Renal cell carcinoma (RCC) accounts for 2% of all new cancer cases worldwide (1). It is estimated that 57,760 new cases will be diagnosed with cancer of the kidney and renal pelvis in the United States in 2009, and 12,980 of these cases will die of their cancer (2). RCC remains a difficult malignancy to treat. Radical nephrectomy can be curative for early stage disease; however, approximately one-third of patients present with metastatic disease, and a further third will subsequently relapse after initial surgery (3, 4). MRCC seems to be resistant to cytotoxic chemotherapies (5), hormone therapies, and radiotherapies with an objective response rate of <10% (6). Additionally, MRCC responds modestly to monoclonal antibodies and tumor vaccines (7). New targeted agents such as tyrosine kinase inhibitors (sunitinib and sorafenib), a mammalian target of rapamycin inhibitor (temsirolimus), and a monoclonal antibody against vascular endothelial growth factor (bevacizumab) have been developed and are currently the standard of care for most patients with MRCC (ref. 8; for generally accepted guidelines see the National Comprehensive Cancer Network Web site).1 Although these agents represent a major advance in the treatment of this disease, they are palliative treatments and rarely, if ever, produce durable complete remissions. Cytokines such as interleukin (IL)-2 and IFN-α were the standard of care before the advent of the above targeted agents and generally also produced only modest benefits (9). It is, however, intriguing that high-dose IL-2 can produce durable complete remissions in small numbers of patients (10). This is at the expense of considerable toxicity and thus careful patient selection by clinical and other criteria is appropriate (9). These limited successes indicate the potential value of optimizing T cell–based immunotherapy for MRCC with the possibility of increasing the number of durable responses.

Here, we review the main strategies of T cell–based cancer immunotherapy in RCC including the adoptive transfer of autologous or allogeneic lymphokine-activated killer cells (LAK), tumor-infiltrating lymphocytes (TIL), as well as genetically modified T cells. More importantly, we discuss potential strategies for improving the efficacy of T cell–based immunotherapy of RCC as learned from successful attempts in melanoma.

**Tumor-Associated Antigens in RCC**

Kessler and Melief (11) have summarized four important criteria to classify tumor-associated antigens (TAA) as ideal potential targets for immunotherapeutic purposes: (a) TAA should be broadly expressed and shared between patients and/or cancer types; (b) they are tumor specific and are rarely expressed in healthy tissues; (c) they play an important role in the oncogenic process and/or cancer survival, and (4) finally, the possible changes in turnover kinetics of the TAA. There are increasing numbers of TAA that have been identified as potential targets

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1 http://www.nccn.org
for immunotherapeutic approaches in various cancers especially melanoma. However, there are relatively few TAA identified in RCC with a striking lack of reports on the use of HLA class I and II–restricted T-cell epitopes in clinical trials of RCC patients (12). TAA in RCC can be classified into four groups: (a) overexpressed antigens shared by many tumors, (b) antigens expressed by the majority of RCC but not by normal tissues, (c) antigens expressed occasionally by RCC and other tumors, and (d) products of gene mutations. Although one of the main limitations for the use of the overexpressed TAA in clinical protocols could be triggering of immunologic tolerance (12), the main limitation for the less expressed TAA (such as RAGE-1, SART1, PRAME, and Hsp70) is the infrequent expression on RCC and restriction to specific HLA background, thus limiting their widespread applications (13).

G250/carbonic anhydrase (CA)-IX is one of the most extensively studied RCC-associated antigens and the first antigen to be defined using a monoclonal antibody approach (WX-G250; ref. 14). It is expressed on >75% of clear cell RCC, and less frequent expression on other malignancies or normal tissues (15). CA-IX antigen has been targeted using many forms of immunotherapy to treat RCC patients. A clinical benefit was achieved in some patients by the administration of chimeric monoclonal antibody G250 either on its own (16) or with low dose of IL-2 (17). A peptide-based dendritic cell vaccination in HLA-A24-positive RCC patients was shown to be safe with 21 months median survival time (18). Although, T cells were successfully transduced with CA-IX chimeric immune receptor (CIR), an early phase trial of the adoptive transfer of these cells was terminated at an early stage due to liver toxicity (19), which seemed to occur as a result of "on-target" effects due to expression of the G250 antigen on bile duct cells.

