Trial Watch: Therapeutic vaccines in metastatic renal cell carcinoma

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Introduction

Kidney cancer accounts for 2.5% of all cancers, with an annual global incidence of 273,000 cases leading to 111,000 deaths. The major histologic subtype (80% of cases) is clear cell renal cell carcinoma (ccRCC) followed by papillary subtype 1 and 2, Bellini tumor and chromophobe carcinoma. ccRCC is a chemoresistant tumor probably because the cells derive from the luminal cells of proximal tubules that express a high intrinsic level of multidrug resistance (MDR-1) protein. About 30% of ccRCCs are diagnosed at the metastatic stage (mRCC).1

Despite the renaissance of cancer immunotherapy, no novel immunotherapy has been approved for the treatment of renal cell cancer (RCC) since the availability of recombinant cytokines (interleukin-2, interferon-α). All vaccine trials have failed to meet their endpoints although they have highlighted potential predictive biomarkers (e.g., pre-existing immune response, hematological parameters, tumor burden). Recent advances in immunomodulatory therapies have prompted the study of combination treatments targeting the tumor immunosuppressive microenvironment consisting of regulatory T-cells (Treg), myeloid suppressor cells, and cytokines. Approaches under investigation are use of inhibitors to curb the overexpression of immune checkpoint ligands by tumor cells (e.g., anti-CTLA-4, anti-PD-1/PD-L1) and exploiting the immunomodulatory effects of anti-angiogenic agents that are the current standard of metastatic RCC care. Phase III trials are focusing on the possible synergy between therapeutic vaccines (e.g., IMA-901 and AGS-003) and anti-angiogenic agents.

Immunogenicity of ccRCC

The immune system has a dual role in cancer development. On the one hand, it can identify and control nascent tumor cells, thereby exerting immunosurveillance.16 On the other, it can...
promote tumor progression through chronic inflammation, immunoselection of poorly immunogenic variants, and suppression of antitumor immunity.\textsuperscript{17} The balance between activation and inhibition is maintained by immuno-editing. Evidence for immunosurveillance, which relies on T-cell response to tumor-associated antigens (TAA),\textsuperscript{16} comes from rare cases of spontaneous complete responses observed in the placebo arm of a phase III trial.\textsuperscript{18} The blood and tumor microenvironment of these cases harbored TAA-specific T-cells; the tumors were highly infiltrated by TAA-specific effector memory CD8 T-cells and CD4 T-cells.

Four classes of TAAs have been identified in RCC

Reactivated embryonic antigens (aka cancer testis antigens), such as MAGE, RAGE, PRAME, SART1, and NY-ESO1. Their expression in RCC is relatively low compared to that in melanoma or squamous cell lung carcinoma. The expression pattern in subtype ccBA of RCC (PRAME, SPANXC, C21orf99, Ssx1) confers a worse prognosis than that (FATE1) of subtype ccAB.\textsuperscript{19} Mutated antigens arising from mutated Von Hippel Landau (VHL) gene (present in >60% of sporadic RCCs)\textsuperscript{20} or mutated p53 gene.

Tissue-specific antigens (e.g., kidney injury molecule (Kim-1), Pax 2) that are expressed in both normal and malignant renal tissue.

Other antigens overexpressed in many tumors (e.g. carbonic anhydrase IX (CAIX), MUC1 (CA15-3), HER2, oncofetal antigen 5T4, peripilin 2, cyclin D1, c-Met and MMP-7).\textsuperscript{21} A genomic approach to TAA identification should hasten steps in the development of cancer immunotherapies, i.e., in the evaluation of the advancement of immune-editing, selection of highly immunogenic tumors for personalized therapy, and identification of TAAs that drive additive oncogenic pathways needed for cancer cell survival.\textsuperscript{16}

TAAs as targets for vaccines

Four criteria have to be met before use of a TAA as a potential target for a vaccine.

Lack of pre-existing immunotolerance. For a vaccine to be effective requires a functional immune system. Vaccines directed against TAAs that might be self-antigens (e.g., HER2) would constitute a means of overcoming immunologic tolerance to self-proteins. However, as cancers are genetically unstable, it might be easier to target TAAs that are neo-antigens through mutation and thus not recognized as “self” by the immune system.

