Therapeutic vaccines in renal cell carcinoma

Metastatic renal cell carcinoma (mRCC) is a lethal disease. The advent of tyrosine kinase inhibitors (TKIs) has changed the disease process, yet the majority of patients will develop treatment-resistant disease. IL-2 based immunotherapy in mRCC is the only US FDA-approved treatment with curative results. Immunotherapeutic vaccine approaches to mRCC have been under investigation for several decades with mixed results. The recent FDA-approval of the first cellular immunotherapy in prostate cancer (Provenge®) has reinvigorated the search for similar vaccines approaches in mRCC. This review introduces the concepts and different features required for a successful anticancer vaccine approach.

KEYWORDS: dendritic cells ▪ immune therapy ▪ renal cell cancer ▪ T cells ▪ tumor vaccine

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Since the late 19th century, and even before, physicians and scientists have been attempting to utilize the power of the host's immune system to treat cancer [1,2]. Today, that hope still exists and is supported by the small but significant number of patients with metastatic cancer and specifically metastatic renal cell carcinoma (mRCC) that have durable complete remissions to therapy, which is designed to manipulate the immune system.

Recently, the management of mRCC has changed significantly with the arrival of VEGF- and mTOR pathway-targeting medications. However, complete and durable unmaintained remissions are rare with agents that target VEGF or mTOR, which differs significantly from the small percentage of patients reaching complete remissions with high-dose IL-2-based immunotherapy. The concept and subsequent development of therapeutic tumor vaccines for patients with mRCC has been under investigation for decades with mixed results [3–5]. Only recently the level of our understanding of the complexity of immune pathways in cancer patients is providing new strategies to enhance this approach. Failures of immune therapies can be, in part, attributed to lack of target tumor-associated antigens, downregulation of tumor antigen-presenting molecules (HLA-ABC), acquired tumor related immunosuppressive environment, including immune inhibitory cell components such as T-regulatory cells, tumor associated macrophages or myeloid derived suppressor cells. The recent US FDA approval of Provenge® (sipuleucel-T) as the first active cellular immunotherapy in advanced prostate cancer and ipilimumab (Yervoy™), an anti-CTLA antibody, in advanced melanoma patients has lead to a renaissance of immunoptherapy approaches. Here, we provide a summary of vaccine strategies in RCC and will outline considerations that will have to be taken into account when formulating an RCC-directed vaccine.

Physiology

The purpose of a vaccine is to induce immunity to a target antigen. For cancers, the tumor-associated antigen needs to be processed and presented by an antigen-presenting cell (APC) to a T cell via physical contact in the appropriate environment. This antigen presentation requires interaction of the T-cell receptor complex with the antigen-presenting complex and once engaged, additional costimulatory signals are required for T-cell activation. One costimulatory signal occurs through the CD28 complex on the T cell with interaction with B7.1, (CD80) and B7.2 (CD86) on the APC. The third signal is the CD40 and CD40 ligand interaction. Evidence suggests that all of these signals are necessary to induce a successful antitumor immune response. In fact, the absence of all or some of these signals in the context of antigen presentation can lead to immune tolerance to this antigen [6–8].

The most potent APC is a dendritic cell (DC). DCs have a key position because they are able to initiate a Th1 cellular or Th2 antibody immune response [9]. Although DCs initiate immunity, it is the DC induced antigen specific cytotoxic T lymphocyte (CTL) that is the effector cell that destroys tumor and maintains immunologic memory. Under certain circumstances, DCs may

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also function as inhibitors of immune response. Thus, the ultimate goal of an anticancer vaccine is to:

- Induce a high quality $CD8^+$ CTL response;
- Induce a high quality $CD4^+$Th1 response and generate memory T cells;
- Reduce the amount of activated regulatory T (inhibitory) cells;
- Reduce the immunosuppressive tumor microenvironment.

Many primary RCC tumors are large and produce a variety of immune inhibitor molecules. The effect of the primary RCC on subsequent immune responses in the setting of metastatic disease can be inferred from two randomized trials that demonstrated significant clinical benefit for upfront cytoreductive nephrectomy in patients with metastatic RCC subsequently treated with IFN-α [10,11]. Another important question is the timing of adjuvant vaccine treatments. Traditionally, vaccines in RCC have been used only in the setting of metastatic disease. There is little data available on the use of vaccines in the adjuvant setting for patients with primary RCC at high risk for disease recurrence. One such report, from a multicenter German study, employed autologous renal tumors exposed to tocopherol acetate and IFN-γ given intradermally [12]. It indicated a survival benefit in the vaccinated group, though there were some methodological concerns regarding the design and analysis. The use of renal cell cancer vaccines and other immune therapies in the adjuvant setting remains unproven but of great interest.

Antigen

Choosing the appropriate antigen is critical to vaccination approaches (Table 1). We differentiate between mutated, cancer-specific antigens and shared nonmutated antigens. Vaccination with a mutated antigen will require induction of immunity to this new and unique epitope in the setting of acquired immune tolerance. Using a shared antigen will require breaking the innate immune tolerance and inducing autoimmunity towards this self antigen, which can lead to severe treatment side effects.

