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Review Article

Role of vaccine therapy for renal cell carcinoma in the era of targeted therapy

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Abbreviations & Acronyms
AE = adverse event
APC = antigen presenting cells
BCG = bacillus Calmette–Guérin
CA = carbonic anhydrase
CA9 = carbonic anhydrase IX
CPA = cyclophosphamide
CR = complete response
CTLA = cytotoxic T lymphocyte antigen
CTL = cytotoxic T lymphocytes
DC = dendritic cell
DFS = disease-free survival
GM-CSF = granulocyte-macrophage colony-stimulating factor
GMTV = genetically modified tumor vaccines
HLA = human leukocyte antigen
HR = hazard ratio
IFN = interferon
IL-12 = interleukin-12
IL-2 = interleukin-2
KHL = keyhole limpet hemocyanin
LMI = large multivalent immunogen
MHC = major histocompatibility complex
MR = minor response
mRCC = metastatic renal cell carcinoma
mTOR = mammalian target of rapamycin
MUC = mucin
MVA = modified vaccinia virus Ankara
N/A = not applicable
NK = natural killer
OS = overall survival
PADRE = Pan-DR-binding peptide
PD = progressive disease
PFS = progression-free survival
PR = partial response
RCC = renal cell carcinoma
RECIST = Response Evaluation Criteria in Solid Tumors
SD = stable disease
TAA = tumor-associated antigens
Th1 = T helper 1
Th2 = T helper 2
Treg = regulatory T cell
TRIST = TroVax Renal Immunotherapy Survival Trial
TUMAP = tumor-associated peptides
VEGF = vascular endothelial growth factor
VHL = von Hippel-Lindau
WT1 = Wilms tumor 1

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Abstract: Renal cell carcinoma is the most common malignant tumor originating from the kidney. Compared with other solid tumors, it does not respond to traditional management modalities, such as chemotherapy and radiotherapy. However, it is well known that renal cell carcinoma represents one of the most immune-responsive cancers and several immunotherapeutic strategies have been investigated in the management of renal cell carcinoma with variable degrees of success. The development of immunotherapy with α-interferon or high-dose interleukin-2 is the best established treatment, and is associated with durable disease control. Although the lack of defined antigens in renal cell carcinoma has hindered more specific vaccine development, research regarding vaccination therapy has been of special interest for the treatment of renal cell carcinoma for more than 30 years. At present, there are three types of cell-based vaccines in renal cell carcinoma treatment: autologous tumor-cell vaccines, genetically modified tumor vaccines and dendritic cell-based vaccines. A further type is peptide-based vaccination with tumor-associated antigens as possible targets, such as carbonic anhydrase IX, survivin and telomerase that are overexpressed in renal cell carcinoma. In the present article, we review data from completed clinical trials of vaccine therapy, and discuss future trials to assess the current knowledge and future role of vaccine therapy for renal cell carcinoma in the era of recently developed targeted therapy.

Key words: autologous tumor cell vaccine, dendritic cell vaccine, genetically modified vaccine, peptide vaccine, renal cell carcinoma.

Introduction

Since the end of the 19th century, and even before, medical scientists have been attempting to utilize the power of the host's immune system to cure cancer.[1] The vaccine therapy developed by Coley in the 1890s is now considered a type of non-specific immune response, which was induced by lipopolysaccharides composed of the bacteria administered, and then cytokines, such as tumor necrosis factor, were produced to elicit antitumor activities. At present, this treatment strategy still exists and is supported by the small but significant number of patients with metastatic cancer, especially mRCC, that have durable disease control, which is designed to manipulate the immune system.

RCC is the most common type of kidney tumor in adults, responsible for approximately 80% of cases. It is well known that when the tumor is confined to the renal parenchyma, the prognosis is relatively favorable, and the 5-year survival rate is 70–80%. In such cases, initial treatment is most commonly a radical or partial nephrectomy, and remains the mainstay of curative treatment. However, the survival rate is lowered considerably when the patient with RCC has regional or distant metastases. It is resistant to conventional treatment modalities, such as chemotherapy or radiotherapy, although some cases respond to immunotherapy, such as IFN or IL-2, which shows limited effects. Therefore, the treatment for patients with mRCC still remains challenging, and multidisciplinary treatment modalities are required to those with mRCC.