Differential TAA expression between tumor and normal tissue. Immunotherapy toxicity and the spontaneous auto-immune paraneoplastic syndrome are thought to arise from TAA expression in normal tissue. For instance, the neurological toxicity of anti-MAGE-A3 TCR gene therapy (also recognizing MAGE-A9 and A12 epitopes) might be related to unreported MAGE-A12 expression in brain.\textsuperscript{22} The uncertainty over antigen expression in normal tissue implies a need for systematic monitoring of potential toxicity.

Immunogenicity. TAA induction of specific memory CD8\textsuperscript{+} T-cell expansion can be assessed by detecting TAA-specific T-cells using ELISPOT or FluoroSpot.\textsuperscript{23}

TAA role in tumorigenesis and tumor cell survival. Because tumors display genomic heterogeneity and constitutive instability, the TAA should preferably be part of an oncogenic additive pathway in order to avoid selecting a poorly immunogenic variant for the vaccine-induced immune response. In a phase I study of a vaccine based on a VHL-mutated antigen, T-cells from 4 out of 5 evaluable patients were reactive against mutated peptides using IFNγ-ELISPOTs.\textsuperscript{24} Unfortunately, the phase II study (NCT00001703) was inconclusive because of poor accrual.

Immune escape mechanisms in ccRCC

The immune cellular response to RCCs is not efficient. Analysis of CD8\textsuperscript{+} T-cell clonality has revealed a lower expansion rate in ccRCC than in other solid tumors.\textsuperscript{25} Only 20% of CD8\textsuperscript{+} tumor infiltrating lymphocytes (TIL) recognize autologous tumor cells,\textsuperscript{26} suggesting default in tumor recognition or resistance to TIL cytotoxicity in RCCs. Unlike in other solid tumors, CD8\textsuperscript{+} TIL in RCC are associated with a poor clinical prognosis.\textsuperscript{27–28} This highlights the probable importance of immuno-editing in RCC oncogenesis.

Modified expression of several proteins in TILs may contribute to tumor immune escape: (i) decreased expression of CD3 zeta chain (which plays a key role in T-cell receptor signal transduction) is associated with a poor prognosis;\textsuperscript{29} (ii) upregulated expression of the inhibitory programmed cell death protein PD-1 in as yet unidentified cells is also associated with a poor prognosis;\textsuperscript{30} (iii) 20–40% of TILs express killer immunoglobulin-like inhibitory receptors (KIR2DL NK-receptors (CD58 a/b) of human leukocyte antigen C (HLA-C).\textsuperscript{31} KIR can interact with major histocompatibility complex (MHC) Class 1 inhibitors before delivering a signal to the cell.

Like other tumors, therefore, RCCs can develop exhaust mechanisms (loss of HLA molecule expression, immunosuppressive signal production). Moreover, some molecules (CD70, HLA-G, PD-L1, B7-H3, B7-H4) are expressed at higher frequencies in RCC, thereby further affecting the phenotype of CD8\textsuperscript{+} TIL in RCC compared to other tumors.\textsuperscript{32–34}

State of the art of cancer immunotherapy in RCC

Currently, recombinant cytokines are the only approved immunotherapies for RCC. The potential of other types of cancer immunotherapy is, however, evidenced by the effectiveness of the cancer vaccine sipuleucel-T in prostate cancer.\textsuperscript{35} Nevertheless, so far, no vaccine has shown proven efficacy in RCC although promising trials are ongoing (see below).

Tumor progression is partly due to escape from immunosurveillance, and targeting immunosuppressive mechanisms is a new thrust in cancer therapy.\textsuperscript{36,37} Immune checkpoint inhibitors, in particular anti-PD-1/PD-L1 antibodies, have been shown to provide impressive tumor responses in metastatic cancers of different histologies (e.g., ipilimumab in metastatic melanoma).\textsuperscript{38} PD-L1 is a cell surface glycoprotein belonging to the B7 family of T-cell costimulatory molecule and is overexpressed in many human tumors.\textsuperscript{39} Activation of the PD-1/PD-L1 pathway induces T-cell anergy. PD-L1 downregulates immune responses by inhibiting both activated and memory T-cells. In a study of 306 patients...
with localized RCC (median follow-up, 11 y), PD-L1 expression correlated with increased risk of disease progression, cancer-specific death, and overall mortality.\textsuperscript{40} Five-year cancer-specific survival rates were higher in patients with PD-L1 negative than positive RCCs (83\% vs. 42\%).