If an antigen is recognized as a 'target antigen' by an APC, it is generally processed and presented within the context of MHC class II, such as HLA–DR-presenting complex to $CD4^+$ T cells. However, in order to generate an effective cytotoxic T-cell response, the target antigen should be presented to $CD8^+$ T cells as well. This process is called 'cross-presentation' and requires MHC class I (HLA–ABC)-antigen complex. The majority of antigen-specific vaccines have been focused on well-defined, immunogenic peptide sequences that allow generation of antigen-specific T-cell clones and the use of MHC tetramers to facilitate immune monitoring *in vitro*. Each immune-dominant peptide has anchor motifs that specify its binding to an explicit HLA-ABC allele molecule. The use of immunogenic peptide as a source of antigen limits its utility to subjects with certain HLA-ABC alleles. Conversely, a full-length peptide is processed to allow for presentation in the context of an individual's MHC allele.

The number of tumor-associated or -specific antigens in RCC has been limited. While a number of tumor-associated antigens (TAAs) have been described, such as RAGE-1. SART, PRAME and HSP-70, these antigens are expressed rather infrequently and have restricted HLA-haplotypes in the preclinical and clinical arena [13]. The most studied peptide antigen for RCC is carbonic anhydrase IX (CA9). CA9-targeted therapy has shown preclinical activity [8,9]. A recent study demonstrated significant antitumor activity in a mouse model using a construct combining anti-CA9 antibody with TNF-α [14]. A similar mouse model recently combined heat-shock proteins with CA9 and showed promising preclinical results [15]. A Phase I clinical trial using pulsed DC with CA-9 peptide demonstrated no significant clinical or immunologic activity [16]. A direct anti-G250 antibody (Rencarex®) combined with systemic IFN-α-2a showed stabilization of progressive disease. One patient even demonstrated a partial response lasting 17 months [17]. An additional antigen of interest has been MUC-1. This antigen has been employed in a Phase II trial using a vaccinia virus construct in combination with IL-2. Clinical results were limited, but some promising immunologic results, such as MUC-1-specific $CD4^+$ and $CD8^+$ T-cell responses. The benefit of using well-defined tumor-specific antigens is the fact that these can be engineered in a commercial fashion at relatively stable and low costs, while any more 'personalized' vaccine approaches are very labor- and therefore cost-intensive. A subgroup of cancer-specific antigens has been described as cancer/testis antigens and have been utilized for cancer vaccines [18]. A recent report demonstrated that the fusion of the highly immunogenic cancer/testis antigen NY-ESO-1 to the DC surface molecule DCE205 significantly improved the uptake and, in particular, the cross-presentation of antigens

to $CD8^+$ T cells [19]. 5T4 oncofetal antigen has also been used in RCC clinical trials. This antigen is delivered via a modified vaccinia ankara (MVA) virus (TroVax®). Two Phase II Trials used this in combination with high-dose IL-2, and an additional two Phase II trials treated patients with or without IFN-α. While clinical responses were mixed, the vast majority of patients showed impressive induction of antigen-specific T-cell responses [20–23].

Other cancer-related proteins are becoming targets for immune therapies. Survivin is an inhibitor of apoptosis, is expressed in most cancers and associated with chemotherapy resistance. In RCC, it has been correlated with worse prognosis. *In vitro* studies demonstrated that survivin expression protected RCC cell lines from apoptosis and has been associated with higher pathologic and clinical stage [24]. Recent data from Roswell Park Cancer Institute support this antigen as promising immunotherapy [25]. Human telomerase reverse transcriptase has been investigated in a number of immunotherapeutic approaches because of its expression as a tumor antigen in a majority of cancers. A small clinical trial using a human telomerase reverse transcriptase-pulsed DC vaccine in metastatic RCC patients showed very limited clinical results [26].

Methods to increase the expression of tumor antigens are also being explored. IFN-α not only upregulates MHC class I molecules, but has also been shown to increase expression of TAAs. Recent data suggest that the expression of this group of antigens can be epigenetically regulated using hypomethylation agents, such as azacitidine or decitabine [27]. This would have the advantage that the density of target antigens would be increased and may convert a subject with very low antigen expression ('negative') on a tumor to one that has significant expression.

Tumor-derived RNA may also be used as a source of immunogenic protein. Preclinical data demonstrate that DC pulsed with autologous tumor-specific RNA coding for tumor antigen can be highly capable of inducing a tumor-specific T-cell response. The advantage of this approach would be that this tumor-specific RNA can be manufactured in an unlimited fashion. In addition, it may induce an immune response directed at several tumor-specific antigens without the need for identification of such antigens [28].

Vaccination could also be 'personalized' to target autologous tumor target antigens. To accomplish this, autologous tumor tissue may be used either as an antigen source, or for an antigen discovery platform. This approach would have the distinct advantage that a larger number of potentially immunogenic, yet not defined, antigens could be presented to the immune system. It would also facilitate vaccination development for patients with variant histologies.