The oncofetal antigen 5T4 has been shown to be expressed on the surface of RCC (20). This TAA is a heavily glycosylated cell surface protein found on human placental trophoblast and on a wide range of human cancer but it is not expressed at significant levels on normal adult human tissues (21). Vaccination of RCC patients with modified vaccinia Ankara delivering the tumor antigen 5T4 (TroVax) administered alongside IL-2 or IFN-α was safe and well tolerated (22, 23), and a phase III study comparing standard treatment with TroVax in combination with standard treatment has been completed. Additionally, an antibody-delivered superantigen therapy targeting 5T4 protein (ABR-214936) produced occasional durable responses and appeared to prolong survival in a phase II study in MRCC patients (24).

Human endogenous retrovirus type E is the most recent RCC antigen to be discovered (25). It was identified using allogeneic T cells from a MRCC patient achieving complete response following hematopoietic stem cell transplantation (HSCT). Human endogenous retrovirus type E is expressed in RCC cell lines and fresh RCC tissue but not in normal kidney or other tissues, which make it a potential target for immunotherapy.

### Strategies of T Cell–Based Immunotherapy

Barnes and colleagues (26) have proved the feasibility and efficacy of the transplantation of homologous bone marrow after irradiation in an animal model of leukemia. These preliminary murine experiments were crucial steps for a large scale of early phase trials in human that eventually lead to the application of allogeneic HSCT to a growing number of hematologic (27) and some solid malignancies (28). HSCT in RCC showed extremely variable response rates, ranging from 0% to 57% (7). In view of the limitations of allogeneic HSCT such as low overall response rate, transplantation-associated complications and the requirement for an HLA-matched family member to donate stem cells (7), the attention was directed to autologous adoptive cell therapy (ACT). A substantial improvement in efficacy of ACT was achieved in 2002 with the introduction of an immunodepleting preconditioning regimen before the adoptive transfer, which improved the clonal repopulation of patient's T cells with antitumor activity (29). The main strategies of T cell–based immunotherapies are illustrated in Fig. 1 and described below.

#### Lymphokine-activated killer cells

Mazumder and Rosenberg (30) reported on the efficacy and feasibility of the adoptive transfer of lymphocytes activated in vitro by IL-2 (named LAK) to mediate cancer regression in an animal model. This was followed by the initiation of many phase I trials in human cancers in which the magnitude of responses in patients with MRCC, melanoma, and colorectal carcinoma were variable (31). Since then, different ACT protocols using LAK have been assessed in many phase I/II trials for the treatment of patients with MRCC. The objective response, as defined by either complete or partial response, of various clinical trials using LAK in RCC varied remarkably. IL-2 administration method and the use of chemotherapy agents with or without surgical intervention posttherapy (32–34) varied between the different clinical trials, and possibly have contributed to the response variation observed. However, the randomized trials revealed no survival benefit of this approach in RCC patients (35) and improved methods of ACT have been investigated.

#### TILs

In murine experiments, TILs have been shown to exert potent antitumor effects in vivo (36) and their adoptive transfer was more effective in eradicating micrometastasis than LAK cells (37, 38). In 1987, Muul and colleagues (39) were the first to report on the feasibility of growing TILs from human melanoma tumors in IL-2–supplemented media and to show the ability of these cells to recognize autologous tumor in a MHC-restricted pattern. Shortly afterwards, this work was...
translated into a clinical trial where autologous TILs were adoptively transferred into melanoma patients alongside high dose IL-2 with or without cyclophosphamide and resulted in almost 30% overall objective response rate (40).

