, sorafenib may inhibit CTL and antigen-presenting cell (APC) function prompting the concern that sorafenib may theoretically attenuate the effects of immunotherapy [65]. Whether the immunostimulatory properties of sunitinib or bevacizumab may potentiate immune strategies and justify combinations with cytokines or newer immunomodulating agents is under investigation in a range of clinical trials in Europe and the USA.

Vaccines

Vaccine-based approaches are based on the concept that RCC cells express tumor-associated antigens capable of generating a CTL response. Unfortunately RCC-specific individual tumor-associated antigens have not been easy to identify. Autologous vaccines utilize the whole tumor cell, cell lysates or tumor-derived DNA or RNA, the aim being to stimulate a host immune response against a wide range of tumor epitopes. In the last 15 years, over 30 trials of cell-based vaccines have been conducted in early and metastatic RCC using autologous tumor cell vaccines, genetically modified tumor vaccines or dendritic cell-based vaccines (reviewed in [66]). Most studies were small and failed to demonstrate significant clinical efficacy. However, two large Phase III adjuvant trials of autologous vaccines have been published. A trial conducted in Germany randomized over 500 patients with resected stage T2–3B, N0–3, M0 to tumor lysate vaccinations or observation [67]. Vaccination improved 5-year PFS from 67.8 to 77.4% ($p = 0.02$); however, benefit was limited to T3 tumors, OS was not reported and 174 patients were not treated because they did not fulfil postoperative eligibility criteria such as histologically confirmed RCC, correct staging or inability to produce a vaccine. A second more recent Phase III trial compared adjuvant vaccination with an autologous tumor-derived heat shock protein (glycoprotein-96)-peptide complex (HSPPC-96, vitespen, Oncophage® Agenus, Lexington, MA, USA) to observation in over 400 patients with high risk resected RCC. There was no significant difference in recurrence-free survival [68]. Most trials of vaccine therapy in metastatic RCC have failed to show a benefit and a recent large Phase III trial of the cancer-associated 5T4 antigen vaccine versus placebo in combination with sunitinib, IL-2 or IFN- α was negative [69]. Practical issues may limit the applicability of autologous vaccines. The feasibility

Table 3. Prognostic model for IL-2 response.

Histological features	CAIX	
	Low	High
>50% alveolar No papillary or granular features	Good risk	Good risk
<50% alveolar <50% granular No papillary	Poor risk	Good risk
Papillary features or >50% granular	Poor risk	Poor risk

This model proposed by Atkins et al. [52] incorporating histological features and CAIX expression levels was prospectively tested in SELECT and failed to predict benefit from high-dose IL-2 [53]. CAIX: Carbonic anhydrase IX.

of autologous RCC tissue collection and vaccine preparation has been shown to be limited by low tissue procurement (samples obtained for <40% of patients) and low rates of viable tumor cells in the specimen (<50%) [70].

As a result of these and other practical considerations, as well as the lack of data supporting this approach, vaccines have not been widely accepted as standard treatment for RCC. However, important therapeutic advances using vaccines in other tumor types have fuelled a renewed interest in vaccine-based immunotherapy for RCC. The first therapeutic vaccine was approved by the FDA in April 2010 for the treatment of metastatic prostate cancer. Sipuleucel-T is a patient-specific vaccine generated through the *ex vivo* stimulation of the patient's own APCs, including dendritic cells, by exposure to a prostate-specific antigen (prostatic acid phosphatase, PAP) fused to GMCSF. The treatment was well tolerated and significantly improved OS for men with metastatic prostate cancer in a Phase III trial [71]. Similar combinations of vaccines with immune stimulation (using GMCSF) or with some of the new modulators of T-cell function discussed below may allow the effectiveness of vaccine therapy in RCC to be optimized. Future clinical trials should explore these approaches and involve both careful patient selection and an attempt to define biomarkers that predict benefit. This is notoriously difficult in trials of vaccine therapy; sipuleucel-T is not associated with any improvement in response rate or PFS and no candidate predictive markers have been identified to date. However, in an increasingly crowded therapeutic field the ability to select the most appropriate patients for such therapy is of increasing importance.

