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SYMPOSIUM PAPER

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Immunotherapy of renal cell carcinoma

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Abstract Carcinomas of the kidney generally have a poor prognosis and respond minimally to classical radiotherapy or chemotherapy. Immunotherapy constitutes an interesting alternative to these established forms of treatment, and indeed, cytokine-based therapies have been used for many years, leading to favorable clinical responses in a small subset of patients. During the past few years, immunotherapeutical trials targeting renal cell tumor-associated antigens have also been reported, with diverse passive or active approaches using antibodies or aimed at activating tumor-directed T lymphocytes. The following review presents the results and the progress made in the field, including classical cytokine treatments, non-myeloablative stem cell transplantation and antigen specific-based trials, with special focus on T-cell studies. In consideration of the few specific molecular targets described so far for this tumor entity, current strategies which can lead to the identification of new relevant antigens will be discussed. Hopefully these will very soon contribute to an improvement in renal cell carcinoma specific immunotherapy and its evaluation.

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Introduction

Renal cell carcinoma (RCC) of the clear cell type accounts for approximately 3% of the adult malignancies and 90–95% of the neoplasms arising from the kidney. In the United States and the EU, about 40,000 patients are diagnosed every year, and its incidence is increasing. Since RCC has only discrete symptoms, most of these new cases are discovered incidentally, and among these, 25–30% are already in a metastatic stage. The overall 5 year survival rate is 60%, but the prognosis for patients with advanced metastatic disease (mRCC stage IV) decreases radically to a rate of less than 10% [1, 2]. Surgical resection, essentially by radical nephrectomy, is the first line of treatment for primary RCC. Despite the fact that half of the patients with primary localized RCC experience metachronous metastasis, there is currently no adjuvant treatment approved for non-metastatic RCC after surgery. Moreover, metastasis is only minimally affected by conventional cytotoxic agents, a feature which has been linked to the high expression of multidrug resistance genes by the tumoral cells, with response rates of less than 10% on average. Novel chemotherapeutical molecules, such as kinase inhibitors (Sunitinib, Sorafenib, both in phase III trials), or combinations of more classical agents, are currently being tested for patients who have undergone immunotherapy without success, and could open the way to new treatments. These clinical trials have been recently presented in detail [1].

The failure of classical chemotherapeutical methods has incited the search for alternative approaches, such as immunotherapy. RCC are indeed considered, along with melanomas, to be the most immunogenic tumors in humans. They are very often infiltrated by cells of the immune system, including T lymphocytes. The occurrence of renal tumors in immunosuppressed patients on one hand [3], and of rare cases of spontaneous metastasis regression on the other [4], led early on to the hypothesis that the immune system may control renal cell tumor

development. Indeed, Interferon alpha (IFN-α) and/or Interleukin 2 (IL-2), which activate diverse immune effector cells non-specifically, are routinely used for the treatment of mRCC patients and constitute the most effective agents currently available, with objective response rates of up to 30%. The discovery of tumor-associated antigens (TAA) recognized specifically by tumor-destroying immune B- [by antibody (Ab) secretion] and T- (by direct, HLA-restricted recognition) cells then rapidly opened the field to specific immunotherapy. So far, the experience is not as extended as for the melanoma tumors, but several clinical trials for RCC are currently testing either Ab or antigens for activation of $CD4^+$ and $CD8^+$ T cells. In the latter case, undefined (tumor-derived products) or selected (TAA-derived peptidic T-cell epitopes) antigens have been used in pilot studies. Although tumor stabilization and even tumor regressions have been observed, clinical benefit is still insufficient for the majority of patients. One of the reasons for such limited success could be the absence of adequate TAA or combinations thereof, which are available for the specific killing of kidney cancer. This review will present the proteins expressed in RCC that could be targeted in clinical trials and the methodologies available to identify new candidate antigens. Finally, new approaches such as non-myeloablative stem cell transplantation have produced some successful results and could also help in defining efficient molecular targets for RCC.