We used autologous tumor preparations to pulse DC and combined this with IL-2 and IFN-α [29]. Clinical and immunologic results from this Phase II trial were very promising. The clinical response rate reached 50% with some of the complete and durable responses lasting several years [30]. Autologous tumor cell vaccine (Reniale®) improved the 5-year progression-free survival for high-risk nonmetastatic RCC patients at all tumor stages when administered after nephrectomy. The benefit was clearer in the T3 group. A per-protocol analysis revealed a statistically significant progression-free survival and overall survival in favor of the vaccine [31]. A subsequent 10-year follow-up analysis showed sustained survival benefit for the vaccine-treated patients [32]. Nonprotein antigens have had limited investigation thus far.

Glycolipids are key-molecules in the cell-surface. They are not gene products and their biosynthesis is rigorously controlled by enzymatic pathways. In RCC, a level of high expression of one form of glycolipids, gangliosides, has been correlated with a higher incidence of metastases. Gylcolipid molecules can be presented as immunogenic antigens in the context of CD1. The molecules of the CD1 family are related in structure to MHC class I and II proteins. Compared with the enormous, almost unlimited number of antigens presented by MHC molecules, the diversity of lipid molecules presented by CD1 is limited secondary to very limited polymorphism. CD1d-mediated antigen presentation leads to activation of invariant natural killer T cells (NKT). Data suggest that invariant natural killer T-cell stimulation may lead to the induction of a Th1-directed immune response.

α-galactosylceramide (α-GalCer), KRN7000, was the first glycolipid antigen to demonstrate expression on CD1d molecules. The affinity of CD1d-α-GalCer and mouse TCRs is one of the highest ever recorded for natural TCR/ligand pairs. Injection of α-GalCer causes a surge in cytokines in mice. α-*C*-galactosylceramide, a synthetic analog to α-GalCer, was found to induce significantly more pronounced NKT-cell proliferation and Th1 responses. α-GalCer-loaded immature DCs present antigens to NKT-cells, leading not only to activation of the

NKT-cells (innate antitumor immunity), but also to subsequent maturation of DCs able to present to T cells and activate them.

In the past we have suggested a unique role for CD1a-positive DCs in primary RCC tumors. We recently demonstrated in an extensive cDNA microarray analysis that immune effector cells isolated from patients undergoing DC vaccine/HD-IL-2 immunotherapy showed upregulation of pathways involving lipid antigen presentation prior to treatment when compared with age-matched healthy donors. Treatment resulted in downregulation of these pathways. Similarly, responders showed upregulation of pathways involving lipid antigen presentation prior to treatment when compared with nonresponders. These data suggest that a lipid-targeted vaccine approach may be of great benefit. However, this research area has been limited by the challenge of isolating lipid antigens in a reproducible fashion.

Antigen delivery

Over the past decade it has become clear that the mode of antigen delivery may be crucial in the failure or success of a vaccine. Not only does the route of vaccine administration play a critical role (e.g., subcutaneous, intravenous, intradermal or direct intranodal injection), but also the context of how the target antigen is presented (i.e., peptide alone, peptide plus vehical, whole cell, DC vaccine and so on) to the immune system is of utmost importance.

The advantage of direct administration of the target antigen into the patient is that DCs will process it *in vivo* and initiate an immune response. Although this approach has had significant benefit in generating protective immunity to viral and bacterial illnesses, it has had little demonstrable benefit to date as a therapeutic approach to cancer. Alternative strategies have been employed including recombinant DNA constructs that incorporate genes that induce cytokine production or expression of costimulatory molecules. Other approaches have used a priming vaccine with antigen-coding RNA, followed by a boost vaccine with protein antigen. Antigens can also be delivered in a viral construct, in particular when combined with costimulatory or immune stimulatory components. This approach allows for standardized monitoring of the antigen-specific effects on the immune system (as discussed later). A recent example is the TRICOM™ vector vaccine in prostate cancer, which consists of a poxviral construct coexpressing prostate-specific antigen (PSA), CD54 (ICAM-1), B7.1 and LFA-3. This Phase II trial demonstrated not only reduction in the target antigen PSA levels, but also significant overall survival when compared with placebo. In addition, significant serologic responses and antigen spread were documented with this vaccine [33,34].

Although different routes of DC administration have successfully induced immunity, preclinical and clinical studies have suggested that intranodal immunization may be best at enhancing protective anti-tumor immunity [35–37]. We have incorporated this approach to elicit immune responses in renal cell cancer patients in addition to systemic therapy with IL-2 and IFN-α.

Dendritic cells

Dendritic cells are the most potent APCs. Not only are they capable of initiating a highly antigen-specific CTL response, but they are also able to enhance central memory and effector memory T-cell responses [38]. In murine models, DC vaccines have been shown to overcome tolerance [39]. DCs take up and process antigens in an immature state. They subsequently mature and migrate to T-cell rich areas where the antigen is

Table 1. Summary of potential target antigens.