Recently, the management of mRCC has drastically changed with the arrival of VEGF and mTOR pathway-targeting agents. However, complete and durable responses are rare with those agents that target VEGF or mTOR, which requires sequential therapy to maintain

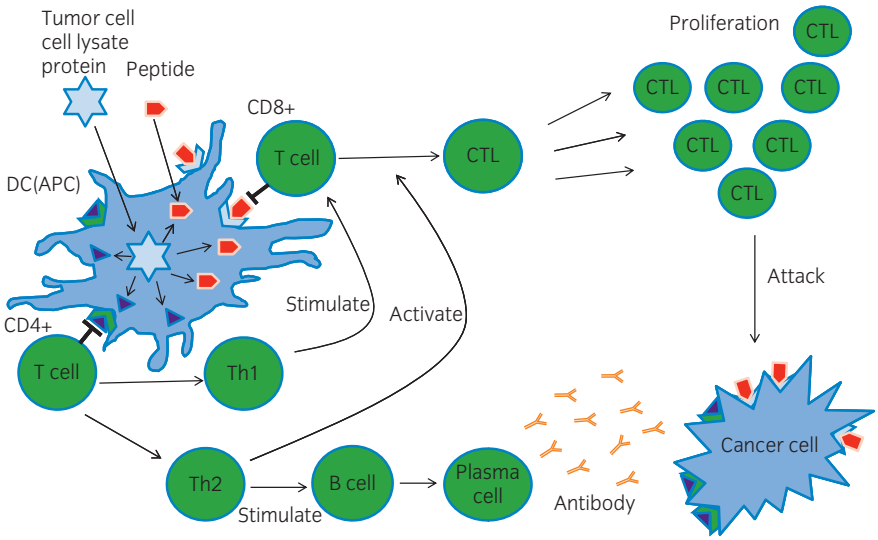

Fig. 1 Schematic presentation of events in the process of tumor vaccine therapy. Peptides presented by MHC class I complexes are recognized by CD8+ T cells. Peptides presented by MHC class II complexes on APC activate CD4+ T cells. This interaction leads to a proliferation of the cytotoxic T lymphocytes, which will attack cancer cells. Th1, T helper 1; Th2, T helper 2. , MHC class I; , MHC class II; , Class I peptide; , Class II peptide.

clinical benefits. The concept and subsequent development of therapeutic tumor vaccines for patients with mRCC has been under investigation for decades with various results.[2–4] The final achievement of newly developing curative RCC vaccines is to stimulate the immune system of the host to recognize and to attack existing tumor cells. RCC vaccines are clinically tried in the metastatic and adjuvant setting. To date, they are only clinically effective in a minority of patients and are still considered experimental. However, the recent USA 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 led to a renaissance of immunotherapy approaches.

In the present review, the PubMed database was searched using a combination of the search items “renal cell carcinoma” or “kidney cancer” and “vaccine” or “vaccination therapy” or “vaccine therapy”. Another search combined the items “dendritic cells” or “active immunotherapy” or “peptide vaccine” or “genetically modified tumor vaccine” or “autologous tumor cell vaccine” with “kidney cancer” or “renal cell carcinoma”. The search was concentrated on articles published in English, and cross-references were used for search completion. Subsequent references were identified from the reference lists of retrieved articles. All articles on the title and abstracts were screened, and selected for the present review. A total of 84 articles were relevant to use. With those articles, we provide an overview of the current role and future options of vaccine therapy for RCC and information on completed clinical studies.

Mechanisms of tumor vaccination, TAA for RCC, and types of therapeutic vaccines

The action of tumor vaccines is shown in Figure 1. The basis for the immune recognition of tumor cells is the presence of TAA. These antigens are glycoproteins expressed by tumor cells, generally at higher expression levels, at altered points during cell differentiation or in mutated forms. This pattern of expression allows the immune system to detect abnormal cell characteristics, which can be used for immune-mediated attack to a malignant cell. The best-studied antigens belong to a class of normal cell differentiation antigens that become overexpressed during malignant transformation. The action of tumor vaccines comprises immunization by genetically modified or irradiated tumor cells, antigen-loaded DC (or tumor–DC cell fusion), and non-cell-based tumor cell lysates and peptides derived from TAA. In general, tumor antigens are presented to the immune system by APC that acquire antigen by uptake of dying tumor cells or circulating proteins.[5] DC are the most potent APC, and process proteins into shorter peptide fragments that are linked to MHC for presentation to T cells.[6] Peptides presented by MHC class I complexes are recognized by CD8+ T cells, which are capable of differentiating into CTL, and can mediate tumor regression with the release of IFN-γ and the production of lytic enzymes, such as perforin and granzyme B[7]. Peptides presented by MHC class II complexes on APC activate CD4+ T cells, which can differentiate into Th1 cells that provide help for the generation of cytotoxic CD8+ T cell responses or Th2 cells that provide help for the generation of B cell and antibody responses.[8] A subset of CD4+ T cells that mediate immune suppression (regulatory T cells) are also activated after exposure to tumor antigens.[9] Although the precise mechanisms that regulate anti-tumor immunity are not completely resolved, there is a report that shows how this process can be used to activate effective therapeutic responses against established tumors in murine tumor models of RCC.[10]

At present, only a few potentially interesting TAA have been identified in RCC, as compared with other immunoresponsive tumors, such as melanoma. Early RCC vaccines were primarily cell-based tumor vaccines in which tumor

Table 1 Clinical trials with autologous tumor cell vaccine in renal cell carcinoma