Cytokine-based immunotherapy

Cytokine-based therapies currently represent the first line treatment for mRCC after surgery. Two cytokines are normally used, IL-2 and IFN-α, produced as recombinant proteins. Since severe toxic effects have been observed early after the intravenous infusion of IL-2, the favored route is now subcutaneous application. IFN-α is administrated subcutaneously. A response rate of about 15% (partial or complete responses) has been reached with IL-2 alone [5, 6], which is better than with IFN-α [7, 8]. When both are combined, tumor stabilization can be observed in 15–30% of the patients [9]. Numerous clinical trials have been published in which dose, mode and schedule of application have been studied and these have been reviewed in detail [1, 10]. However, the success reported for these treatments is generally of short duration, tumor regression is rare, and most of the patients will eventually undergo progression. Application of GM-CSF together with IL-2 alone or with IFN-α has revealed various effects [11, 12], whereas the combination of IL-2 with IFN-γ did not increase clinical responses [13].

To improve response rates, cytokines are also being tested in combination with various chemotherapeutical agents. Most of the trials report on the use of vinblastine, 5-FU or more recently 13-*cis*-retinoic acid together with IFN-α alone or IFN-α plus IL-2, respectively. Results obtained in such combined therapies vary, but generally show an increased response rate and a survival benefit of patients over chemotherapy or immunotherapy alone [1, 2, 14, 15]. Overall, they encourage further evaluation and indicate that selected patient groups should clearly benefit from chemoimmunotherapy adjuvant treatments [16, 17].

The in vivo effects of cytokines are probably related to the non-specific activation of T lymphocytes ($CD4^+$ and $CD8^+$) and possibly of natural killer (NK) cells, but the recent evidence that IL-2 also regulates the homeostasis and function of $CD4^+CD25^+$ regulatory T (T regs) cells is puzzling [18]. IFN-α acts on various immune effectors and also shows anti-angiogenic properties [19]. However, the mechanisms involved in the positive effects of cytokine therapy have not yet been investigated in detail. A correlation between clinical response and the infiltration of tumoral tissue by activated T cells and dendritic cells (DC) has been reported [20, 21]. Investigations into the mode of action of cytokine treatment on immune cells might help to predict and evaluate clinical responses in treated patients.

Non-myeloablative allogenic transplantation therapy

Allogenic stem cell transplantation has proved to be efficient against several hematological malignancies. The curative effect of this treatment correlates with a graft-versus-host disease whereas the anti-tumor, or graft-versus-tumor activity, is thought to be mediated through the recognition of minor histocompatibity antigens by donor T lymphocyte effectors. Because of the immunogenic properties of RCC, several phase I and II clinical trials testing the effect of allogenic transplantation in nephrectomized patients have been performed. To decrease transplantation-related complications, non-myeloablative conditioning regimens were applied, followed by the transplantation with stem cells derived from HLA-identical sibling donors (NST). Encouraging results were first reported from 10/19 patients (52%) who achieved a partial or complete tumor regression including 3 patients with prolonged remission [22]. Subsequent trials using similar regimens confirm a clinical benefit but only rarely demonstrate complete remission [23–25].

It can be hypothesized that the activity of a "naïve" (allogenic), fully functional immune system, the depletion of autologous T regs and the existence of strong immunogenic antigens are responsible for the tumor regressions which have been observed under NST. Indeed, the peripheral expansion of functional $CD8^+$ T cells [26] and the specific recognition of minor antigens [27] have been reported in responders. However, the limitations of this kind of approach are numerous. First, allogenic stem cell transplantation, even after non-myeloablative regimens, can lead to severe or even fatal complications and patients have to be carefully and continuously monitored during treatment. Second, metastatic regression following transplantation has been

reported to occur relatively late (up to 6 months post-transplantation), thus excluding individuals suffering from rapidly progressing disease [22]. Finally, patients require an HLA-matched volunteer donor. Taken together, published studies show that NST can be successfully applied to a subset of patients and emphasize the role of the immune system in controlling renal cell tumor growth. Further development of allogenic transplantations could concentrate on the infusion of preselected anti-tumor T-cell populations.