The protocol of adoptive transfer of TILs requires the *ex vivo* isolation of TILs from tumor tissue fragments cultured in the presence of IL-2. Reactive cells are then selected by their IFN-γ secretion when cultured with autologous or allogeneic MHC-matched tumor cell lines. These cells are then rapidly expanded using IL-2, anti-hCD3, and irradiated allogeneic peripheral blood mononuclear feeder cells. The expanded cells are then reinfused with systemic IL-2 given to complete the course of treatment (41). Recent protocols use intensive lymphodepleting chemotherapy with fludarabine and cyclophosphamide, sometimes with total body irradiation, to enhance survival of adoptively transferred T cells (29, 41). In view of the easy accessibility of melanoma tumor tissue and the high frequency of tumor-specific T cells, this approach is attractive for melanoma patients and seems effective for patients with metastatic melanoma refractory to other treatments (42). Nonetheless, there are number of practical limitations with TIL adoptive therapy for other tumors. It is not always possible to isolate TILs from all patients, nor all tumor types, and it is also a very labor-intensive process requiring the expansion of a specific set of cells for each individual patient (43). There are, however, new approaches that are less complex as they expand all TILs rather than selecting reactive cells. These approaches seem effective and could simplify TILs therapy to make it applicable to more centers (44, 45). Few trials of ACT with TILs have been done in RCC with poor objective response, as shown in Table 1, whereas others are yet to report including those incorporating preconditioning chemotherapy (identifier, identifier).
Adoptive cell therapy is often not feasible because of the difficulties in generating sufficient number of T cells with antitumor reactivity. To overcome this problem, genetic programming of T cells toward a desired reactivity against a targeted antigen has been developed with some promising results (49). This approach enables the generation of therapeutic quantities of T-cell populations with defined antitumor characteristics in a relatively short period of time. It also allows the introduction of tumor specific TCRs that are not present naturally, and hence provide a strategy to overcome the limitations of the endogenous T-cell repertoire (50). In gene transfer approach, virus vectors are used to transduce autologous or allogeneic lymphocytes to express either a tumor antigen-specific T-cell receptor (TCR) or a CIR.

The first successful clinical trial of gene-modified T cells in the treatment of solid cancers was conducted in melanoma patients. Sixteen patients with MART1-positive tumors received ACT of MART1 gene–modified T cells with two complete responders (51). More recently, other receptors have been explored with good responses seen, although at the expense of some “on-target” toxicity (52). Lymphocytes genetically engineered to express TCRs against a broad array of cancer antigens (53, 54) are now available and they may be of value for the adoptive immunotherapy of patients with a variety of common malignancies. To date, there are no completed clinical trials using TCR-transduced T cells for treating RCC patients; however, the successful transduction of human T cells with cloned RCC-antigen–reactive TCR has been achieved in vitro (49) and a trial targeting TRAIL bound to the DR4 receptor has commenced (identifier, NCT00923390).2

As an alternative to the use of full-length TCR genes, several groups have developed CIR for gene transfer into T cells. CIR comprise of a single-chain antibody fragment, which recognizes tumor-associated cell-surface protein, fused to a transmembrane region and a cytoplasmic sequence with signaling function (most commonly used are CD3ζ, the intracellular tail of the Fc receptor γ chain or MHC II domain; ref. 55). Although the only CIR trial in RCC to date had to be stopped early due to liver toxicity (19), several trials using this approach for other cancers have been initiated, and several others are in various stages of planning. However, results from only a few of these studies have been published thus far (56). In renal cancer, a CIR that targets ST4 has been tested in vitro and in preclinical models both alone and in combination with a vaccine (57).

One in vitro restriction of the CIR is the inadequate activation of the T cells resulting in compromised antigen-dependent IL-2 production, proliferation, and survival (58). To overcome this limitation, second generation CIR T cells have been developed. By introducing the CD28 domain into the single chain scFvζ or γ, these second generation receptors enable T cells to proliferate upon stimulation with the relevant TAA-positive tumor cells, even without exogenous IL-2. Furthermore, these transduced T cell also exhibit enhanced cytokine secretion and protection against apoptosis upon antigen stimulation (59).