Investigator	Year	No. patients	Phase	Stage	Vaccine	Adjuvant	Clinical outcomes
Adjuvant setting							
Galligioni *et al.*[19]	1996	120	2	I to III	Autologous irradiated tumor cells	BCG	5-year DFS 63%
Repmann *et al.*[22]	1997	222 (116 vaccines)	2	I to III	Autologous tumor cells	None	Significantly improved OS; significantly better OS in Robson II and III, not in I and IV
Jocham *et al.*[21]	2004	379	3	pT2-3b pN0-3	Autologous irradiated tumor cell lysate	None	5-year PFS 77.4% (control 67.8%, $P = 0.0204$)
May *et al.*[23]	2010	692	Matched-pair analysis	pT2-3 pNx-2 M0	Autologous irradiated tumor cell lysate	None	Significantly improved OS (HR = 1.28, $P = 0.030$) and in subgroup with pT3 tumors (HR = 1.67, $P = 0.011$)
Metastatic setting							
Kurth *et al.*[16]	1987	33	2	mRCC	Autologous ($n = 22$) and allogeneic ($n = 4$) irradiated tumor cells	Corynebacterium parvum	8 objective response Trend to better OS (insignificant statistically)
Schwaab *et al.*[20]	2000	14	2	mRCC	Autologous irradiated tumor cells	BCG, IFN-α and β	5 SD, 3 MR
Dillman *et al.*[24]	2004	25	2	11 II to IV 14 mRCC	Autologous irradiated tumor cells	BCG, IFN-β, GM-CSF and CPA	No objective response Median PFS reached more than 7 years
Dudek *et al.*[25]	2008	31	2	IV	Autologous LMI	None, CPA, CPA + IL-2	5 SD 4 SD 1 PR, 3 SD

cells provided sources of unknown TAA for immunization. The tumor antigen preparation can be based on whole tumor cells, tumor-derived cellular lysates, whole apoptotic or necrotic tumor cells, or tumor-derived total RNA, mRNA or DNA.[11,12] This approach aims to stimulate a polyclonal T cell immunoresponse against a broad range of tumor-derived epitopes, thereby reducing the possibility that tumors escape immune surveillance and destruction. There are three types of cell-based RCC vaccines: autologous tumor cell vaccines, GMTV and DC-based vaccines.[13] Another type is the peptide-based vaccines with tumor antigens. Studies of the most promising vaccine therapy in development for RCC are described here.

Autologous tumor cell vaccines

Cell-based vaccines are basically comprised of non-viable autologous tumor cells or some form of preparation that provides antigens to activate an immune response. Autologous tumor vaccine is based on the knowledge that RCC themselves express TAA that will induce CTL responses. It has already been recognized that additional treatment must be used to enhance the immune response necessary for a strong therapeutic effect. For this reason, traditional adjuvants, such as incomplete Freund's adjuvant, IL-2, IL-12, GM-CSF and BCG, have been used.[14,15] Several studies reported various results, in which toxicities were relatively mild.[16–21]

Clinical trials using autologous tumor cell vaccines in the metastatic and adjuvant setting are shown in Table 1.

As far as studies in the adjuvant setting are concerned, the first report on vaccine therapy in the advanced setting in localized and surgically resected RCC was reported by Galligioni *et al.* in 1996.[19] A total of 120 RCC patients (stages T3a-bN0M0 or T2-3N1M0) were randomized in a phase II trial to receive either adjuvant BCG-activated vaccine therapy or no adjuvant vaccine treatment. Vaccination comprised of three intradermal administrations of 10^7 autologous irradiated tumor cells mixed with/without 10^7 colony-forming units of BCG. A total of 38 out of 54 treated patients showed a significant response to autologous tumor, but not to normal renal cells, 1 month after vaccination. The 5-year PFS was enhanced, with 63% for the treated patients and 72% for the control group after a median follow up of 61 months.[19]

After Galligioni's report, Repmann *et al.* showed the results of a non-randomized trial evaluating the outcome of 116 patients treated with an autologous tumor cell vaccination in the adjuvant setting compared with 106 control patients in 1997.[22] Significantly prolonged OS was observed in the vaccine group ($P = 0.0007$). Patients at Robson stages II and III showed significantly improved survival rates ($P = 0.02$ and 0.04, respectively). However, there was no significant difference for patients at stages I and IV, possibly because of the short follow up and the limited number of patients.

In 2004, Jocham *et al*. reported a phase III trial showing significant benefit for RCC patients by adjuvant vaccination therapy.[21] In this randomized trial, PFS of patients with RCC at stage T2-3bN0-3M0 after nephrectomy followed by an adjuvant autologous tumor cell vaccination (Reniale; Vaccentis AG, Zurich, Switzerland) was significantly prolonged compared with control patients. After 5 years and 70 months, respectively, the HR for PFS were 1.58 (95% CI 1.05–2.37) and 1.59 (95% CI 1.07–2.36) in favor of the vaccine group ($P = 0.0204$), respectively. PFS rates at these time-points were 77.4 and 72.0% in the vaccine group and 67.8 and 59.3% in the control group, respectively ($P = 0.0204$). However, the results of that study have been questioned and have raised criticism because of some methodological pitfalls.