Tumor antigen-specific immune responses

Immune reactivity against tumors in patients

It is well known that kidney tumors are frequently infiltrated by cells of the immune system. The majority of these are $CD3^+$ T cells, predominantly of the $CD8^+$ rather than the $CD4^+$ subset and NK cells which can represent up to 15% of the lymphocyte population. These tumor infiltrating lymphocytes (TIL) express activation markers such as CD69 or HLA-class II and can bear functional NK inhibitory receptors [28–30]. Oligoclonal expansions of certain TCR-Vbeta regions have been observed, suggesting the in situ selection of an anti-tumor T-cell repertoire [28, 31]. On the functional level, freshly isolated $CD8^+$ TIL showed a reduced cytotoxic potential, but after in vitro culture, the T cells display various effector functions such as cytokine secretion and the killing of autologous tumor cells [28, 29]. Thus, T lymphocytes infiltrating RCC seem to be similar to those described in melanomas, i.e. they have most likely been activated in the vicinity of tumor cells but are functionally impaired. A detailed picture of the phenotype and functionality of these TIL is, however, still missing. Nevertheless, it is clear that HLA-restricted T cells can be found in the TIL and in the peripheral blood of RCC patients [32, 33]. Several TAA-derived epitopes expressed by kidney cancers have been identified in mixed TIL-tumor cultures. The RCC-associated antigens recognized by not only $CD8^+$ but also $CD4^+$ effectors in a peptide and HLA-restricted fashion are detailed in the following paragraph.

TAA-specific Ab arising spontaneously in tumor patients have also been detected in RCC. The application of the SEREX method, which is based on the screening of cDNA expression libraries with human serum, has successfully identified antigens exclusively recognized by the patient´s Ab, but not by healthy individuals [34, 35]. In an extensive study, 20/65 described renal tumor gene products were recognized exclusively by autologous sera, although the majority was actually broadly expressed in normal tissues [36]. Other methods, such as serum Ab detection array (SADA) or proteomics-based analysis (SPEAR), have implemented the list of immunogenic antigens [37–39]. For example, members of the carbonic anhydrase family (CA-I and CA-XII) or thymidine phosphorylase, which is upregulated in the majority of the tumor samples analyzed, constitute immunogenic proteins frequently recognized by the patient´s natural Ab. Overall, 25–77% of the individuals suffering from RCC develop Ab against tumor-associated proteins, with a reactivity pattern highly variable among individuals. Since the Ab detected by these methods are of the IgG isotype, it is most probable that target antigens are also recognized by $CD4^+$ T-helper cells, and possibly also by $CD8^+$ T lymphocytes as already shown for the melanoma-associated antigen NY-ESO-1 [40, 41]. Thus, besides their potential role in diagnosis and disease monitoring, analysis of the anti-cancer humoral response in cancer patients can also support the identification of new tumor-specific HLA-class II and HLA-class I T-cell epitopes. Provided that the recognized protein is membrane-associated (which is, however, rare for SEREX identified antigens) it could also help to identify target structures for Ab-based immunotherapies.

RCC-associated tumor antigens and known derived T-cell epitopes

As compared to melanoma, only few TAA expressed by kidney tumor cells have been described so far. They can be classified into four categories: (a) overexpressed antigens which are shared by many tumor types, (b) antigens expressed by the majority of the RCC tumors, but not or minimally by normal tissues, (c) antigens expressed occasionally by RCC and other tumors and (d) products of gene mutations or aberrant reading frames. These antigens and derived T-cell epitopes are listed in Table 1. Those belonging to the categories (a) and (b) appear at the first sight to be the most interesting for broad immunotherapy, but the targeting of overexpressed proteins has to fight against immunological tolerance as already demonstrated in mouse models. Antigens from groups (c) and (d) might be more immunogenic, although expressed either in a subset of patients or possibly only individually. Prescreening of individual tumors before vaccine design could help in this case [42]. Alternatively, immunization with a cocktail of antigens/peptides should increase the chance to generate a beneficial immune response in many patients.

Several of the listed antigens recognized by T cells have been found using a direct approach in which patient peripheral lymphocytes or TIL were stimulated in vitro with the autologous tumor cells. This is the case, in particular, for T-cell epitopes resulting from point-mutations or abnormal translation products (Table 1) [43–48]. Moreover, an HLA-A3-restricted epitope derived from a post-translational protein splicing product of the FGF-5 gene was shown to be recognized by $CD8^+$ TIL [49]. Although it remains unclear whether such unconventional products constitute a frequent and repetitive source of MHC-ligands in tumors, they considerably increase the field of potential targets for T-cell recognition.