Future Perspectives

Factors contributing to the limited success of T cell–based immunotherapy in MRCC are being addressed, and strategies are being suggested to improve the outcome of this therapy, as illustrated in Fig. 2 and discussed below.

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2 http://www.clinicaltrial.gov

### Table 1. TIL-based clinical trials in RCC

<table>
<thead>
<tr>
<th>Reference</th>
<th>Trial phase and tumor group</th>
<th>Administration method</th>
<th>Patient number</th>
<th>Number of objective responders</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topalian et al. (38)</td>
<td>Phase I; RCC and other solid</td>
<td>TIL+ HDIL-2+ CPM</td>
<td>12</td>
<td>2 (I RCC)</td>
<td></td>
</tr>
<tr>
<td>Kradin et al. (94)</td>
<td>Phase I; RCC and other solid</td>
<td>TIL+ CI IL-2</td>
<td>28</td>
<td>5 (2 RCC)</td>
<td></td>
</tr>
<tr>
<td>Bukowski et al. (95)</td>
<td>Phase I; RCC</td>
<td>TIL+CIIL-2 (escalating dose) vs TIL alone</td>
<td>18</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Dillman et al. (96)</td>
<td>Retrospective analysis. RCC, melanoma, and colorectal cancer</td>
<td>TIL+ CI IL-2/HDIL-2</td>
<td>55</td>
<td>1 (RCC patient)</td>
<td></td>
</tr>
<tr>
<td>Thionn et al. (97)</td>
<td>Phase II; RCC with previous IL-2 treatment</td>
<td>TIL</td>
<td>6</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Goegegebuure et al. (98)</td>
<td>Phase I; RCC and melanoma</td>
<td>TIL+ moderate dose IL-2</td>
<td>20</td>
<td>2 (non RCC)</td>
<td></td>
</tr>
<tr>
<td>Figlin et al. (99)</td>
<td>Phase III, RCC</td>
<td>CDB TIL + CI IL-2 vs CI IL-2 alone</td>
<td>81</td>
<td>9.9%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>79</td>
<td>11.4%</td>
<td></td>
</tr>
</tbody>
</table>

**Remarks:** Dose/benefit relationship between the total number of TIL infused and survival. CD4 TIL may improve an initially response induced by IL-2 therapy. Treatment with CDB TIL is not superior to IL-2 in MRCC.

**Abbreviations:** HDIL-2, high dose IL-2; CI IL-2, continuous infusion IL-2; CPM, cyclophosphamide.
Identification and selection of TAA-specific T cells. One important aspect of cell-based immunotherapy is the in vitro identification of tumor-reactive T cells to be selected and expanded to numbers sufficient to exert antitumor activity in vivo. Identification of tumor-specific T cells has proven to be difficult in RCC. This is mainly due to the limited number of TAA identified in RCC and the low immunogenicity of these antigens. Strategies that can lead to the identification of new and more immunogenic TAA in RCC are needed. HLA class I and II epitopes identified for RCC-associated antigens have recently been described by Gouttefangeas and colleagues (12), and the common TAA such as hTERT, MUC-1, Survivin, and Her2/neu can be a source of well characterized class I epitopes. In our laboratory, we have identified some HLA class I and II–restricted 5T4-derived epitopes, and we are currently investigating the isolation of 5T4-specific T cells for further expansion and application in immunotherapeutic settings. Clearly, the lack of well-defined RCC-associated antigens limits the possibilities for ex vivo identification and selection of RCC-specific T cells before administration to patients (60). Alternatively, nonselected ex vivo-expanded TILs may be used for adoptive cell transfer in RCC, as will be discussed later.

It is vital to develop protocols that may improve selection of tumor reactive T cells. CD137 (4-1BB) expression (61, 62) and IFNγ (63) release have been used recently for the identification and selection of antigen specific T cells in melanoma and other tumors, and could be used for the selection of RCC-reactive T cells before expansion.