In 2007, a second updated intention-to-treat analysis including a higher number of patients ($n = 477$) reported a significantly prolonged PFS ($P = 0.0476$), but not OS ($P = 0.1185$), after vaccination. In a per-protocol analysis ($n = 352$), both PFS ($P = 0.024$) and OS ($P = 0.0356$) were significantly enhanced after vaccine treatment with Reniale.[26]

In 2010, May *et al*. confirmed the efficacy of adjuvant therapy with Reniale. They reported the results of a 10-year survival analysis of patients treated with Reniale in the retrospectively designed matched-pair adjuvant setting.[23] The study group comprised 692 patients with complete follow up (stages pT2–3, pNx–2, M0). Adjuvant treatment with autologous vaccination therapy resulted in a significantly improved overall survival in pT3 stage RCC patients, suggesting benefit, especially in this subgroup.

With regard to trials for metastatic RCC, one of the first trials using autologous tumor cell vaccination in the metastatic setting was carried out by Kurth *et al*. Of the 33 patients with metastatic disease, eight patients had objective responses with a median survival of 32 months compared with the overall survival of 17 months. Although the results were not statistically significant, a favorable trend was observed and toxicity was minimal.[16]

The results of two trials using irradiated tumor cells in 14 patients with mRCC were published by Schwaab *et al*. in 2000 (applied in addition to BCG and IFN-α and IFN-β)[20] and Dillman *et al*. in 2004 (in addition to BCG, IFN-β, GM-CSF and cyclophosphamide).[24] Although five patients in the trial by Schwaab featured SD and a further three patients had MR, in the trial by Dillman, no objective responses were observed.

In 2008, Dudek *et al*. have investigated the safety and tolerability of autologous LMI in stage IV RCC.[25] LMI results from preparation of immobilized autologous tumor cell plasma membrane on 5-μm diameter silica beads and is used to augment a tumor-specific CTL response. A total of 31 patients received LMI monotherapy every month (group 1) or were randomized to treatment with LMI in combination with cyclophosphamide (group 2) or LMI, cyclophosphamide and IL-2 (group 3). Low-dose cyclophosphamide was applied to downregulate suppressor T cell activity and to enhance immune response. Clinical outcomes were five SD in group 1, four SD in group 2, and one PR and three SD in group 3. Although a favorable clinical response was observed, there was no validated tool to establish immune response monitoring.[25]

Although devitalized tumor cell vaccines have proven to be safe, there has been a lack of data to support any significant clinical benefit from this type of vaccine therapy. However, especially in the adjuvant setting, favorable results have been shown to warrant further research.

Genetically modified tumor cell vaccines

To increase the immunogenic response, tumor cell-based vaccines using autologous or allogeneic tumor cells have been genetically modified. By incorporating genes encoding immunostimulatory cytokines or costimulatory molecules of the B7 family, such as GM-CSF, CD80, IL-2, IL-12 and IFN-γ, into tumor cells, several GMTV have been designed.[13,27–29] GMTV strategy is based on the idea that local cytokine secretion can elicit T cell and NK cell activation, and can also induce inflammatory responses against tumors.[30]

There are two basic strategies for using GMTV. One utilizes autologous tumor cells transfected with a costimulatory gene.[13,30] The second strategy utilizes genetically modified cells from well-established RCC cell lines, which dissolves the limitation that only limited amounts of tumor cells are available and lifelong immunization is required with autologous tumor cells.

Clinical trials using GMTV are shown in Table 2. In 1997, Simons *et al*. reported the first phase I trial showing safety as well as bioactivity of an autologous GM-CSF GMTV in mRCC patients.[27] They used a replication-defective retroviral vector to transfer the GM-CSF gene into irradiated autologous tumor cells, and an inclination towards increased delayed-type hypersensitivity with increased macrophage, eosinophil, neutrophil and T cell infiltration in the injection site was observed in the vaccine group. However, no significant difference in clinical response was achieved.

In 2002, Antonia *et al*. reported the results of GMTV using costimulatory B7.1 (CD80) gene in combination with IL-2. Of the 13 patients, two PR and two SD were observed. Three out of these four patients showed delayed-type hypersensitivity skin test reaction.[28] Pizza *et al*. evaluated the clinical efficacy of irradiated allogeneic GMTV producing IL-2 mixed with formalin-treated autologous tumor cells for mRCC patients after failure of IL-2 treatment.[29] Of the 30 mRCC patients, one CR and four PR were observed, and nine cases had SD. Although it is not clear that these clinical

Table 2 Clinical trials with genetically modified tumor cell vaccine in renal cell carcinoma