Table 1 RCC-associated antigens and the described T-cell-derived epitopes

Antigens	Tumor expression[a]	HLA-class I epitopes[b]	HLA-class II epitopes[b]
Broadly overexpressed			
EphA2	> 90%	A2 [103, 104]	
Her2/neu	30%	A2, A3, A24	DR1, DR52, DR53 [105, 106]
c-Met	100%	A2 [59]	
MUC1	> 60%	A2	
p53	10–35%	A2, A24	DR1, DR4, DR14, DP5 [107–109]
Survivin	100%	A2 [110], A24 [111], B35 [112]	
hTERT (telomerase)	> 70%	A1, A2, A3, A24	DR1, DR4, DR7, DR11, DR15 [113]
OFA-iLR[c]	> 100%	A2	
Expressed in the majority of RCC			
G250/CA-IX	90%	A2	DR
Expressed in a proportion of RCC			
ADFP	45%	A2 [60]	
MAGE-A3	60%	A1, A2, A24, B35, B37, B40 [114], B44, B52	DPw4, DR1, DR4, DR7, DR11, DR13 [115]
MAGE-A6	30%	A34, B37	DR4
MAGE-9	> 30%	A2 [116]	
PRAME	> 40%	A2, A24	
RAGE-1	2–21%	A2 [116], B7	
SART1(259)	25%	A24, A26	
SART-3	57%	A24	
Aberrant gene products			
ICE[d]	3/4	B7 [45]	
FGF-5	NA[e]	A3 [49]	
HLA-A2	NA	[46]	
Hsp70	1/9	A2 [47]	
M-CSF	6/10	B35 [43]	
RU-2	10/10	B7 [44]	
TRP-1 (gp75)	11%	A31 [48]	

[a] Reported expression on RCC primary tumors by RT-PCR or immunohistochemistry
[b] HLA restriction—for details including peptidic sequences, see http://www.syfpeithi.de and references
[c] Oncofetal antigen immature laminin receptor
[d] Intestinal carboxyl esterase
[e] Not available

During the past few years, reverse immunology has also been used extensively to identify many T-cell epitopes suitable for T-cell-based immunotherapy and/or monitoring and applicable for diverse cancers. In this method, candidate antigens known to be expressed in tumor cells—but not or only weakly in normal cells—are screened for the presence of potential HLA-binding sequences which are used in the form of synthetic peptides to stimulate and expand specific T lymphocytes in vitro. Using continuously improving prediction programs (see, for example, http://www.syfpeithi.de), many groups have been successful in describing new tumor-associated $CD8^+$ T-cell epitopes. Thus, common TAA such as telomerase (hTERT), Survivin, Mucin1 (MUC-1) and Her2/neu are overexpressed in the majority of the RCC, and are a source of well characterized class I epitopes (essentially restricted to the common allele HLA-A2). Other TAA that are found in a variable proportion of renal cell tumors, such as MAGE-3, PRAME or RAGE-1, have also been screened for $CD8^+$ T-cell recognition. Importantly, MHC-class II epitopes are known for several of these antigens (see Table 1). Members of the carbonic anhydrase family are also found in RCC, especially CA-IX and CA-XII. The G250 surface protein (CA-IX) is described to be expressed in approximately 90% of the primary renal carcinomas, and also in some other tumor types. Its expression is inducible by hypoxia and decreased expression levels have been proposed to represent a predictive factor of poor survival in advanced RCC, whereas high expression has been linked to favorable outcomes after IL-2 therapy [50, 51]. One HLA-class I epitope specific for the common allele HLA-A2, and one HLA-DR epitope (predicted to bind to HLA-DR1, -DR4 and -DR11) have been identified.

New strategies to identify a larger number of target antigens

Rapid progress in functional genomic analysis has opened the way for identifying new TAA. Thus, gene expression analysis using genechips can be applied in order to list cellular products either overexpressed or exclusively expressed in a variety of tissues including tumors [52]. Two-dimensional gel electrophoresis followed by mass spectrometry (MS) shows an increasing sensitivity for detecting cell proteome components [53]. However, these methods do not allow the selection of antigens that are targets for T-cell recognition. In this respect, elution and sequencing of peptides binding to HLA molecules of fresh tumor cells selects those particular peptides which can actually be processed and presented at the cell surface [54, 55]. Direct and quantitative

comparison of HLA-ligands in pairs of samples can be achieved by differential isotope labeling of tumoral and normal autologous tissues followed by MS analysis [56].