Target antigen	Advantages	Disadvantages	Clinical data	Immune data
CA IX	Highly expressed in clear cell RCC; promising preclinical (mouse) data; significant prognostic marker	Only expressed on clear cell RCC Lacks immunogenicity	No significant clinical responses	Induction of antigen-specific T cells
MUC-1	Very immunogenic	Limited expression on RCC	No significant clinical responses	Induction of antigen-specific T cells
Tumor-specific RNA	Individualized; unlimited supply for booster shots	Need for autologous tumor material	Promising early clinical results	Induction of tumor-specific T cells
Tumor lysate	Individualized; multiple target antigens	Can be labor and cost intensive Need for tumor material	Varied; some very impressive clinical results; only Phase III trial with improved PFS	Varied; some trials with promising early immunologic changes related to therapy

PFS: Progression-free survival; RCC: Renal cell carcinoma.

presented in the context of costimulatory molecules (signal 1 and 2, as previously discussed) to antigen-specific T cells, which will subsequently proliferate. Although tumor-infiltrating DCs have been described [40], their function in the cancer-bearing host appears impaired [41,42]. While DC could be stimulated *in vivo* by providing the necessary cytokine cocktail systemically, the rationale for *ex vivo* generation of therapeutic DC preparations has been provided by murine tumor models [39,43] that have shown reduced T-cell tolerance and improved anti-tumor immunity. Similar data has been generated in several human studies [30].

Two major subsets of DC have been described (myeloid and plasmacytoid DCs). The majority of human anticancer trials have employed myeloid (also called 'classical') DCs. The challenge in myeloid DC-based vaccines is that a number of different functions and phenotypes have been described. In addition, it has been demonstrated that the combination of cytokines used to generate *ex vivo* DCs may be of utmost importance in generating phenotypically and functionally desirable product [44,45]. The initial and clinically most commonly utilized DCs maturation cocktail is the combination of GM-CSF and IL-4 (from 3 up to 9 days). The addition of TNF-α has been shown to generate DCs highly capable of priming T cells [30,46]. However, some data exist suggesting that DCs generated with GM-CSF and IL-15, instead of IL-4, will generate stronger $CD8^+$ T-cell responses [47]. A number of clinical trials utilizing incompletely matured DCs failed to show clinical results, mostly due to the fact that vaccination may induce immune tolerance rather than tumor cytotoxicity. Two adjuvant studies using melanoma antisense vaccine in high-risk melanoma patients suggest that induction of tolerance by vaccine may be real as patients who received treatment had a trend toward worse overall survival [48,49]. DCs can be loaded with antigen either by allowing for phagocytosis, endocytosis or peptide interaction with MHC molecules. Recent data suggest that antigen loading onto DCs may be improved by using a construct that combines a target antigen with the mannose receptor on DCs. This will facilitate antigen uptake and processing [19].

In summary, the advantage of DC as vehicles of tumor target antigen delivery is that they induce a highly capable and cytotoxic immune response. Their position and role in innate immunity is crucial and the means of maturation and delivery are crucial.

Additional immune signaling

The use of immunostimulatory cytokines in RCC has been well documented and reviewed in the past [50]. IL-2 and IFN-α have been shown to not only provide immune stimulatory and T-cell proliferative effects but will also overcome immune regulatory pathways [51]. Recent murine data are emerging demonstrating that memory T-cell responses can be augmented by the addition of rapamycin, a mTOR inhibitor and considered an immune inhibitor, as a systemic adjuvant [52].

We, as well as others, have shown in the past that patients who respond to immunotherapy may have a predisposition to respond as compared with nonresponders [29]. The concomitant use of immunostimulatory cytokines may provide the additional signals that enhance response to therapeutic cancer vaccines. In fact, recently we have not only demonstrated a high level of Treg cells in RCC patients when compared with healthy donors, but also found that patients who will ultimately respond to DC vaccine plus IL-2 immunotherapy will have a significantly reduced induction of circulating Treg cells than patients who failed therapy [30]. This observation is further supported by the finding that the number of tumor-infiltrating Treg cells correlate with treatment outcome [53].

In order to succeed, vaccine approaches will have to incorporate treatments that will counteract tumor-derived immunosuppressive factors and T-cell inhibitor molecules, such as CTLA4 and PD1 (Table 2). Candidate immunology targets are myeloid-derived suppressor cells, tumor-associated macrophages, Th2-directed cytokines, such as IL-10, IL-13 and TGF-β. Selective check point inhibitors, such as anti-CTLA4 and anti-PD1 antibodies may enhance T-cell activity and have shown early clinical activity in RCC [54].

Response evaluation

Clinical

The primary reason why the vast majority of previous immunotherapeutic vaccine trials were labeled as clinical failures was that direct clinical tumor regression was rare. The data from the use of the immunotherapeutic Provenge® in prostate cancer, where response is not seen but survival benefit is observed, has challenged us to rethink clinically relevant end points. Another example of this necessary paradigm shift was demonstrated in recent immunotherapy trials using an anti-CTLA4 antibody ipilimumab, the overall survival was significantly improved, yet response rate was low. This may be explained in part by the fact that the immune system may take time to mount

an appropriate cytoreductive anti-tumor response. The use of survival as an end point will necessitate a change in the design of trials and the sample size required to demonstrate relevant benefit.