Investigator	Year	No. patients	Phase	Stage	Vaccine	Adjuvant	Clinical outcomes
Simons *et al.*[27]	1997	16	1	mRCC	Autologous irradiated GM-CFS	None	1 PR No significant statistical difference
Wittig *et al.*[31]	2001	5	1/2	mRCC	Autologous irradiated tumor cells transfected with GM-CFS and IL-7	Oligonucleotides	1 CR, 1 PR, 2 SD
Antonia *et al.*[28]	2002	13	1	mRCC	Autologous irradiated B7.1 gene	IL-2	2 PR, 2 SD
Tani *et al.*[32]	2004	6	1	mRCC	Autologous irradiated tumor cells transfected with GM-CSF	None	1 SD, 1 MR
Pizza *et al.*[29]	2004	30	2	mRCC	Allogeneic irradiated IL-2-producing tumor cells with autologous formalin-treated tumor cells	None	1 CR, 4 PR, 9 SD
Fishman *et al.*[33]	2008	39	2	mRCC	Autologous irradiated B7.1 transduced tumor cells	IL-2	1 CR, 2 PR, 25 SD

benefits are a result of a response elicited by the use of allogeneic or autologous tumor antigens, the fact remains that there was some useful achievement in the patients.

Recently, Fishman *et al*. investigated the use of irradiated B7.1-transduced, cultured autologous tumor cells plus subcutaneous IL-2 in a non-randomized trial. This trial could not show a higher rate of tumor regression; however, one CR, two PR and 25 SD were noted.[33] Further investigations with regard to GMTV therapy in clinical trials are required to verify the aforementioned relatively small number of trials.

DC-based vaccines

The recent trend in cell-based vaccine therapy has been directed to DC-based vaccines. DC are potent APC derived from CD34+ bone marrow cells and CD14+ monocytes. These cells are naturally found in peripheral tissues and migrate into the lymphoid organs to induce T cell immune response.[34,35] Infiltration of DC into primary tumor lesions has been associated with improved survival in a wide range on malignancies. Major steps have been taken towards the development of culture methods to differentiate and expand DC populations. DC pulsed in culture with various TAA from tumor cells or tumor cell lysate transferred back to the patient play a major role to present antigens to native T cells and induce primary immune responses.[30] Monocyte-derived DC are mostly used for clinical applications. However, isolation procedures of peripheral blood mononuclear cells, the differentiation towards DC and maturation are very different between the published clinical studies.[36]

Clinical trials using DC-based vaccines are summarized in Table 3. In 2009, meta-analysis by Van Poppel *et al*. on recently published trials using DC vaccines was reported. In this article, 37% of all patients (95 out of 256 patients) achieved a clinical response (4 CR, 12 PR and 79 SD).[57] Höltl *et al*. showed specific immune responses in the trial using autologous DC loaded with autologous or allogeneic tumor cell lysate and keyhole limpet hemocyanin.[37] A total of 35 patients with mRCC were enrolled in this trial, and 10 achieved a clinical response (2 CR, 1PR and 7 SD). Furthermore, an association between immune response and clinical response was detected.[39]

In 2006, Wierecky *et al*. reported the results of DC vaccine therapy pulsed with HLA-A2-binding peptide mucin 1 for mRCC patients. In this phase I trial ($n = 20$), immune or clinical responses were found in six patients (clinically, 1 CR, 2 PR and 2 MR) during treatment duration of 14 months.[49] A significant correlation between clinical and immune response was noted ($r = 0.791$) with higher induction of immune response in the case of SD or reduction of metastatic lesions ($P = 0.046$). In another phase I/II trial with an autologous tumor cell lysate-pulsed DC vaccine, Kim *et al*. showed five SD and one PR at a median follow up of 17.5 months in nine patients with mRCC.[52] Except for one patient, all patients showed an antigen-specific lymphocyte proliferation response after the first cycle, and patients with PR or SD had higher responses by day 42. Similar findings have been published suggesting the value of DC vaccines.[50,51,54] In these trials, an association between clinical and immunological responses was observed. Recently, Wei *et al*. reported vaccine therapy using hybrids of DC fused with tumor cells (dendritomas) in 10 patients. They concluded that their therapy is safe and effective when administered alongside escalating doses of IL-2. Immune, as well as clinical, responses (1 PR and 3 SD) were achieved.[53] In another trial by Oosterwijk-Wakka *et al*. using DC pulsed with autologous tumor cell lysate in combination with IL-2, no regression of metastases was noted. Although a measurable immunological response was not induced, there was extended disease stabilization.[58]

In 2009, Schwaab *et al*. reported clinical outcomes and immune response after DC vaccine therapy in combination with IL-2 and IFN-α in 18 mRCC patients.[55] The overall clinical response rate using RECIST was 50%, and three