We have developed an approach for identifying as many new targets as possible for T-cell-based immunotherapy of RCC. The same tumor is analyzed here by MS to identify a large number of HLA-ligands and by genechips to obtain a gene expression profiling [52]. Combining the results obtained with these two methods enables the identification of HLA-binding peptide sequences derived from antigens overexpressed or exclusively expressed in individual tumor tissue compared with autologous normal renal tissue or with a panel of healthy organs. One advantage here is the direct identification of ligands for a variety of HLA alleles, including HLA-A, -B as well as -class II molecules. All these sequences may not represent relevant targets for tumor-directed effector T cells, but they can be then synthesized and used in vitro to induce specific cells and to check their functionality. In particular, the capacity of these T-cell lines tumor cells expressing a physiological amount of the antigen can be examined. The main drawback is the amount of material which should be available for peptide extraction and the efforts involved. However, even with just a small amount of tumor material, RNA extraction for genechip analysis is generally still possible. In this case, known epitopes derived from individual overexpressed genes can be selected as T-cell targets.

Using this combined strategy, we recently identified several new RCC-associated antigens and derived HLA-class I ligands [52, 57, 58]. Two particularly interesting tumor antigens were the adipose differentiation-related protein (adipophilin, ADFP) and the c-met protooncogene (c-met) (see Table 1).

ADFP is a protein associated with lipid droplets in diverse cell types such as adipocytes or some macrophages. We found an overexpression of ADFP mRNA in 5/11 fresh RCC tumors analyzed, all of the clear cell subtype, whereas the expression in a panel of 25 healthy tissues including the normal kidney was either absent or very weak. The only organs expressing relevant amounts of ADFP were the placenta, mammary gland and liver, but were always less than twofold as compared to the normal kidney. ADFP is a strikingly abundant protein source for HLA-ligands, since we found seven different ADFP-derived peptides associated with HLA-A2, -A3, -A31, -A68 and -B7 in 5 of the 13 tumors analyzed. The HLA-A2-restricted peptide was synthesized, loaded onto DC and used to prime specific cytotoxic cells from healthy donor PBMC. The cytotoxic T-cell line obtained was able to kill peptide-loaded targets as well as tumor cell lines expressing ADFP in an antigen and HLA-restricted manner, demonstrating that the ADFP-derived ligand represents a tumor-associated T-cell epitope [59].

Overexpression of the c-met has been described in many tumor types. In line with these results, we observed a high level of c-met mRNA in all the tumors analyzed (11/11) as compared to the normal kidney tissue. Gene expression profiling of other healthy tissues showed a generally very weak expression [57]. From c-met we could identify one HLA-A2-restricted epitope [60]. Other candidate T-cell epitopes are currently under investigation, including some regulator of G protein signaling protein 5- or apolipoprotein L1-derived ligands. Using HLA-tetramers refolded with selected peptides, we have also screened the PBMC of RCC patients for the presence of CD8 populations specific for described or candidate epitopes derived from RCC-associated antigens, including ADFP and c-met. Specific stainings were only marginally observed, suggesting that these peptides do not generally induce peripheral T-cell responses (C. Gouttefangeas, unpublished data). However, one can hypothesize that the description of pre-existing responses against one particular antigen or epitope in cancer patients does not represent a *sine qua non* condition for its use in vaccination. Rather, the stimulation of a "naïve" or subdominant anti-tumoral T-cell repertoire might prove to be more efficient in clinical settings.

Tumor escape mechanisms

In many cases as exemplified above, the immune system of patients is able to recognize and kill the autologous or HLA-matched tumor cells specifically, at least in vitro. Why these T-cell effectors are finally unable to eliminate in vivo the tumors that they infiltrate, and more generally which inhibitory mechanisms are involved in renal cancer, as well as in other tumors, is the object of intensive investigations.

Downregulation of surface HLA expression by tumor cells

Partial or complete loss of HLA expression at the cell surface has been described in many cancer types. However, this does not seem to be a major cause of tumor immune escape in RCC, at least in clear cell type tumors. In particular, only 3/45 tumors analyzed were found to have lost HLA heterozygosity [61]. Immunostaining with an anti-HLA Ab detecting HLA-A, -B and -C molecules (specific for complexed, but also free heavy chains) revealed expression of HLA molecules in almost all the tumors analyzed, regardless of their histological subtype [62]. In this study, the HLA-class II expression by some tumors was also reported. Confirming these results, we have recently detected the HLA-class II expression not only by tumor infiltrating immune cells but also by the RCC cells themselves (J. Dengjel, submitted). In contrast to these observations, various defects in the components of the HLA-class I antigen processing system have frequently been detected, such as a transporter associated with antigen processing 1 or LMP2 deficiencies occurring in the majority of the samples analyzed. At least in established tumor cell lines, these defects are not always irreversible and expression can be restored by treatment with IFN-γ [63].