Expansion of TAA-specific T cells. Tumor-specific T cells compromise only a small proportion of the total lymphocytes with a frequency generally much less than 1% of total peripheral blood lymphocytes and between 2% and 15% of total TILs reported for melanoma patients (64). These cells should be expanded to sufficient numbers before transfusion. Polyclonal and antigen-specific T cells can be expanded using autologous antigen-presenting cells (APC). However, autologous APCs have some disadvantages that limit their practical use, and currently artificial APCs (aAPC) represent a potentially

Fig. 2. Schematic diagram showing main aspects of concern of cell-based immunotherapy and potential solutions.
cost-effective and less labor-intensive approach to expand T cells \textit{ex vivo} for ACT (65). aAPCs are genetically modified to express selective costimulatory molecules that induce T-cell expansion. Bead-based anti-CD3/CD28 aAPCs induce the expansion of CD4+ T cells efficiently, but not CD8+ T cells (66). Expansion of antigen-specific cytotoxic T cells was achieved using aAPCs coated with HLA-Ig and CD28-specific antibody (67). In a recent study, it has been shown that aAPCs expressing anti-CD3/4-1BBL preferentially expand memory CD8+ T cells, resulting in superior enrichment of antigen-specific cells (65). Another study reported the efficient expansion of antitumor CD8+ T cells, which specifically lyse melanoma cell lines, by using aAPCs expressing HLA class I, CD54, CD58, CD80, and CD83 (68).

IL-2 is currently the main cytokine used for \textit{in vitro} expansion and \textit{in vivo} activation of tumor-specific T cells. However, other cytokines have recently shown promising activity. IL-15 was found to play an essential role in the development, homeostasis, function, and survival of natural killer, NKT, and CD8+ T cells (69). Furthermore, it has been shown that RCC-reactive T cells expand better using IL-15 compared with IL-2 (70). IL-7 is another cytokine that plays a role in early T-cell development (71) as well as mediating the homeostasis of naïve and memory T cells in mice (72). It is also essential for the development of T cells in humans (73) with a preferential expansion of naive T-cell subsets (74) and relative decrease in Treg levels being shown after IL-7 administration (75). These cytokines, either individually or in combinations, may contribute positively to improving the expansion of T cells for cellular immunotherapy of RCC.

Shortening the expansion time could be vital for the success of cellular immunotherapy as long cultures often lead to cell exhaustion and may be the clinical deterioration of patients while waiting for TILs preparation (76). A new approach has recently been developed by the adaptation of “young TILs” for ACT in melanoma (44, 45). This approach uses the entire resected tumor to rapidly expand TILs for infusion without testing or selecting cells based on their reactivity for tumor recognition (45). This successful protocol in melanoma was shown to be adoptable for the development of TILs from RCC. In a recent study, RCC-derived TILs were isolated and expanded efficiently with an antitumor activity as confirmed by cytotoxicity and IFN-γ release against autologous targets in a MHC class I-restricted and nonrestricted manners (76).

\textbf{Significance of the transferred T-cell characteristics.} As inferred from melanoma trials, certain characteristics of the transferred cells had a profound effect on the outcome of ACT. Antitumor T cells that express lymph node–homing surface markers CCR7 and CD62L and costimulation markers CD28 and CD27, which are characteristic of central memory cells, were more effective than highly differentiated cells that had lost expression of these markers (77). The age of the transferred TILs has also correlated with their efficacy. TILs with longer telomerases and expression of CD27 and CD28 have a greater proliferative potential and longer \textit{in vivo} persistence (45). Furthermore, the absence of an objective response following administration of highly selected tumor-reactive CD8+ T-cell clones suggests that the polyclonal nature of tumor reactivity and possibly the presence of CD4+ T cells are necessary to mediate tumor rejection (78, 79). Another important factor in the success of ACT is the phenotype of the transferred cells. Although CD8+ T cells have been identified as potent effectors of the adoptive antitumor immune response, tumor-specific CD4+ T cells were also identified as an equally critical component (80). The importance of CD4+ T cells was further shown with the successful treatment of a melanoma patient using the adoptive transfer of autologous tumor-specific CD4+ T cells (81).