Table 3 Clinical trials with dendritic cell vaccine in renal cell carcinoma

Investigator	Year	No. patients	Phase	Antigen	Adjuvant	Clinical outcomes
Höltl *et al.*[37]	1999	4	1/2	Tumor lysate + KHL	None	1 PR
Märten *et al.*[38]	2002	15	1/2	Tumor lysate + KHL	None	1 PR, 7 SD
Höltl *et al.*[39]	2002	35	2	Tumor lysate + KHL	None	2 CR, 1 PR, 7 SD
Märten *et al.*[40]	2003	12	1/2	Tumor cells	None	4 SD
Oosterwijk *et al.*[41]	2003	12	1	Tumor lysate + KHL	IL-2	8 SD, No measurable immune response
Gitlitz *et al.*[42]	2003	14	1	Tumor lysate	None	1 PR, 3 SD
Su *et al.*[43]	2003	15	1	Tumor RNA	None	N/A
Arroyo *et al.*[44]	2004	5	1	Tumor lysate + KHL	None	3 SD
Pandha *et al.*[45]	2004	5	1/2	Tumor lysate	KHL	2 SD
Avigan *et al.*[35]	2004	13	1	Tumor cells + KHL	None	5 SD
Dannull *et al.*[46]	2005	11	1/2	Tumor RNA	Treg depletion	N/A
Barbuto *et al.*[47]	2005	22	1/2	Tumor cells	None	2 PR, 14SD
Höltl *et al.*[48]	2005	22	1/2	Tumor lysate + KHL	CPA	2 MR, 3 SD
Wierecky *et al.*[49]	2006	20	1/2	MUC1 peptide + PADRE	IL-2	1 CR, 2 PR, 5 SD
Matsumoto *et al.*[50]	2007	3	N/A	Tumor lysate + KHL	None	1 SD
Bleumer *et al.*[51]	2007	6	1/2	CA 9 peptide + KHL	None	No clinical response
Kim *et al.*[52]	2007	9	1/2	Tumor lysate + KHL	None	1 PR, 5 SD
Wei *et al.*[53]	2007	10	2	Dendritomas†	IL-2	1 PR, 3 SD
Avigan *et al.*[54]	2007	20	1/2	Tumor cells	None	2 PR, 8 SD
Schwaab *et al.*[55]	2009	18	2	Tumor lysate	IL-2, IFN-α2a	50% clinical response rate
Soleimani *et al.*[56]	2009	17	1/2	Telomerase and survivin/ allogeneic tumor cell lines	IL-2	No CR, specific T cell response in SD

†Hybrids of DC with tumor cells.

patients achieved CR. The median time to progression was 8 months, and the median survival had not been reached within a median follow up of 37 months. Soleimani *et al.* also evaluated DC vaccine therapy for 27 mRCC patients in phase I/II clinical trials.[56] In the first trial, HLA-A2$^+$ patients were treated with autologous DC pulsed with telomerase and survivin, whereas HLA-A2$^-$ patients were administered with autologous DC pulsed with allogeneic tumor cells. In the second trial, immune responses in HLA-A2$^-$ patients were evaluated during vaccine therapy to identify potential response biomarkers. As a result, tumor lysate specific T cell response was induced, and predominant Th1 response with tumor lysate-specific IFN-γ T cell responses before and during vaccine therapy was found to correlate to disease stabilization. However, serum concentrations of cytokines were comparable in both SD and PD patients during treatment. The authors concluded that the future of DC-based vaccines might be a combination with current cancer treatment regimens to attenuate regulatory T cells and to expand effector T cells.

These reported studies show that DC-based vaccines are safe in mRCC patients and feasible to induce antigen-specific immune response, and clinically achieve tumor regression in several patients. However, the results should be viewed with caution, because of the relatively small number of patients enrolled in these trials and the multiplicity of vaccination strategies used.

Peptide-based vaccines

With regard to vaccine design, the use of restricted antigens is more relevant to tumor vaccine therapy than the use of tumor cells or cell lysates. Tumor cell and tumor lysate vaccines contain unknown antigens including normal self-proteins, which might result in unexpected host immune responses. Other disadvantages of tumor cell or lysate vaccine are the *ex vivo* preparation of cells and the limitation of tumor materials as the source of antigens. In contrast to these obstacles of using these types of vaccines, synthetic peptide-based vaccines have several advantages, such as easy production, stability, safety, no tumor tissue required and cost effectiveness.[59] However, despite these advantages, only a limited number of clinical studies using peptide-based RCC vaccines have been reported to date. Clinical trials with peptide-based vaccines in the metastatic and adjuvant setting are summarized in Table 4.