Suppression of T-cell function by tumor cells

Another escape mechanism is the "silencing" of T cells by tumor cells, which can be mediated by direct interaction or by secretion of immunosuppressive factors. This could lead to the low expression levels of the CD3ζ chain which have been detected in peripheral T lymphocytes or TIL [64, 65]. Renal tumor cells have been shown to express diverse molecules with potential modulatory effect on immune effectors, for example NK receptor ligands for NK inhibitory receptors (e.g. HLA-G, the ligand of ILT2, ILT4 and possibly CD158d, all present in a subset of activated T cells), FasL or more recently B7-H1. Their possible involvement in TIL suppression is actively explored [62, 66–68].

Role of regulatory T cells

Since $CD4^+CD25^+$ T regs have recently emerged as a crucial subset for regulating immune responses, several groups have examined their prevalance in cancer patients. This is rendered difficult by the fact that not only T regs, but also activated $CD4^+$ do express CD25 (the α-chain of the IL-2 receptor) and until now, functional assays are crucial to unambiguously characterize T regs. Increased numbers of T regs are found in patients with various cancers, including ovarian tumors, where they have been associated with poor prognosis [69–71]. Only one very recent report has examined these cells in RCC ($n = 12$) as well as in melanoma ($n = 45$) patients before and after treatment with a high dose of IL-2 [72]. First, the frequency of peripheral T regs defined by their phenotype ($CD4^+CD25^{high}$) and their suppressive function was elevated in RCC as compared to healthy volunteers (7.2 vs. 2.2%). Second, two groups could be established following IL-2 administration: patients with objective clinical responses (eight melanoma and three RCC) experienced a decrease in the number and frequency of T regs which reached the level observed in healthy donors, whereas an increase in T regs was observed in patients with progressive disease ($n = 37$ including nine RCC). These interesting data question the possible dual role of IL-2 in vivo and suggest that T regs could impair the response to IL-2 immunotherapy. To which extent tumor cells are able to induce or recruit T regs, and how this can contribute to the immune tolerance of cancer is a challenging question. Recently, T regs specific for the TAA LAGE1 have been detected in the TIL population of a melanoma patient showing that this subset could develop inside tumors and probably participate in the local dysfunction of effector lymphocytes [73].

Antibody-mediated clinical trials

Considerable efforts have been made over the last years to improve the efficacy of monoclonal Ab-based immunotherapy of cancer. New reagents including Ab coupled to toxins or radioactive components as well as bispecific Ab are currently being tested in many adjuvant trials worldwide. Few constructs, targeting three antigens, have been tested for RCC. They are listed in Table 2. Two of them are currently under phase III development.

Targeting of the tumor cells

Using Ab binding to the G250 (CA-IX) protein and coupled to iodine 131, earlier studies have demonstrated efficient and specific tumor targeting in patients [74]. These studies provided the impetus for further development of anti-G250 Ab for in vivo application. WX-G250 (Rencarex) is a chimeric monoclonal Ab (75% human, 25% mouse sequence) which is specific for G250 and acts by ADCC induction. An initial multicentric phase II study on 36 patients with mRCC who received several infusions of the Ab has established feasibility of the approach. Ten patients experienced temporary tumor stabilization after the first Ab cycle, eight of whom had a documented progressive disease before entering into the trial. One complete response was observed after conclusion of treatment, whereas the median survival time was 15 months [75]. In a follow-up study, the combination of a low dose of IL-2 with WX-G250 was found to improve the clinical benefit and prolong the median time of survival to 22 months [76]. Overall, the Ab showed very good safety and tolerability levels, no severe adverse effects were reported, and a low immunogenicity was noted. The Wilex company registers a 39% survival rate at 2 years for patients included in the first trial. A multicenter phase III study using WX-G250 Ab as monotherapy for non-metastatic RCC is currently under clinical development.