Immune monitoring

If vaccine approaches fail to show immediate early clinical tumor responses, the success of a vaccine will have to be measured in validated surrogate biomarkers. This is, in particular, true of small Phase I or II trials. Yet, the Phase III Provenge® trial failed to identify a relevant biomarker, emphasizing the barriers to this approach as well.

Recent vaccine approaches have all included an immune monitoring component that serves a number of purposes:

- Monitoring of immune parameters early on in the trial will provide the investigators with a glimpse at the potential for subsequent clinical success;
- Immune monitoring will provide clues to effective antigen;
- Immune monitoring will provide insight into the role of regulatory pathways;
- Such data will, over time, allow prognostication for the patient's future clinical course; it will also provide the clinician with indication as to whether or not the patient may benefit from additional ('booster') vaccines.

Conclusion

In summary, we provide here an overview of vaccine strategies and success of vaccines in RCC. We demonstrate why historic vaccine approaches may have failed to show clinical efficacy. Vaccine approaches have largely been limited to the metastatic setting, in particular in the face of previously failed treatment regimens. The advantage of vaccines in the advanced disease setting is that the side effects in general are quite low. However, the true benefit for cancer vaccines may be in the adjuvant setting, following surgical debulking. The reason for this is a minimal disease burden with low immune inhibitory tumor-derived factors. Patients tend to be of better performance status than patients with end-stage disease. As outlined earlier in this article, the concept of a vaccine is to induce a long-lasting memory immune effect preventing disease recurrences. Therefore, the adjuvant cancer vaccine may be the most appropriate clinical setting. This may require intermittent booster vaccines. Given the recent advances in treatment of metastatic RCC with tyrosine kinase inhibitors, it is of great interest to address their role in immunotherapy. Early data suggest an ambivalent role for TKIs in this context. The VEGF pathway is important in regulation of DC maturation, function and development. Anti-VEGF directed therapies (such as sunitinib, sorafenib or bevacizumab) may lead to a Th1-directed anti-tumor effect. In fact, the immune-stimulatory effects of sunitnib may be a marker for therapy effect. On the contrary, sorafenib is not only a VEGF-receptor inhibitor, but also a B-Raf inhibitor. The Raf–kinase pathway is partially responsible for immune downregulation [55]. Therefore, the role for TKIs in general and some of these drugs in particular may hold great promise, but will have to be the target of intense future investigations.

Current vaccine approaches will have to carefully determine the impact of different vaccine components (e.g., antigen, antigen delivery vehicle and immune costimulants) on the immune system. Future vaccines will balance induction of antitumor immunity, autoimmunity and generation of immune tolerance. It appears that DC-based vaccines hold promise to achieve a global, cancer-specific and clinically meaningful immune stimulation. A large variety of different aspects in this complicated system have to be assessed in a systematic manner. Yet, vaccine approaches to RCC are seeing a well-deserved renaissance with the recently improved understanding of tumor immunology.

Table 2. Advantages and disadvantages of cytokines and check point inhibitors.

Immune adjuvant	Advantages	Disadvantages
IL-2	Global immune stimulant; T-cell proliferation, activation; US FDA approved for mRCC	Highly toxic as high dose IL-2; increases Treg cells
GM-CSF	T-cell proliferation; leukocyte activation	Nonspecific, no proven clinical benefit
IFN-α	Macrophage/NK cell activation; US FDA-approved for mRCC	–
Anti-CTLA4	Treg depletion, good results in melanoma	Limited results in RCC
Anti-PD1	Treg depletion	In clinical trials

mRCC: Metastatic renal cell carcinoma; NK: Natural killer; RCC: Renal cell carcinoma.

Future perspective

Vaccine-based immunotherapy in RCC holds great promise. Curative approaches to a highly lethal cancer can be envisioned in the future with better understanding of the immune system. The role of vaccine target antigens, target delivery and immune stimulants will have to be systematically evaluated in the future.

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Executive summary

Physiology

- Tumor-associated antigens (TAAs) are processed and presented by antigen-presenting cells to T cells.
- T-cell activation requires direct antigen-presenting cell/T cell contact and at least three signaling mechanisms.
- Dendritic cells are the most potent antigen-presenting cells, but also hold a key position in the induction of effective anticancer immune mechanisms or tolerance.

The antigen & delivery

- Antigenic structures can be peptides, full length protein, tumor lysate, tumor-specific RNA or lipid molecules.
- TAAs can be delivered within viral constructs (coexpressing potential vaccine stimulants).
- Route of administration subcutaneous, intravenous or intramodal.
- TAA-specific autologous T cells can be manufactured and expanded extracorporeally.
- TAAs can be presented to the immune system via dendritic cells as antigen-presenting cell vehicles.