Studies in the metastatic setting

CA9 antigen is a tumor-associated glycoprotein expressed in a variety of malignancies, such as cervical, colorectal, esophageal and lung cancers.[69–73] Approximately 90% of any type of RCC and 99% of clear cell RCC express CA9, whereas CA9 expression in normal tissues including kidney tissue is limited. Therefore, CA9 antigen is a suitable target

Table 4 Clinical trials with peptide-based vaccine in renal cell carcinoma

Investigator	Year	No. patients	Phase	Stage	Vaccine	Adjuvant	Clinical outcomes
Metastatic setting							
Uemura *et al.*[60]	2006	23	1	mRCC	CA9 derived peptide	Freund's adjuvant	3 PR, 6 SD, Clinical response rate 39%, at least 12 vaccination required to elicit CTL
Bleumer *et al.*[51]	2007	8	1	mRCC	CA9 derived peptide	KHL	5 injections did not elicit CTL
Iiyama *et al.*[61]	2007	3	1/2	mRCC	HLA-A2402 restricted 9-mer WT1 peptide	Montanide ISA51	2 SD, 12 weekly vaccination elicited CTL
Suekane *et al.*[62]	2007	10	1	mRCC	4 peptides	None, IL-2 or IFN	6 SD, Induction of humoral immunity
Patel *et al.*[63]	2008	60	2	mRCC	Heat shock Mycobacterium vaccae (SRL 172)	None	1 PR, 7 SD
		36	2	mRCC	Heat shock Mycobacterium vaccae (SRL 172)	With/without IL-2	No survival advantage, fewer AEs
Jonasch *et al.*[64]	2008	60	2	mRCC	Autologous HSPCC96 (vitespen)	None	2 CR, 2 PR, 7 SD
Amato *et al.*[65]	2010	733	3	mRCC	TroVax	IFN-α, IL-2 or sunitinib	Immune response in 60%, no significant improved survival in whole group, significant survival advantage in good-prognosis patients
Rahma *et al.*[66]	2010	6	1/2	mRCC	Mutant VHL peptide	Montanide	Median OS and PFS: 30.5 and 6.5 months
Walter *et al.*[67]	2012	96	1/2	mRCC	IMA901 (10 TUMAP)	CPA	Improved OS, Immune responses to TUMAPs were associated with longer OS
Adjuvant setting							
Wood *et al.*[68]	2008	728	3	cT1b-T4N0M0 or cTanyN1-2M0	Autologous HSPPC96 (vitespen)	None	In patients with stage I/II, recurrence rare 15.2% (control 27%, $P = 0.056$), post-hoc analysis: significant risk reduction in intermediate risk group ($P = 0.004$)

for active specific immunotherapy. Furthermore, new TAA and tumor-derived HLA class I ligands have been identified.[74]

In 2006, Uemura *et al*. reported the results of a phase I trial using CA9 vaccine.[60] In that trial, they showed that vaccination with three CA9 peptides is well tolerated and feasible in 23 HLA-A24+ patients with progressive cytokine-refractory RCC. Approximately 70% of evaluable patients showed CA9-specific CTL, as well as immunoglobulin G responses, and the clinical response rate was 39%. At least 12 vaccinations were required to elicit specific CTL, which possibly suggests the low immunogenicity of these peptides. In 2007, Bleumer *et al*. reported the results of a similar trial using a HLA-A0201-restricted 9-mer peptide, CA 9p254, and a HLA-DR-restricted 20-mer peptide, CA 9p249-loaded DC vaccine.[51] Five intradermal vaccine injections did not induce peptide-specific CTL and immunoglobulin G. Although it is difficult to compare these two different vaccine trials, it is suggested that induction of immune response might be associated with the number of vaccinations.

Iiyama *et al*. presented the results of a phase I/II study of peptide vaccine therapy for various malignancies in 2007. In this trial, of the three patients with progressive mRCC, two cases showed long SD and peptide-specific CTL immune response after vaccination of an HLA-A2402-restricted 9-mer genetically modified WT1 peptide.[61] WT1 peptide vaccine induced grade 3–4 leukocytopenia in two patients with myelodysplastic syndrome, which might indicate a sufficient induction of specific CTL to eradicate target cells in bone marrow. Suekane *et al*. showed that personalized peptide vaccines with four different peptides administered every second week were well tolerated and induced peptide-specific humoral immune response in a small phase I study. Six out of 10 patients with cytokine-refractory mRCC showed SD after vaccination.[62] Recently, Patel *et al*. reported the results of two completed phase II trials that evaluated heat-killed Mycobacterium vaccae SRL172 for mRCC patients. In the first non-randomized trial, SRL172 was feasible, as well as IL-2 or IFN-α, and more effective than chemotherapy in mRCC patients. The second randomized trial showed that SRL172 in combination with IL-2 had no clinical advantage with regard to efficacy over IL-2 alone.[63]

In 2010, Rahma *et al*. reported the results of a phase I/II trial with six mRCC patients receiving mutant VHL peptide mixed with the immune-stimulating adjuvant montanide subcutaneously every 4 weeks until disease progression or utilization of all available peptide stock. Four patients showed specific immune responses against the corresponding mutant VHL peptides. Median OS and PFS rates were 30.5 and 6.5 months, respectively. Three of the six treated patients were still alive 57, 87 and 88 months after starting treatment without further conventional treatment. The

vaccine was well tolerated without any grade 3 or 4 adverse events.[66]