Targeting of the tumor vasculature

Recent results indicate that the tumor suppressor gene von Hippel-Lindau (VHL) is frequently mutated in RCC. Following this mutation, the hypoxia-inducible factor alpha is dysregulated, which finally leads to an overproduction of vascular endothelial growth factor (VEGF),

Table 2 RCC-associated antigens targeted in the published Ab-based clinical trials

Antigen	Tumor expression[a]	Ab name	Addional drug	Clinical trials
G250/ CA-IX	90%	Rencarex	–	[75]
		Rencarex	Low dose IL-2	[76]
VEGF	80–100%	Bevacizumab	–	[78]
		Bevacizumab	Thalidomide	[79]
		Bevacizumab	Erlotinib	[80]
		Bevacizumab	IFN-α	[81] (ongoing)
EGFR	85%	ABX-EGF	–	[82]
		Cetuximab	–	[83]

[a] Reported expression on RCC primary tumors by RT-PCR or immunohistochemistry

stimulating tumor angiogenesis [77]. Bevacizumab (Avastin), a humanized Ab directed against soluble VEGF, is the first angiogenesis inhibitor approved for the treatment of colon cancer (in combination with 5-FU chemotherapy). A first clinical trial recruiting 116 pre-treated patients with mRCC showed a prolonged time to disease progression for the group receiving a high dose of Ab as compared to placebo controls (median time of 4.8 and 2.5 months, respectively). However, only 4/39 patients treated with the high dose of Bevacuzimab experienced objective partial clinical responses, whereas no difference in the overall survival between groups was observed [78]. Ab was well tolerated in this study, although serious associated effects have been noted in other trials. Additional non-Ab agents neutralizing the VEGF pathway (including the promising small molecule kinase inhibitors) are currently under clinical investigation and have been reviewed recently [77]. Several studies have investigated the combination of Bevacizumab with other drugs, such as thalidomide [79] or the EGFR-small molecule inhibitor [80], with variable success. A randomized phase III trial combining Bevacizumab with standard IFN-α immunotherapy vs IFN-α alone is ongoing [81]. This and further studies should help to determine optimal combinations, dose and schedule application, and the overall benefit for RCC patients.

Other targets

The epidermal growth factor receptor (EGFR) is reported to be overexpressed in approximately 85% of the renal cell tumors. Two EGFR specific Ab have been tested for RCC patients, ABX-EGF, a fully human anti-EGFR Ab, and Cetuximab, which is a chimeric construct. The ABX-EGF Ab was well tolerated but led only to marginal clinical responses [82]. Cetuximab has been shown to be efficient against different epithelial-derived tumors and is now used frequently in combination with irinotecan for the treatment of advanced colorectal cancer. Disappointing results reported for this Ab in the first clinical assay do not support its further evaluation in the case of RCC [83].

Immunotherapy based on tumor targeting by T cells

T lymphocyte infusion

Adoptive transfer of ex vivo expanded autologous T-cell populations has been continuously tested and improved for many years, especially for melanoma patients. Early studies revealed no or only minor partial responses in mRCC patients after TIL transfer [84, 85]. In a later pilot trial, polyclonally activated $CD8^+$ cells derived from autologous tumoral tissues were infused in combination with a low dose of IL-2. Survival rate was 65% 1 year after nephrectomy and the overall median survival for all the patients was 22 months [86]. Unfortunately, this was not confirmed in a following multicentric phase III trial, where treatment with autologous $CD8^+$ TIL plus IL-2 did not improve the response and the survival rate of patients as compared to IL-2 alone [87]. The logistical problems associated with such strategies as well as the disappointing results obtained in the last study do not encourage the further use of adoptive T-cell transfer for RCC, unless combined approaches are tested, as recently shown for melanoma patients [88].

Antigen-undefined mixtures

Several phase I/II studies have been published in which mixtures of TAA in the form of irradiated whole tumor cells, tumor-derived cellular lysates or tumor-derived total RNA were applied. In the latter two cases, in vitro expanded monocyte-derived DC are fed with the tumoral products (by spontaneous uptake or electroporation) and subsequently injected into the patients. This concept has the advantage of being independent of the described T-cell epitopes. In theory, it also allows simultaneous immunization against several tumor antigens, including rare or individual ones in autologous settings, as well as T-cell stimulation against diverse HLA-presented class I and also class II-helper peptides. Major drawbacks are the application of undefined cellular products, including potential tolerizing T-cell epitopes, and the difficulty involved in the in vitro immunomonitoring of the anti-vaccine response. Nevertheless, all investigators report on the safety of these approaches, with only rare adverse effects.