Novel immunomodulators

The identification of positive and negative regulators of T-cell activation has generated research efforts aimed at expanding the number and function of tumor-specific CTLs or depleting circulating Tregs. Therapeutic immunomodulatory strategies involving either stimulation of innate immunity or the inhibition of negative regulators of host immunity are under investigation and are illustrated in **FIGURE 1**.

For example, T-cell function can be activated using agonist antibodies against CD-137 or subcutaneous IL-21 [72,73] and dendritic cells can be stimulated using Toll-like receptor agonists [74]. CD137 is a T-cell costimulatory receptor that, upon ligand binding, results in increased T-cell proliferation, maturation and survival. Agonist antibodies to this receptor

provoke marked tumor-specific T-cell responses capable of eradicating tumor cells in preclinical models [75]. Unfortunately, preliminary results from a Phase I trial failed to demonstrate objective responses among 22 patients treated with a CD137 antibody [72].

IL-21 is a cytokine that stimulates the effector functions of CTLs and NK cells. Although structurally similar to IL-2, IL-21 does not increase circulating Tregs and renders CD4⁺ T cells resistant to regulatory cell suppression [76]. Human recombinant IL-21 has been evaluated in early trials and shown encouraging activity in metastatic RCC, including objective responses among IL-2 treated patients [77].

Another immunomodulating strategy involves reversing tumor-mediated immunosuppression using antibodies against TGF- β [78] or Treg depletion. CD25⁺ Tregs can be targeted by antibodies against CD25 with resulting RCC tumor eradication in mice models [79]. The recombinant anti-CD25-diphtheria conjugate (Ontak[®]) is approved for use in T-cell lymphoma and has been shown to reduce circulating Tregs in patients with metastatic RCC and enhance subsequent vaccine-mediated anti-tumor immunity [80].

At present, one of the most promising approaches involves T-cell activation using immune checkpoint inhibitors. The CTL-associated antigen-4 (CTLA-4) and programmed death (PD)-1 pathways are two critical immune checkpoints; these negative regulators of T-cell function are involved in immunotolerance and have therefore been identified as important anti-tumor immunologic targets.

Checkpoint inhibitors

■ Cytotoxic T-lymphocyte-associated antigen antibodies

Antigenic T-cell stimulation is generated by interactions with major histocompatibility complexes and by binding of the CTL receptor, CD28 to ligands on the APC. CTLA-4 is an inducible receptor that attenuates CTL activation by competing with CD28 for APC ligand binding, thus resulting in suppressed CTL immune responses and APC function [81]. Antibodies against CTLA-4 promote CD28-APC interactions and sustained CTL activation. Two fully human monoclonal antibodies against CTLA-4 have been developed, ipilimumab and tremelimumab. Ipilimumab was first in its class [82]. As discussed below, the mode of action, response pattern and toxicity profile of these novel immunomodulating agents differ from conventional chemotherapy as well as IL-2 and IFN- α .