Early trials of anti-PD1 and anti-PD-L1 antibodies in mRCC patients have reported a 30\% ORR and 20–25\% prolonged response rate.\textsuperscript{41–42} The anti-PD1 antibody pidilizumab (CT011) has a particularly good safety profile and lasting clinical activity.\textsuperscript{63} Two ongoing phase III trials in mRCC patients are comparing first-line sunitinib to the anti-PD1 antibody nivolumab either alone (NCT01668784) or combined with ipilimumab. ORRs in early phase trials are low and highlight the need for a standard method of determining PD-L1 expression to improve prediction.\textsuperscript{44}

Another promising new approach is adoptive cell therapy using engineered T-cell Chimeric Antigen Receptor (CAR).\textsuperscript{45}

\textbf{Clinical trial results for therapeutic RCC vaccines}

We reviewed 6 clinical trials of RCC-specific therapeutic vaccines administered in an adjuvant or a metastatic setting: three completed phase III trials (Reniale,\textsuperscript{46–47} TroVax,\textsuperscript{48–50} and Vitespen\textsuperscript{51}) and three phase II trials (TG4010,\textsuperscript{52} AGS003,\textsuperscript{53–54} and IMA901\textsuperscript{55}), as well as a meta-analysis of dendritic cell (DC)-based vaccines in mRCC\textsuperscript{55} (Table 1). The publications were retrieved from a Medline search (from January 2004 to November 2014).

Reniale\textsuperscript{5} is an autologous RCC-tumor lysate cell-based vaccine. It was administered post nephrectomy in an adjuvant setting in a phase III trial that had tumor progression risk (defined as progression or death) as primary endpoint. The goal of a significant reduction in risk was not met. However, the subgroup of patients with pT3 tumors showed significantly improved progression-free survival (PFS) compared to placebo (5-year PFS: 71.2\% vs 65.4\%, \(p = 0.02\); 10-year PFS: 53.6\% vs 36.2\%, \(p = 0.022\)) as did patients with Fuhrman grade 3 tumors (5-year PFS: 71.9\% vs 60.3\%, \(p = 0.008\)). There was no significant improvement in patients with pT2 or grade 2 tumors. Strong criticism relating to methodological bias (choice of primary endpoint, between-arm imbalance) meant that drug approval was not granted despite the promising results.\textsuperscript{46–47}

TroVax\textsuperscript{6} (MVA-5T4) is a therapeutic vaccine targeting a heavily glycosylated 5T4 antigen chiefly expressed in human placental trophoblasts but also expressed in various human cancer cells. This TAA is overexpressed in most RCCs.\textsuperscript{56} The TRIST phase III trial of MVA-5T4 in combination with IFN\(\alpha\), IL2 or sunitinib as first-line mRCC therapy did not meet its objective of a significant increase in OS.\textsuperscript{48} However, patients with a vaccine-induced antigen-specific immune response did show higher OS, and those with a good MSKCC prognostic score receiving MVA-5T4/IL2 had significantly better OS than patients on placebo/IL2 (Hazard Ratio (HR): 0.54 [0.30–0.98], \(p = 0.046\)). As MVA-5T4 targets a single TAA, each patient has to be evaluated for tumor TAA expression and for any preexisting specific immune response.

Vitespen (formerly Oncophage\textsuperscript{8}) consists of a heat-shock protein (HSP)–glycoprotein 96 peptide complex derived from autologous tumor. Because HSPs bind strongly to peptides, they provide a tumor antigenic fingerprint. Vitespen showed a specific CD8\(^+\)T-cell immune response and NK-cell expansion in 50\% of patients in a preliminary study.\textsuperscript{57} However, in the phase III trial, it did not meet its primary endpoint of a significant increase in relapse-free survival (37.7\% vs. 39.8\% in the observation arm (\(p = 0.5\)), median follow-up= 1.9 y).\textsuperscript{51} In a planned subgroup analysis (stage I or II disease), a non-significant reduction in disease recurrence was observed (Hazard Ratio (HR): 0.576 [0.324–1.023], \(p = 0.056\)). Vitespen has been approved in Russia for intermediate-risk patients in an adjuvant setting.