A randomized phase III study for high risk non-metastatic RCC patients vaccinated with autologous devitalized tumor cells showed a slight benefit for treated patients over the control group, with a 5-year disease-free survival of 77 and 68%, respectively [89]. GM-CSF-transduced tumor cells have been reported to generate T-cell responses including multiple $CD8^+$ antigen-specific reactivities [90, 91]. Intradermal or intranodal injections of monocyte-derived DC loaded with autologous tumor cell lysate induce also immunological responses (including DTH and anti-tumoral T-cell activity), but only rare clinical responses, mostly partial, were reported so far for mRCC patients [92–94]. Combining DC vaccination with low dose IL-2 did not seem to improve benefit [95]. The most promising results were described after vaccination with RNA-transfected DC in a small cohort of mRCC patients ($n = 7$) [96]. T-cell activity (IFN-γ production) was detected in the majority of patients evaluated after vaccination, including specific recognition of RCC-TAA such as G250, telomerase or the oncofetal antigen immature laminin receptor (OFA-iLR), but induction of an efficient $CD4^+$ T-cell immunity remains to be investigated. Seven out of ten patients were still alive after a mean follow-up of 19 months. Although it should be noted that most of them were given additional treatment (essentially IL-2) after vaccination was completed, these results are very encouraging.

Other groups have concentrated on the use of allogenic products for vaccination, such as preselected RCC tumor cell lines (either irradiated or as lysates), allogenic DC loaded with allogenic or autologous tumor cell lysates, or hybrid cell products [97]. The underlying concept is to induce a strong T-cell allogenic response against the foreign HLA molecules applied which should provide help for the self-restricted anti-tumoral activity. Here again, vaccination is well tolerated, but clinical benefit does not seem to be superior to autologous products [98, 99].

Vaccination with defined T-cell epitopes

In contrast to the other tumors, including melanoma, there is a striking lack of reports on the use of T-cell HLA-class I and/or -class II epitopes for clinical trials in RCC patients.

Only one completed study has been presented very recently using autologous DC preloaded with two class I epitopes derived from the MUC1 protein as well as a pan HLA-class II-binding peptide. Based on the previous encouraging results for breast and ovarian cancer patients, 20 HLA-$A2^+$ patients suffering from mRCC received repeated subcutanous injections of DC in combination with low dose IL-2 [100]. Vaccinations were well tolerated with no side effects. The authors report on metastasis regression in six patients (complete or partial responses), which was accompanied by an enhancement of post-vaccination T-cell-specific activity against MUC1 peptides and MUC1-expressing tumor cell lines. Another specific vaccination attempt was reported briefly using synthetic peptides derived from the mutant VHL sequences (Achtar et al. ASCO annual meeting 2004, abstract no. 2589); detailed results are pending. Finally, a phase II trial for HLA-$A2^+$ patients with advanced RCC but without evidence of metastatis is currently in the recruiting stage, where the HLA-A2-class I epitope derived from G250 will be applied in adjuvant (Montanide) in combination with GM-CSF and different doses of IL-2. First data of this ongoing study are urgently awaited.

Concluding remarks

Adjuvant cytokine therapies with IL-2 or IFN-γ have shown relative efficacy for a subset of 10–20% of the patients with metastatic RCC. Despite various efforts, including the application of Ab or tumor-derived products for activating T cells, progress in immunotherapy is still modest. For RCC, the TAA identified so far are often not tumor cell specific or are expressed in a small proportion of the tumors only. There is thus a need for more adequate $CD8^+$- and importantly also $CD4^+$-specific TAA-derived epitopes for use in immunotherapy and its monitoring. In this regard, the identification of target antigens for T cells, and also for Ab-based trials, should be pursued further. However, based on the experience with melanoma, it seems unlikely that tumor antigen-specific immunotherapy alone will be able to increase clinical benefit for treated patients to a great extent. Instead, combined therapies are being designed, for example Ab treatment plus chemotherapy, as already tested for the other cancer types. Various adjuvants for activating the immune system are also available. Finally, other alternatives, such as CTLA4 blockade [101], depletion of T regs [102], or even the very promising small molecules kinase inhibitors (Sunitinib, Sorafenib) [77], can be combined with antigen-targeting immunotherapy. Such new combinations are hoped to bring major improvements for the majority of patients.

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