Immune signaling

- Additional immunostimulants can increase the therapeutic and immunologic value of the vaccine. The role of tyrosine kinase inhibitors and other VEGF pathway-targeting agents is not well understood in this context.

Response evaluation

- Clinical responses may be limited or not apparent until several months into the treatment. Vaccine therapy may significantly inhibit the growth rate of disease.
- Careful assessment of immune parameters may serve as predictor for response and as early proof of principle for vaccine strategies in early development.

Bibliography

1 Hoption Cann SA, van Netten JP, van Netten C, Glover DW. Spontaneous regression: a hidden treasure buried in time. *Med. Hypotheses* 58(2), 115–119 (2002).

2 Coley WB. The treatment of malignant tumors by repeated inocolulations of erysipelas: with a report of ten original cases. *Am. J. Med. Sci.* Volume 5, Paris, 487–511 (1893).

3 Neidhart JA, Murphy SG, Hennick LA, Wise HA. Active specific immunotherapy of stage IV renal carcinoma with aggregated tumor antigen adjuvant. *Cancer* 46(5), 1128–1134 (1980).

4 McCune CS, O'Donnell RW, Marquis DM, Sahasrabudhe DM. Renal cell carcinoma treated by vaccines for active specific immunotherapy: correlation of survival with skin testing by autologous tumor cells. *Cancer Immunol. Immunother.* 32(1), 62–66 (1990).

5 Sahasrabudhe DM, deKernion JB, Pontes JE *et al*. Specific immunotherapy with suppressor function inhibition for metastatic renal cell carcinoma. *J. Biol. Response Mod.* 5(6), 581–594 (1986).

6 Bonifaz L, Bonnyay D, Mahnke K, Rivera M, Nussenzweig MC, Steinman RM. Efficient targeting of protein antigen to the dendritic cell receptor DEC-205 in the steady state leads to antigen presentation on major histocompatibility complex class I products and peripheral $CD8^+$ T cell tolerance. *J. Exp. Med.* 196(12), 1627–1638 (2002).

7 Hawiger D, Inaba K, Dorsett Y *et al*. Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions *in vivo*. *J. Exp. Med.* 194(6), 769–779 (2001).

8 Barth RJ Jr, Fisher DA, Wallace PK *et al*. A randomized trial of *ex vivo* CD40L activation of a dendritic cell vaccine in colorectal cancer patients: tumor-specific immune responses are associated with improved survival. *Clin. Cancer Res.*. 16(22), 5548–5556 (2010).

9 Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat. Rev. Immunol.* 4(10), 762–774 (2004).

10 Mickisch GH, Garin A, van Poppel H, de Prijck L, Sylvester R. Radical nephrectomy plus interferon-α-based immunotherapy compared with interferon α alone in metastatic renal-cell carcinoma: a randomised trial. *Lancet* 358(9286), 966–970 (2001).

11 Flanigan RC, Salmon SE, Blumenstein BA *et al*. Nephrectomy followed by interferon α-2b compared with interferon α-2b alone for metastatic renal-cell cancer. *N. Engl. J. Med.* 345(23), 1655–1659 (2001).

12 Jocham D, Richter A, Hoffmann L *et al*. Adjuvant autologous renal tumour cell vaccine and risk of tumour progression in patients with renal-cell carcinoma after radical nephrectomy: Phase III, randomised controlled trial. *Lancet* 363(9409), 594–599 (2004).

13 Shablak A, Hawkins RE, Rothwell DG, Elkord E. T cell-based immunotherapy of metastatic renal cell carcinoma: modest success and future perspective. *Clin. Cancer Res.* 15(21), 6503–6510 (2009).

14 Bauer S, Oosterwijk-Wakka JC, Adrian N *et al*. Targeted therapy of renal cell carcinoma: synergistic activity of cG250-TNF and IFNγ. *Int. J. Cancer* 125(1), 115–123 (2009).

15 Kim HL, Sun X, Subjeck JR, Wang XY. Evaluation of renal cell carcinoma vaccines targeting carbonic anhydrase IX using heat shock protein 110. *Cancer Immunol. Immunother.* 56(7), 1097–1105 (2007).

16 Bleumer I, Tiemessen DM, Oosterwijk-Wakka JC *et al*. Preliminary analysis of patients with progressive renal cell carcinoma vaccinated with CA9-peptide-pulsed mature dendritic cells. *J. Immunother.* 30(1), 116–122 (2007).

17 Siebels M, Rohrmann K, Oberneder R *et al*. A clinical Phase I/II trial with the monoclonal antibody cG250 (RENCAREX(R)) and interferon-α-2a in metastatic renal cell carcinoma patients. *World J. Urol.* 29(1), 121–126 (2011).

18 Scanlan MJ, Gure AO, Jungbluth AA, Old LJ, Chen YT. Cancer/testis antigens: an expanding family of targets for cancer immunotherapy. *Immunol. Rev.* 188, 22–32 (2002).