TroVax (Oxford BioMedica, Oxford, UK) is a novel vaccine based on a MVA vector engineered to express 5T4 TAA, found in more than 95% of clear cell and papillary RCC tumors. 5T4 antigen is a transmembrane glycoprotein overexpressed in a variety of malignant tumor cells, but with a limited expression in normal tissues. The safety and efficacy of TroVax have been evaluated in several phase I/II clinical trials.[75–80] Results from nine completed phase I and II trials in colorectal, renal and prostate cancer in approximately 190 patients showed that TroVax is safe and well tolerated. A cross-trial analysis of all evaluable patients showed a statistically significant association between immune responses to 5T4 and OS.[80] TRIST study (a randomized, double-blind, placebo-controlled Phase III trial in patients with advanced or mRCC) was completed in 2009. The primary endpoint was OS. TroVax was well tolerated when administered with IL-2, IFN-α or sunitinib. Although significant survival advantage was seen in good-prognosis patients treated with IL-2 plus TroVax compared with patients treated with IL-2 alone, there was no statistical difference in OS between the two groups (median 20.1 months MVA-5T4 *vs* 19.2 months placebo; $P = 0.55$).[65]

IMA901 (Immatics Biotechnologies GmbH, Tübungen, Germany) is the first therapeutic vaccine for RCC consisting of multiple TUMAP confirmed to be naturally presented in human cancer tissue. Results from a completed phase II trial of IMA901 combined with standard care showed significantly enhanced survival rates and time to progression in patients treated with IMA901 vaccine therapy. Furthermore, enhanced survival was seen in patients additionally treated with one application of cyclophosphamide before commencing vaccination. Recently, Walter *et al*. reported the outcomes of phase I/II clinical trials using IMA901.[67] They treated a total of 96 HLA-A02 positive patients with mRCC with IMA901 in two consecutive studies. In the phase I study, they showed that the T cell responses of the patients to multiple TUMAP were associated with better disease control. The randomized phase II trial showed that a single dose of cyclophosphamide administration before IMA901 immunotherapy reduced the number of regulatory T cells, and confirmed that immune responses to multiple TUMAP were associated with longer OS. A randomized phase III study to determine the clinical benefit of treatment with IMA901 is now ongoing (NCT 01265901). IMA901 multipeptide vaccine, such as sipuleucel-T, is one of the most available peptide vaccines in clinical practice in the near future.

Studies in the adjuvant setting

In 2008, Wood *et al*. published the clinical outcomes of adjuvant vaccine therapy with autologous tumor-derived heat-shock protein (glycoprotein-96) peptide complex (HSPCC-96; vitespen; Oncophage) in 361 patients compared with observation alone in 367 patients at high risk for recurrence after nephrectomy in a multicenter, open-label, randomized phase III trial.[68] PFS was not significantly improved after vaccine therapy with recurrence reported in 37.7% in the vaccine group and 39.8% in the observation group ($P = 0.506$; HR 0.923; 95% CI 0.73–1.17). This investigation represents the largest study completed to date regarding vaccination strategies in RCC. However, OS data were not enough to be favorably evaluated. An insignificant trend towards a better PFS was only noted for the less advanced RCC patients. Another trial has been commenced to follow the remaining 500 patients to obtain mature data on OS. Furthermore, among 33 PD patients on vitespen alone, two patients showed SD after the addition of IL-2.[68] This peptide vaccine has also been applied to those with mRCC.[64]

Although DC-based vaccine has been the major stream in the vaccine therapy, peptide-based vaccination still remains as an attractive method because of its technically easy approach. At present, the most promising approach in vaccine therapy in patients with RCC seems to be peptide-based vaccination with CA9, MVA-5T4 or IMA901.

Present limitations of vaccine therapy

One of the greatest obstacles for the development of vaccine therapy, especially in RCC patients, is the lack of specific TAA. The concept of immunotherapy is theoretically simple; however, it has been shown that the immune system is very complex from the practical experiences. Tumor surveillance derives from over 1000 tumor antigens that could enhance the adaptive immune response.[81,82] As there are still few TAA identified from RCC, many investigators have chosen to use DC-based or autologous tumor cell lysate vaccines to overcome this problem. It has also been proposed that because of the complicated nature of cancer cells and their genetic instability, expression of molecules targeted by effector T cells, such as TAA, MHC molecules and molecules associated with antigen processing and presentation, might be reduced or lost.[83,84]

Conclusion

Clinical investigations of vaccine therapy in RCC patients have shown that this treatment modality is safe and less toxic than other currently available molecular targeted therapies. Adverse events are less frequent and they are mostly grade 1 or 2. However, although immune and clinical responses have been noted in phase I/II trials among smaller patient cohorts, clinical benefits have not yet been fully proven in randomized phase III trials.

Despite small confirmative evidence-based data supporting vaccine therapy for RCC patients in clinical practice,

vaccine therapy might have an important role of treatment for those with RCC in the future, even in the era of targeted therapy. Further well-designed clinical trials including optimally selected patients (nephrectomized, with non-massive metastases and good performance status) should be required to warrant vaccine therapy for RCC.

Conflict of interest

None declared.

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