TG4010 is a modified vaccinia virus expressing MUC1. MUC1 is overexpressed in RCC and is associated with a poor prognosis.\textsuperscript{58} No objective clinical response was observed in the phase II study evaluating TG4010 efficacy and tolerability, alone or in combination with cytokines, as first-line mRCC therapy. Stable disease for \(\geq 6\) months was reported in 5/27 of evaluable patients (18\%) with TG4010 alone and 6/20 of patients (30\%) with TG4010 plus cytokines. MUC1-specific CD8\(^+\) T-cell responses were associated with longer OS.\textsuperscript{50}

AGS-003 is a DC-based vaccine in which mature DCs are electroporated with amplified total tumor mRNA and CD40-ligand (CD40L). CD40L expression on the DC surface is thought to induce a costimulation signal, counteract T-cell anergy induced by inhibitory receptors like PD-1, and reestablish a balance in favor of T-cell activation. AGS-003 was tested in combination with sunitinib in a phase II study in poor or intermediate-risk mRCC patients eligible for nephrectomy. Median PFS was 11.2 month; median OS was 30.2 months. No additive toxicity was reported other than grade 1 local reactions.\textsuperscript{53} Peripheral blood mononuclear cells (PBMCs) showed decreased Treg levels and increased levels of CD28\(^+\) memory cytotoxic T-cells (CTLs) that were positively correlated with improved PFS.\textsuperscript{59} A phase III trial is ongoing.

IMA-901\textsuperscript{5} is a 10 tumor-associated peptide (TUMAP) pool combined with granulocyte macrophage colony-stimulating factor (GM-CSF) injected after a single low dose of cyclophosphamide. The phase I and II trials had two objectives.\textsuperscript{21} The first was to identify strong immunogenic TUMAPs (using the XPRESSIDENT platform to screen for overexpressed genes corresponding to HLA ligands) and specific anti-TUMAPS T-cells in blood. Nine HLA-A*02-restricted and one HLA-DR-restricted TUMAPs were selected (see footnote to Table 1). The studies showed that multi-anti-TUMAP T-cell responses were associated with better disease control and that lower pre-vaccine infiltration of Tregs was correlated with a better T-cell response. On the other hand, preexisting type 2 (CD15+IL-4Ra+) and type 4 (CD14+HLA-DR-/Lo) myeloid derived suppressor cells (MDSC) seemed to worsen prognosis. The second objective was to identify an agent (single low cyclophosphamide dose) capable of depleting Tregs. A vaccine vector was not used for reasons of cost, safety, and simplicity of clinical use. An efficient immune response was achieved, with disease control rates of 31\% and
| Vaccine developer | Phase | Patients (N) | TAA or vaccine type | Vector | Combined with | Notes | Reference |
|-------------------|-------|--------------|---------------------|--------|---------------|-------|-----------|
| Reniale LipoNova, Hannover, Germany | III | 379* RCC | Tumor lysate | None | 0 | Endpoint: Tumor progression risk (NS) Subgroups: pT3 and G3 better 5 and 10-y PFS Strong methodological bias | [46–47] |
| TroVax Oxford Biomedica, Oxford, UK | III | 733 mRCC | ST4 | Modified Ankara virus | IFNα, IL2, or sunitinib | Endpoint: OS (NS) Subgroup Immune responders with normal hemogram: higher OS | NCT00397345 [48–50] |
| Vitespen (formerly Oncophage) Agenus (formerly Antigenics), Lexington, USA | II III | 818 RCC | gp96 HSP-peptide complex (HSPPC-96) | None | 0 | Endpoint: RFS (NS) Subgroup Stage I & II: NS decrease in recurrence Marketed in Russia | NCT01147536 NCT00126178 NCT00033904 [51] |
| TG4010 Transgene, Illkirch-Graffenstaden, France | II | 37 mRCC | MUC1 | None | ± cyto-kines | Endpoint: Clinical response (NS) | [52] |
| AGS-003 Argos Therapeutics Durham, NC, USA | II | 21 mRCC | Tumor mRNA (+CD40L) | DC | Sunitinib | Good safety | NCT00678119 [53-54] |
| IMA-901 Immatics Biotechnologies, Tübingen, Germany | II | 30+68 mRCC | 10 peptides* | Adjuvant: GM-CSF | 0 | Subgroup benefit: Immune response (multi-TUMAP responder) | NCT00523159 [21] |
| DC-vaccines | Meta-analysis (n = 12) | 148 mRCC | DC | DC | 0 | ORR 12.7%, CBR 48% Subgroup benefit: Immune response and High dose DC | (55) |

* 379 eligible patients for assessment (intention to treat) on 558 enrolled.