19 Tsuji T, Matsuzaki J, Kelly MP *et al.* Antibody-targeted NY-ESO-1 to mannose receptor or DEC-205 *in vitro* elicits dual human $CD8^+$ and $CD4^+$ T cell responses with broad antigen specificity. *J. Immunol.* 186(2), 1218–1227 (2011).

20 Amata RJ, Shingler W, Naylor S *et al.* Vaccination of renal cell cancer patients with modified vaccinia ankara delivering tumor antigen 5T4 (TroVax) administered with interleukin 2: a Phase II trial. *Clin. Cancer Res.* 14(22), 7504–7510 (2008).

21 Kaufman HL, Taback B, Sherman W *et al.* Phase II trial of modified vaccinia Ankara (MVA) virus expressing 5T4 and high dose interleukin-2 (IL-2) in patients with metastatic renal cell carcinoma. *J. Transl. Med.*7, 2 (2009).

22 Hawkins RE, Macdermott C, Shablak A *et al.* Vaccination of patients with metastatic renal cancer with modified vaccinia Ankara encoding the antigen 4T4 (TroVax) given alongside interferon-α. *J. Immunother.* 32(4), 424–429 (2009).

23 Amato RJ, Shingler W, Goonewardena M *et al.* Vaccination of renal cell cancer patients with modified vaccinia Ankara delivering the tumor antigen 5T4 (TroVax) alone or administered in combination with interferon-α (IFN-α): a Phase 2 trial. *J. Immunother.* 32(7), 765–772 (2009).

24 Okamura K, Koike H, Sekine Y, Matsui H, Suzuki K. Survivin and its spliced isoform gene expression is associated with proliferation of renal cancer cells and clinical stage of renal cancer. *Cancer Epidemiol.* 33(2), 137–141 (2009).

25 Ciesielski MJ, Ahluwalia MS, Munich SA *et al*. Antitumor cytotoxic T-cell response induced by a survivin peptide mimic. *Cancer Immunol. Immunother.* 59(8), 1211–1221 (2010).

26 Marten A, Sievers E, Albers P *et al.* Telomerase-pulsed dendritic cells: preclinical results and outcome of a clinical Phase I/II trial in patients with metastatic renal cell carcinoma. *Ger. Med. Sci.* 4, Doc02 (2006).

27 Woloszynska-Read A, James SR, Link PA, Yu J, Odunsi K, Karpf AR. DNA methylation-dependent regulation of BORIS/CTCFL expression in ovarian cancer. *Cancer Immun.* 7, 21 (2007).

28 Heiser A, Maurice MA, Yancey DR, Coleman DM, Dahm P, Vieweg J. Human dendritic cells transfected with renal tumor RNA stimulate polyclonal T-cell responses against antigens expressed by primary and metastatic tumors. *Cancer Res.* 61(8), 3388–3393 (2001).

29 Schwaab T, Heaney JA, Schned AR *et al*. A randomized phase II trial comparing two different sequence combinations of autologous vaccine and human recombinant interferon γ and human recombinant interferon α2B therapy in patients with metastatic renal cell carcinoma: clinical outcome and analysis of immunological parameters. *J. Urol.* 163(4), 1322–1327 (2000).

30 Schwaab T, Schwarzer A, Wolf B *et al*. Clinical and immunologic effects of intranodal autologous tumor lysate-dendritic cell vaccine with Aldesleukin (interleukin 2) and IFN-{α}2a therapy in metastatic renal cell carcinoma patients. *Clin. Cancer Res.* 15(15), 4986–4992 (2009).

31 Van Poppel H, Joniau S, Van Gool SW. Vaccine therapy in patients with renal cell carcinoma. *Eur. Urol.* 55(6), 1333–1342 (2009).

32 May M, Brookman-May S, Hoschke B *et al.* Ten-year survoival analysis for renal carcinoma patients treated with an autologous tumor lysate vaccine in an adjuvant setting. *Cancer Immunol. Immunother.* 59(5), 687–695 (2010).

33 Kantoff PW, Schuetz TJ, Blumenstein BA *et al*. Overall survival analysis of a Phase II randomized controlled trial of a poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. *J. Clin. Oncol.* 28(7), 1099–1105 (2010).

34 Gulley JL, Arlen PM, Madan RA *et al*. Immunologic and prognostic factors associated with overall survival employing a poxviral-based PSA vaccine in metastatic castrate-resistant prostate cancer. *Cancer Immunol. Immunother.* 59(5), 663–674 (2010).

35 Schwaab T, Tretter CP, Gibson JJ *et al*. Immunological effects of granulocyte-macrophage colony-stimulating factor and autologous tumor vaccine in patients with renal cell carcinoma. *J. Urol.* 171(3), 1036–1042 (2004).

36 Lambert LA, Gibson GR, Maloney M, Durell B, Noelle RJ, Barth RJ Jr. Intranodal immunization with tumor lysate-pulsed dendritic cells enhances protective antitumor immunity. *Cancer Res.* 61(2), 641–646 (2001).