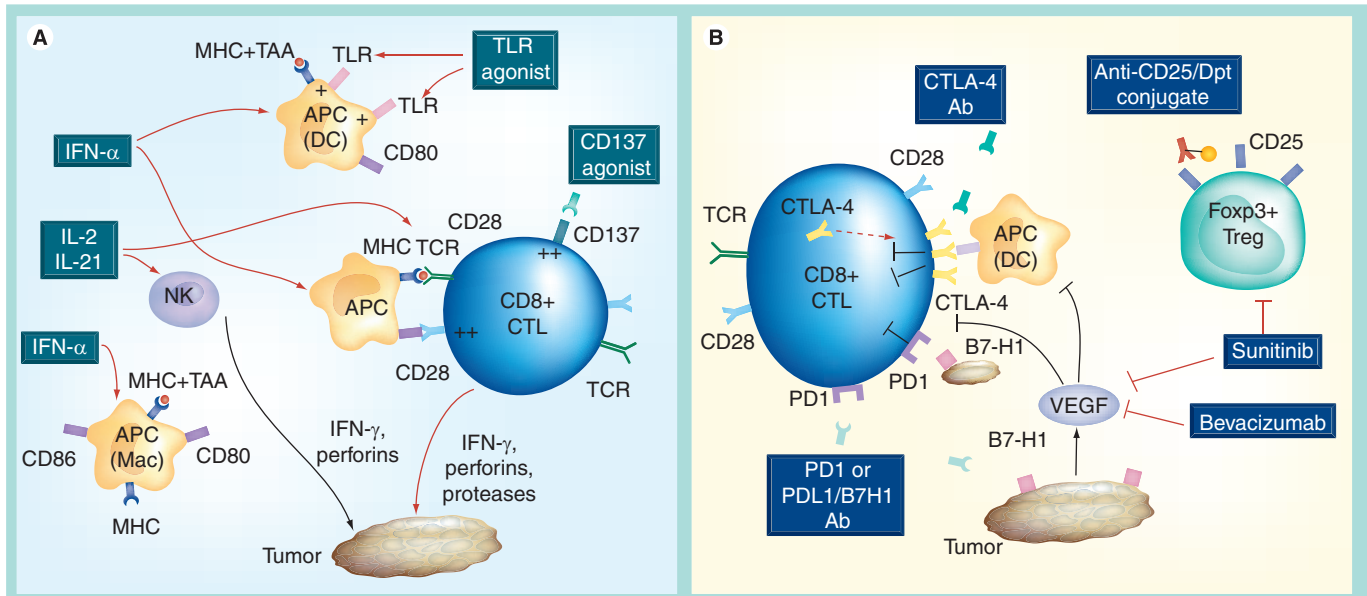


Figure 1. The balance of immune effector/suppressor signaling can be altered by immunomodulatory therapeutic strategies to promote a host anti-tumor immune response. (A) Therapeutic strategies to enhance immune effector function. The major immune effector is the CD8⁺ CTL, which is equipped to recognize and induce apoptosis of target antigenic tumor cells. These innate effector cells are stimulated by antigen-presenting cells such as DCs and macrophages that process and present tumor antigens, CTL activation is then specifically triggered by binding of T-cell receptors to antigen-presenting cells via major histocompatibility complexes I and II, and by interaction between the main CTL stimulatory molecular, CD28, with antigen-presenting cell ligands (CD86 and CD80). This generates a signaling cascade resulting in increased CTL numbers and release of cytokines (IFN- γ) and toxins such as perforins, promoting an antigen-specific CTL response. Other immune potentiating molecules include the costimulatory receptor CD137 on CTLs, or TLRs expressed on DCs. Therapeutic strategies to augment CTL effector functions include IL-2, IFN- α , IL-21, CD137 agonists or TLR agonists. **(B)** Strategies to block immune suppression. A number of immune factors and cells exert a reciprocal negative regulatory effect on innate immunity. CTL activation can increase membrane translocation of the cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), which competes with CD28 for APC ligand binding, thus downregulating CTL effector immune response. Similarly, PD-1 is expressed on CTLs, and binding to its ligands, such as B7H1 expressed on tumor cells, results in CTL apoptosis [97]. Inhibitors of these two CTL checkpoints have been developed: antibodies against CTLA-4, PD-1 or B7H1. VEGF can be secreted by tumor cells or surrounding stroma and has been shown to suppress DC and CTL activity [98]. Tregs are the main immune suppressor cell. These CD25⁺/forkhead transcription factor, Foxp3⁺ cells suppress CD4⁺ and CD8⁺ T cells. The tyrosine kinase inhibitor, sunitinib, has been shown to suppress Treg maturation and may also exert negative pressure on innate immunity by reversing VEGF-mediated inhibition of CTLs and DCs [10,60,63]. Other Treg depletion strategies include the recombinant anti-CD25-diphtheria conjugate (Ontak[®]) [80]. CTL: Cytotoxic T lymphocyte; DC: Dendritic cell; TLR: Toll-like receptor; Treg: T regulatory cell.