Abbreviations: CBR, clinical benefit rate; DC, dendritic cells; GM-CSF, granulocyte macrophage colony-stimulating factor; HSP, heat-shock protein; NS, not significant; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; RCC, renal cell carcinoma; TAA, tumor associated antigen; TUMAP, tumor associated peptide

*These are APO-001, PLIN2 and APOL1 (overexpressed in lipid droplets in RCC); 91 CCND1 (modified cell cycle regulator showing aberrations in RCC); 92 GUCY1A3 (involved in cGMP synthesis and angiogenic in RCC); 93 PRUNE2 (overexpressed in RCC); 94 MET (hepatocyte growth factor receptor); 95 RGS (cell cycle regulator involved in angiogenesis); 96 MUC1 (cell surface glycoprotein with modified glycosylation pattern in tumors leading to potential antigens (e.g., CA15-3); MMP7 (involved in invasion and metastasis). 97
14\% after cytokines or anti-angiogenic agents TKIs, respectively. A Phase III trial is ongoing.

DCs-vaccines

DCs are potent antigen-presenting cells. Trials of small mRCC patient numbers have evaluated DC-based vaccines to boost specific antitumor responses but with few significant results. A meta-analysis of 12 trials reported an ORR of 12.7\% and a clinical benefit of 48\%. The trials differed in DC subtype (9 used mature and 3 immature monocyte-derived DCs) and type of antigen (6 tumor lysate, 2 peptide pulsing, 1 either cell lysate or peptide, 2 mRNA coinubation, 1 cell fusion with autologous tumor cells). The cellular immune response and DC dose (mean= 38.7×10^6 cells) were the main predictors of response.55

Ongoing clinical trials of therapeutic RCC vaccines

We selected five ongoing trials: two ongoing phase III trials, one Phase II trial and two Phase I trials (Table 2). The trials were retrieved from a NCT Database search in November 2014 on Clinicaltrial.gov.

IMA-901

In the IMPRINT phase III trial, 340 mRCC patients with HLA-A*02 haplotype were randomized to a combination of first-line sunitinib and IMA-901 (with single dose cyclophosphamide and GM-CSF as adjuvant) or sunitinib alone. Recruitment is closed.

AGS-003

The ADAPT phase III trial is comparing the combination AGS-003 plus sunitinib versus sunitinib alone, in newly diagnosed unfavorable-risk mRCC patients. Recruitment will stop once 450 patients have been included.

DC-RCC is a tumor vaccine in which patient-derived tumor cells are fused with autologous DCs. In a phase I trial of 16 mRCC patients, DC-RCC vaccination post-cytoreductive nephrectomy was associated with antitumor immunity in all 16 patients and good tolerance, with 7 patients achieving a partial response or prolonged stable disease.60 The efficacy of DC vaccination might be enhanced by targeting effector cell inhibition induced by the tumor microenvironment. The anti-PD1 antibody pidilizumab (CT011), which promotes Th1 polarization of vaccine-induced T-cells and decreases Treg number, has shown promising results when combined *ex vivo* with an autologous DC/myeloma fusion vaccine.61 The combination DC-RCC/CT011 is being studied in a phase II trial (NCT01441765) in patients who have undergone nephrectomy or cytoreductive metastasis surgery.