37 Bedrosian I, Mick R, Xu S *et al.* Intranodal administration of peptide-pulsed mature dendritic cell vaccines results in superior $CD8^+$ T-cell function in melanoma patients. *J. Clin. Oncol.* 21(20), 3826–3835 (2003).

38 Wiethe C, Dittmar K, Doan T, Lindenmaier W, Tindle R. Enhanced effector and memory CTL responses generated by incorporation of receptor activator of NF-κ B (RANK)/RANK ligand costimulatory molecules into dendritic cell immunogens expressing a human tumor-specific antigen. *J. Immunol.* 171(8), 4121–4130 (2003).

39 Fields RC, Shimizu K, Mule JJ. Murine dendritic cells pulsed with whole tumor lysates mediate potent antitumor immune responses *in vitro* and *in vivo*. *Proc. Natl Acad. Sci. USA* 95(16), 9482–9487 (1998).

40 Schwaab T, Schned AR, Heaney JA *et al.* *In vivo* description of dendritic cells in human renal cell carcinoma. *J. Urol.* 162(2), 567–573 (1999).

41 Gabrilovich DI, Corak J, Ciernik IF, Kavanaugh D, Carbone DP. Decreased antigen presentation by dendritic cells in patients with breast cancer. *Clin. Cancer Res.* 3(3), 483–490 (1997).

42 Troy AJ, Summers KL, Davidson PJ, Atkinson CH, Hart DN. Minimal recruitment and activation of dendritic cells within renal cell carcinoma. *Clin. Cancer Res.* 4(3), 585–593 (1998).

43 Lou Y, Wang G, Lizee G *et al*. Dendritic cells strongly boost the antitumor activity of adoptively transferred T cells *in vivo*. *Cancer Res.* 64(18), 6783–6790 (2004).

44 Slingluff CL Jr, Petroni GR, Yamshchikov GV *et al*. Clinical and immunologic results of a randomized Phase II trial of vaccination using four melanoma peptides either administered in granulocyte–macrophage colony-stimulating factor in adjuvant or pulsed on dendritic cells. *J. Clin. Oncol.* 21(21), 4016–4026 (2003).

45 Giermasz AS, Urban JA, Nakamura Y *et al*. Type-1 polarized dendritic cells primed for high IL-12 production show enhanced activity as cancer vaccines. *Cancer Immunol. Immunother.* 58(8), 1329–1336 (2009).

46 Lapenta C, Santini SM, Logozzi M *et al.* Potent immune response against HIV-1 and protection from virus challenge in hu-PBL-SCID mice immunized with inactivated virus-pulsed dendritic cells generated in the presence of IFN-α. *J. Exp. Med.* 198(2), 361–367 (2003).

47 Dubsky P, Saito H, Leogier M *et al.* IL-15-induced human DC efficiently prime melanoma-specific naive $CD8^+$ T cells to differentiate into CTL. *Eur. J. Immunol.* 37(6), 1678–1690 (2007).

48 Eggermont AM, Suciu S, Rutkowski P. Randomized Phase III trial comparing postoperative adjuvant ganglioside

GM@-KLH/QS-21 vaccination versus observation in stage II (T3-T4N0M0) melanoma: final results of study EORTC 18961. *J. Clin. Oncol.* 28(15S) (2010) (Abstract 8505).

49 Morton DL, Mossillo N, Thompson JF. An international, randomized, Phase III trial of bacillus Calmette-Guerin (BCG) plus allogeneic melanoma vaccine (MCV) or placebo after complete resection of melanoma metastatic to regional or distant sites 2007. ASCO Annual Meeting Proceedings Part I. *J. Clin. Oncol.* 25(Suppl. 18) (2007) (Abstract 8508).

50 McDermott DF. Immunotherapy of metastatic renal cell carcinoma. *Cancer* 115(10 Suppl.), 2298–2305 (2009).

51 Anderson PO, Sundstedt A, Yazici Z *et al*. IL-2 overcomes the unresponsiveness but fails to reverse the regulatory function of antigen-induced T regulatory cells. *J. Immunol.* 174(1), 310–319 (2005).

52 Rao RR, Li Q, Shrikant PA. Fine-tuning $CD8^+$ T cell functional responses: mTOR acts as a rheostat for regulating $CD8^+$ T cell proliferation, survival and differentiation? *Cell Cycle* 9(15), 2996–3001 (2010).

53 Jensen HK, Donskov F, Norsmark M *et al.* Increased intratumoral FOXP3-positive regulatory immune cells during interleukin-2 treatment for metastatic renal cell carcinoma. *Clin. Cancer Res.* 15,1052–1058 (2009).

54 Maker AV, Attia P, Rosenberg SA. Analysis of the cellular mechanism of antitumor responses and autoimmunity in patients treated with CTLA-4 blockade. *J. Immunol.* 175(11), 7746–7754 (2005).

55 Laschos KA, Papazisis KT, Kontovinis LF *et al.* Targeted treatment for metastatic renal cell carcinoma and immune regulation. *J. BUON* 15(2), 235–240 (2010).