Ipilimumab's most encouraging activity to date has been reported in melanoma, another immunoresponsive tumor. Objective response rates to the CTLA-4 antibody in a large Phase III trial in 676 patients with previously treated melanoma were modest (<11%) but associated with a significant improvement in survival (median OS = 10.1 vs 6.4 months for ipilimumab vs placebo, $p = 0.001$) [83]. Among tumors that did regress with ipilimumab, sustained response for over 2 years as observed. In addition, patients who benefited from treatment included patients exhibiting delayed responses as well as durable stable disease after initial progression. Immune-related response criteria have thus been proposed instead of conventional response assessments in order to take into account the heterogeneous kinetics of response and avoid premature treatment discontinuation [84]. A Phase II trial in metastatic RCC showed a response in 5 of

40 patients treated at the higher dose (3 mg/kg). A third of patients experienced grade III or IV immune-related adverse events (IRAEs) such as enteritis and hypophysitis and the presence of immune toxicity was strongly associated with response (RR = 30% with IRAE vs RR = 0% without IRAE) [85]. Other IRAEs associated with ipilimumab included hepatitis, rash, iritis, vitiligo or nephritis and most were reversible with steroid treatment. Despite encouraging activity, there are currently no ongoing trials of anti-CTLA-4 antibodies in RCC which may be attributable to the significant grade III and IV IRAEs reported in the Phase II trial.

■ Programmed death 1 antibodies

The PD-1 protein is expressed on activated T-cells. Upon binding to its ligand, B7H1 within the tumor, PD-1 can downregulate T-cell function [86]. As previously mentioned, B7H1

expression has been described in nephrectomy specimens from patients with RCC and shown to be associated with a poor prognosis [8,87]. A number of antibodies are in development against PD-1 or its ligand, programmed death 1 ligand (PD-1L/B7H1). The initial Phase I trial of an anti-PD-1 antibody (MDX 1106) demonstrated a favorable toxicity profile and a durable objective partial response in a patient with heavily pretreated metastatic RCC [88]. The pretreatment tumor specimen from this patient demonstrated substantial B7-H1 expression. A follow-up Phase I trial recently reported a response rate of 31% (including one CR) among 16 patients with clear cell metastatic RCC [89]. At preliminary analysis, all responses were ongoing including one at 17 months. In line with the ipilimumab data, response patterns were variable with some patients demonstrating initial tumor growth with subsequent regression. Antibodies against both PD-1 and its ligand PD-1L/B7H1 are in early trials in solid tumors including RCC.

Challenges of novel immune modulators: response assessment & predictors of benefit

■ Immune-related response criteria

Immune-related response criteria (irRC) have been formulated to capture all of the response patterns associated with benefit from new antibodies such as ipilimumab [90]. These guidelines are based on the observation that four distinct types of response were observed in clinical trials of anti-CTLA-4 antibodies among patients who ultimately went on to achieve durable stable disease or tumor shrinkage:

- Shrinkage of existing disease with no new lesions;
- Durable stable disease;
- Response after initial progression;
- Response in target lesions but appearance of new sites.

The latter two response patterns would have been labeled as progressive disease using conventional RECIST criteria. The irRC differ from RECIST in the following way: the first assessment occurs later (at least 12 weeks into treatment) and progressive disease is defined as more than 25% increase in the sum of all lesions (including new lesions) maintained for at least 4 weeks [90]. The rationale for irRC is that immunomodulating agents exert an indirect anti-tumor effect dependent on activation of the host immune system, making them ill-suited to

RECIST criteria designed to detect the immediate cytotoxic response expected from conventional chemotherapy. In line with the unconventional responses observed with ipilimumab, similar observations were made with the sipuleucel-T vaccine in prostate cancer; despite an increase in OS, PFS was not improved by the vaccine [71]. Immune-based treatment may result in delayed shrinkage, an effect that will be missed by early response assessments and may not even take hold until after treatment completion, but persist longer. Alternatively, immune therapies may not decrease objective tumor size, but significantly alter tumor growth rate. Regardless, this could translate into a meaningful increase in survival.