AdGMCAIX is another DC-based vaccine. A fusion gene construct of GM-CSF and CAIX is transduced by a replication deficient adenovirus into autologous DC. CAIX is a hypoxia-induced protein. Its expression in ccRCC is ubiquitous because of the mutational loss of VHL that is variable in ccRCC (> 95\%) but rare in normal cells.62 It is used to activate a specific immune response whereas GM-CSF is used to boost antitumor immunity. AdGMCAIX has shown activity in preventive and therapeutic preclinical models63 and is under study in a phase I trial of mRCC patients (NCT01826877). All six patients included had a good tolerance profile. Four patients were evaluable for response, one had progressive disease, two had stable disease lasting < 6 months, and one still had stable disease at 6 months. Issues pertaining to the conduct of the study have temporarily stopped patient accrual.64

DC-CIK

Autologous DCs pulsed with tumor lysate in order to generate a specific antitumor response have been combined with an infusion of Cytokine Induced Killer (CIK) cells (a heterogeneous

| Vaccine Trial | Indication | Phase | Status | TAA or vaccine type | Combined with | Reference |
|---------------|------------|-------|--------|---------------------|---------------|----------|
| IMA-901 (IMPRINT) | mRCC (HLA-A*02 haplotype patients) | III | Active, completed | 10 peptides* (adjuvant: cyclophosphamide + GM-CSF) | Sunitinib | NCT01265901 |
| AGS-003 (ADAPT) | mRCC | III | Recruiting | DC transfected with CD40L and tumor mRNA | + sunitinib vs. sunitinib alone | NCT01582672 |
| DC-RCC | RCC | II | Recruiting | Tumor cells fused with autologous DC | Anti-PD1 (CT-011) combined with vaccine or alone. | NCT01441765 |
| AdGMCAIX | mRCC | I | Recruiting | DC transduced with GM-CSF+CAIX fusion protein | 0 | NCT01826877 |
| DC-CIK | mRCC | I | Recruiting | DC loaded with tumor lysate + CIK cells | 0 | NCT 00862303 |

Abbreviations: CAIX, carbonic anhydrase IX; CIK, cytokine induced killer; DC, dendritic cells; GM-CSF, granulocyte macrophage colony-stimulating factor; PD1, programmed cell death; RCC, renal cell carcinoma; TAA, tumor associated antigen

*See footnote to Table 1.
population of non-MHC restricted CD3+ CD56+ T-cells) on the premise that the vaccine might enhance CIK cell activity and the CIK cells might enhance the vaccine-induced antitumor immune response. In a phase I trial (NCT00862303) of 28 patients with mRCC, the ORR was 39% and the disease control rate was 75%. No serious adverse event was reported.

Strategies for the development of therapeutic RCC vaccines

No vaccine-based treatment has been approved in RCC therapy because of lack of proven efficacy, but promising trials are ongoing.

Optimal choice of TAA

With the exception of cancer cell-derived vaccines (based on tumor lysate or fusion cells) which embrace all antigens expressed by tumor cells, most therapeutic vaccines target only one or a few TAs. Choosing a single TAA provides a stronger directional immune response but downsides are variable TAA expression on tumor cells and tumor heterogeneity among patients. For instance, the IMA-901 vaccine is exclusively destined for HLA-A*02 patients, i.e., about 50% of Caucasians. It is thus essential to test each tumor for the TAA targeted by the potential vaccine. Choosing a TAA involved in an addictive oncogenesis pathway can help prevent secondary resistance to treatment by down-regulation of antigen expression.

Choice of TAA vector

A potent anti-TAA immune response is elicited by viral delivery systems, such as the modified vaccinia virus Ankara (MVA) or poxvirus, as they abate self-tolerance. However, to avoid an anti-vector immune response that might curb the efficiency of repeat administrations of the same recombinant vaccine, either a strategy using different viral vectors for the prime and boost is used or the peptide of interest is loaded ex vivo directly on DCs (e.g., sipuleucel-T). The latter method has a theoretical benefit even if the exact nature of the cells used as DC precursors (mononuclear?) may not be known. An option to be considered in DC-based therapies is use of non-replicative vectors to avoid the intrinsic immunogenicity of live vectors and to reduce treatment complexity and cost. Non-replicative vectors have been shown to be effective in preclinical models. In practice, TAA-specific immune responses and clinical efficacy should be compared using different vectors but it may also be feasible to do away altogether with vectors for peptide vaccines (see IMA-901). This would lower cost and might even be safer.

Choice of adjuvant to boost immune response

Choice of TAA and vector is followed by the difficult choice of best adjuvant to boost and drive the immune response. The ideal adjuvant should trigger an effective immune response and ensure strong immunogenicity. A variety of agents can be defined as adjuvants (e.g., water-in-oil emulsions, GM-CSF, toll-like receptor (TLR) agonists).