\textbf{Removal of suppressive factors.} Clinically, the success of ACT depends on number of factors that support the effector function of the transferred cells. Lymphodepletion before adoptively transferring cells has been shown to be an effective strategy in maintaining the persistence of transferred cells in vivo (82). Although the explanation of this observation is still not fully understood, the removal of Tregs and subsequently the suppressive effect on the effector T cells, and the removal of other lymphocytes that compete with the transferred cells for the homeostatic cytokines “cytokine sinks” (41, 83) have been proposed as possible mechanisms. The increased persistence and function of the transferred cells may be enhanced by the administration of cytokines. IL-2 is the standard cytokine used for this purpose; however, in view of its toxicity and ability to expand the Tregs population, alternative methods such as other cytokines or stimulating vaccines (82) could be useful.

The selective removal of suppressive factors from the transferred cells is an attractive strategy. ACT regimen may be improved by depleting CD4+ cells or Tregs, or using antibodies to block inhibitory signals on lymphocytes such as CTLA4, PD-1, or transforming growth factor β. The B7-H1/PD-1 interaction seems to be particularly important in RCC and needs to be considered (84). We and others have reported increased levels of Tregs in peripheral blood mononuclear cells of RCC patients, compared with healthy donors, and their frequency was significantly higher in TILs than peripheral blood mononuclear cell (85–87). In addition, we found that higher Treg level was associated with adverse overall survival (87). Based on these findings, we have done a phase I clinical trial by the adoptive transfer of Treg-depleted (using anti-CD25 magnetic beads) autologous T cells in patients with advanced RCC following conditioning chemotherapy (88). However, this approach provided only a transient reduction of circulating Treg levels, which was associated with improved antitumor immune response. Interestingly, the patient with increased antitumor response had the highest baseline Treg level. We have previously discussed the limitations associated with Treg targeting and the striking need for specific surface markers expressed solely on Tregs, and novel agents targeting Tregs selectively (89). Curiel (90) has also summarized the main strategies for managing Treg in cancer immunotherapy and the advantages for inhibiting Treg activity over their depletion (91).

\textbf{T-cell immunotherapy–induced autoimmunity.} A major problem of the transfer of genetically modified T cells is the possible induction of either on-target or off-target autoimmune reaction (19, 50). Although this is a difficult problem to tackle, a readily available technique might represent a feasible solution. The suicide gene is a newly developed safeguard method aimed at the selective inhibition of the transferred T-cell function. This is achieved by introducing a specific gene into the genetically modified T cells. In case of autoimmunity, the suicide gene is activated \textit{in vivo}, which results in the transduced lymphocyte lysis (92, 93).

\textbf{Concluding Remarks}

Treatment of MRCC represents a great dilemma in clinical practice. Although there are currently number of therapeutic options...
available such as immunomodulating cytokines and the antiangiogenic targeted agents, these are commonly toxic and generally only of palliative benefit. High-dose IL-2 can produce occasional durable responses and give hope that this can be built upon with improved cellular immunotherapy. Cellular immunotherapy has been used to treat cancer patients, and has been shown to be a powerful therapeutic tool in melanoma. RCC is an immunosensitive tumor but unlike melanoma, ACT has not yet been successful and more work is still required to improve its efficacy in RCC. Recent developments in generating sufficient numbers of TILs together with current lymphodepletion protocols are being investigated and warrant further development. Identifying new TAA, optimizing the identification, isolation/modification, and expansion of tumor-specific T cells are all important areas to be considered for improving T-cell-based immunotherapy. Finally, the considerable success of cellular immunotherapy in some cancers especially melanoma warrants further efforts in applying this treatment to metastatic renal cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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