■ Predictors of response

As with high-dose IL-2, it is likely that the benefit of these new immunotherapies may be limited to a minority of patients; in order to take these new therapies forward it is imperative that predictive markers be identified early on in drug development. The lack of patient selection for future Phase II and III trials of immunomodulators may dilute any real effect in a subset of patients and risk falsely negative trials. Unfortunately, the identification of reliable predictors has eluded us so far. A correlation between treatment-induced host autoimmunity and tumor response to treatment has been suggested. Data regarding an association between immune toxicities such as vitiligo or hypothyroidism and response to IL-2 is conflicting [91,92] raising the possibility that the higher rate of toxicities in responders simply reflected more prolonged administration of IL-2. In the case of ipilimumab there is compelling evidence that on treatment IRAEs predict for response. In one early trial in melanoma, the response rate was significantly higher among patients experiencing IRAEs (36 vs 5%, $p = 0.008$) [93]. A similar relationship between immune toxicity and relapse-free survival has been reported in several other studies [94,95]. Increased circulating levels of inducible CD4⁺ T helper-17 cells from baseline to 6 months or increased intratumoral CD8⁺ CTL infiltrates have also been correlated with disease-free survival and response, respectively [94,96]. The occurrence of IRAEs and immune changes in peripheral blood or tumor with treatment may prove to be surrogate markers of response; however, baseline pretreatment predictors would be more useful. Early studies suggested that single nucleotide CTLA-4 polymorphisms associated with low T-cell CTLA-4 expression were associated with enhanced immune response to ipilimumab and improved prognosis [95].

The identification of pretreatment predictors appears more promising for anti-PD-1 treatments. The presence of PD-1 ligand, B1H7 may identify patients with a poor prognosis who may be more likely to respond to PD-1 inhibition. The predictive value of B7H1 positivity in RCC pathological specimens was suggested by early trials of MDX 1106. In addition, soluble B7H1 has been recently detected in the serum of patients with clear cell RCC and its levels shown to correlate with increased risk of cancer related death [9]. Whether this may provide a valuable and more readily accessible serum biomarker of response to PD-1 or PD-1L inhibition is unknown.

Conclusion & future perspective

New molecularly targeted therapies against VEGF and mTOR have markedly altered the landscape of systemic therapy for metastatic RCC. These tyrosine kinase inhibitors result in significant clinical benefit in the majority of patients, but are associated with side effects; they are given with palliative intent and require continuous treatment until progression. The need for curative therapies remains; high-dose IL-2 is still the only systemic therapy shown to induce durable complete responses or cure in a minority of patients with metastatic RCC. The advent of angiogenesis and signal transduction inhibitors has increased

the number of therapies in the armamentarium against RCC, but there may still be an important role for immunotherapy. There are promising new immunomodulating agents that block immune checkpoints, such as the antibodies against CTLA-4 and PD-1. These drugs depend on promoting host immunity; as monotherapy they may prove to have a greater role in the adjuvant setting in patients with minimal residual disease and less developed immune tolerance. In addition, combining or sequencing immunotherapies with molecularly targeted therapies to maximize host immune response and overall clinical benefit should be investigated. Clinical trials investigating these approaches must include parallel biological studies that aim to identify immunologic predictive markers and, particularly, to identify the subset of patients who should be targeted with immunotherapy with potentially curative intent.

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Executive summary

- Renal cell carcinoma (RCC) is a chemoresistant malignancy and for many years the only treatment for advanced disease was cytokine treatment with IFN- α or IL-2.
- IFN- α and IL-2 were widely used but offered limited objective response rates of 10–20% and only modest survival benefits in the majority of cases.
- The last decade has witnessed a marked increase in the availability of novel targeted therapies against VEGF or mTOR demonstrating anti-tumor activity and more importantly providing a meaningful impact on overall survival.
- However, to date, high-dose IL-2, remains the only treatment modality able to induce durable complete remissions and/or cure in metastatic RCC in a small minority of patients (7–10%).
- Unfortunately, reliable biomarkers to select the subset of patients for this toxic, but potentially curative treatment are lacking; the use of high dose IL-2 is therefore limited to young, fit patients treated in a small number of specialized centers.
- A number of approaches are therefore being investigated to augment the effectiveness of immunomodulation and enable a greater proportion of patients with advanced RCC to benefit from this approach.
- VEGF receptor-targeted therapies may stimulate host immune response and justify combinations with cytokines or newer immunomodulating strategies.
- Immune checkpoint inhibitors such as cytotoxic T-lymphocyte-associated antigen-4 and programmed death-1 antibodies are showing encouraging activity in early clinical trials and vaccines are of renewed interest.
- Taking these and other novel immunomodulatory agents forward will require the use of immune related response criteria in clinical trial design and, most importantly, the ability to identify reliable predictors of benefit so that appropriate patients may be selected for immunotherapeutic treatments.

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