Several water-in-oil emulsions were tested after Freund incomplete adjuvant (FIA) was abandoned. Like FIA, Montanide ISA 51 - an experimental adjuvant for protein and multi-peptide vaccines such as the dual-adjuvant telomerase vaccine GX301 is composed of a light mineral oil and a mannide monooleate emulsifier but the emulsifier is highly refined and the emulsifier-to-oil ratio is lower than in FIA. The resultant emulsion is more consistent and controllable, and is effective and in general well tolerated in humans. The main reported adverse reactions are transient local reactions, including local swelling and pain with or without fever.

GM-CSF is used as a local adjuvant for IMA-901, and is expressed within a fusion protein in the sipuleucel-T and AdGM-CAX-transduced autologous DC-based vaccines. GM-CSF plays a critical role in DC maturation and T-cell proliferation and activation, and increases DC-mediated responses to tumor cells.

When used as an adjuvant, it might, however, increase MDSCs in the tumor micro-environment and in blood. This deleterious effect remains, however, controversial and its clinical relevance is doubtful.

In the absence of a "danger signal", tolerization may be induced instead of a proper antitumor immune response. Danger-associated molecular patterns (DAMPs) can reverse DC inhibition in the tumor microenvironment, increase the efficiency of antigen presentation to cytotoxic T-cells (CTLs), and prevent tolerization to tumor antigens. DAMPs interact with TLRs, and TLR agonists are in increasing use in cancer therapy. The one in most common use is poly-ICLC, a TLR3 agonist, which has shown clinical benefit, either alone or in combination, in a variety of cancers.

Imiquimod, a TLR7 agonist, has been approved for the treatment of superficial basal skin carcinoma and precancerous lesions. Tasquinimod, a TLR4 agonist with anti-angiogenic activity, targets S100A9 protein and affects regulatory myeloid cell function. A phase II trial of tasquinimod in several cancers including mRCC is ongoing (NCT01743469).

Identification of biomarkers

Several potential biomarkers have emerged during trials of vaccines against RCC.

Hematologic impairment (low hemoglobin level, high neutrophil count and low lymphocyte count) predicted a poor response to TroVax.

The neutrophil-lymphocyte ratio (NLR) has also shown prognostic value in mRCC (HR = 1.59 [1.10-2.31], p = 0.014 for NLR >3.3). An Immune Response Surrogate (IRS) predictive score for vaccine efficiency (anti-5T4 antibody, hemoglobin, and hematocrit) has been proposed but needs validation.

Among potential serum biomarkers, serum apolipoprotein A-1 (ApoA1) and chemokine (C-C motif) ligand 17 (CCL17) predicted immune response to TAA and OS in the IMA-901 trial. Both factors were significant positive predictors of immune response (p = 0.016, p = 0.032, respectively) and multipeptide responses (p < 0.0001, p = 0.0028, respectively). High levels of these biomarkers identified patient populations with significantly longer OS (p < 0.007, p < .011, respectively) but only in the cyclophosphamide arm of the trial.

Pretherapy immune response (i.e., amount of TAA-specific CTL detected by ELISPOT) seems to be a good predictor of response to immunotherapy.
Immunosuppressive cells such as Tregs (CD45+CD3+CD4+FoxP3+CD25hiCD127low), MDSC type 4 (CD14+HLA-DR-/lo) or type 5 (CD11b+CD14-CD15+) and Th17 cells (IL-17+CD4+T cells) are elevated in several cancers and may predict metastatic progression.\(^7^6\) TILs should be more informative than PBMCs as they reflect tumor immune response better but caution is needed as technicalities (e.g., isolation methods) might generate artifacts.

In short, there is an urgent need for effective immune-monitoring both pretreatment in order to select patients and specifically counteract the immunosuppressive pathways involved and also during treatment for early prediction of clinical response to therapeutic vaccines.

**Countering the immunosuppressive environment**

After boosting the immune response with adjuvants, it is necessary to loosen the brake on the immunosuppressive environment from Tregs, MDSCs, overexpression of immune checkpoint proteins and other mechanisms.

Several approaches to deplete or block Treg function have been successfully tested in preclinical models and in humans.\(^7^7–^8^0\)

For instance, a low cyclophosphamide dose decreased Tregs in a preclinical model\(^7^7–^7^8\) and in the phase I-II trials of IMA-901 in mRCC.

Anti-angiogenic agents are the current standard of care for mRCC. They inhibit cancer-related immunosuppression thus justifying combination with immunotherapy. Some induce a less immunosuppressive tumor microenvironment by decreasing Tregs and MDSCs.\(^8^1\) However, it is unclear whether they affect VEGF-dependent DC maturation or Treg proliferation.\(^8^2\) In a prospective study of 28 mRCC patients receiving first-line sunitinib, the number of Tregs (defined as CD3+CD4+CD25(hi) Foxp3+) in blood and tumor fell after each sunitinib cycle. OS was significantly longer in patients with a decrease in Treg after two or three cycles of sunitinib (\(p < 0.05\)).\(^8^3\) Treg depletion has also been observed with axitinib.\(^8^3\) In murine models, combining anti-angiogenic agents with immunotherapy enhanced the effectiveness of immunostimulation. Treg number may thus be a predictor of anti-angiogenic response.

There is an inverse association between angiogenesis and PD-L1 expression in primary RCC.\(^8^4\) The mechanism of escape from immunosurveillance might depend on tumor biology. Increased VEGF expression decreases immune infiltration and might thus lessen the adaptive pressure on the tumor which would thus not need to express PD-L1. PD-L1 expression, which often reflects a preexisting immune response, may be an adaptation mechanism to immune selection pressure but, depending upon the tumor, might also be due to early oncogenic events.\(^8^5\)

Vaccination results in upregulation of immune checkpoint receptors (e.g., PD1 receptor and T-cell membrane protein 3 (TIM3) that suppresses Th1 cell activation) and induction of T-cell anergy.\(^8^6\) Studies are being conducted on immune checkpoint inhibitors combined with anti-angiogenic agents or DC-based vaccines in mRCC. Synergy between an anti-PD-L1 antibody and a vaccine against human papillomavirus was shown.\(^8^7\)

**Assessing clinical response to immunotherapy**

Most therapeutic vaccines are considered failures because direct clinical tumor regression, as evaluated by radiological response according to RECIST criteria (Response Evaluation - Criteria in Solid Tumors), is rare. A survival benefit can be observed in the absence of an objective response, as demonstrated by the efficacy of sipuleucel-T in prostate cancer.\(^8^8\) Moreover, the immune checkpoint inhibitor, ipilimumab, showed distinct radiological response patterns in metastatic melanoma (long stabilization, pre-response flare-up, and delayed response). An early response might not always be a good surrogate for survival. New radiological criteria – the immune response related criteria (irRC) – have been proposed for solid tumor response to immunotherapy but have not undergone validation.\(^8^9\) A simple threshold percentage reduction in tumor burden was successfully used as a criterion in a trial of nivolumab plus ipilimumab in malignant melanoma.\(^9^0\) Until the clinical relevance of new end-points is established and a robust surrogate indicator is developed, OS must remain the endpoint in immunotherapy trials.

**Concluding Remarks**

Reactivating the immune response after an escape from immunosurveillance is challenging. A shrewd choice of TAA and vector, as well as the evaluation of pretreatment specific immune response, are necessary to target an immunogenic tumor but are not sufficient to halt escape mechanisms. In short, boosting the immune response with vaccines without counteracting immunosuppressive mechanisms is not enough. Avenues that are being explored are cyclophosphamide-induced Treg depletion and immunomodulation by immune checkpoint inhibitors and anti-angiogenic agents. Therapeutic vaccines combined with immunomodulators that will boost antigen presentation and DC maturation, offset the immunosuppressive micro-environment and orient Th1 polarization of the immune response are being investigated in attempts to achieve an efficient antitumor immune response with significant clinical benefit.

However, an efficient immune response might be hard to achieve in the absence of immune infiltration and in a context of an extrinsic resistance mechanism. The absence of immune infiltration and PD-L1 overexpression, not because of immune pressure but as a result of loss of a tumor suppressor gene (phosphatase and tensin homolog (PTEN)) in RCC, are documented adverse prognostic factors. The impact of the immune micro-environment on immunotherapy outcome should thus be assessed and used to stratify patients in clinical trials. Such an approach could lead to the development of more relevant biomarkers and to the translation of the latest advances in immunotherapy to kidney cancer.

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