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 extensively explored in melanoma and RCC as they poorly respond to conventional cytotoxic agents but show responses to a variety of immunologic agents. The recent considerable success of T cell–based immunotherapy in melanoma warrants further efforts to apply this treatment to other cancers including MRCC. Although RCC is an immunosensitive cancer, similar attempts in MRCC have shown a very limited success. In this review, we summarize the clinical data on T cell–based immunotherapies for MRCC showing the modest success that has been achieved to date. More importantly, we discuss potential strategies for improving its efficacy for the treatment of MRCC in light of the important achievements for treating metastatic melanoma. In particular, the growing evidence of success by combining expanded tumor-infiltrating lymphocytes with lymphodepletion merits investigation in MRCC. Identifying new RCC-associated antigens, optimized methods, and conditions for detection, isolation, and/or modification and expansion of tumor-specific T cells are all important strategies to be pursued for improving T cell–based immunotherapy of MRCC. (Clin Cancer Res 2009;15(21):OF1–8)

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...
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 MRCC 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 MRCC 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 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.

**Translational Relevance**

The treatment of metastatic renal cell carcinoma (MRCC) remains a therapeutic challenge. Although the recently developed targeted agents are currently the standard of care for most MRCC patients, the benefit is relatively limited and there are few durable remissions once treatment is stopped. Durable complete remissions in some MRCC patients treated with interleukin-2 indicates the potential role of immunotherapy in this cancer. T cell–based immunotherapy using natural or gene-modified T cells has shown success for treating patients with metastatic melanoma. Similar attempts in MRCC have not yet been encouraging, and it is vital to address factors contributing to this limited success and highlight potential strategies to improve the efficacy of T cell–based 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,
Although the difficulty in generating sufficient numbers of RCC reactive T cells in vitro remains the main drawback of a successful TILs adoptive therapy in RCC, several other factors also contribute. They include resistance of RCC cells to TIL-mediated apoptotic pathways (46), loss of CTL function following tumor infiltration (47), poor definition of the target antigen expressed on tumor cells, and poor localization of T cells in tumor sites (48).

**Gene-modified T cells.** 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). Gene transfer methods both alone and in combination with a vaccine (57). Studies have been published thus far (56). In renal cancer, a CIR that targets 5T4 has been tested in vivo in preclinical models both alone and in combination with a vaccine (57). One in vivo 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.
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
cost-effective and less labor-intensive approach to expand T cells 
*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 *in vitro* expansion and 
*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, homeosta-
sis, 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 naive 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 indi-
vidually or in combinations, may contribute positively to improv-
ing 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 test-
ing or selecting cells based on their reactivity for tumor recogni-
tion (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 effi-
ciently with antitumor activity as confirmed by cytotoxicity 
and IFNγ release against autologous targets in a MHC class I–
restricted and nonrestricted manners (76).

**Significance of the transferred T-cell characteristics.** As in-
ferred from melanoma trials, certain characteristics of the trans-
ferred cells had a profound effect on the outcome of ACT. Antitumor T cells that express lymph node–homing surface mar-
kers 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 corre-
lated with their efficacy. TILs with longer telomeres and expression 
of CD27 and CD28 have a greater proliferative potential and 
longer *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 pa-
tient using the adoptive transfer of autologous tumor-specific 
CD4+ T cells (81).

**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). Al-
though the explanation of this observation is still not fully un-
derstood, 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 trans-
ferred cells is an attractive strategy. ACT regimen may be im-
proved 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 inter-
action seems to be particularly important in RCC and needs to 
be considered (84). We and others have reported increased le-
vels of Tregs in peripheral blood mononuclear cells of RCC pa-
tients, 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 pro-
vided 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 dis-
cussed 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 inhibit-
ing Treg activity over their depletion (91).

**T-cell immunotherapy-induced autoimmune.** A major prob-
lem 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 sui-
cide 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 mod-
ified T cells. In case of autoimmunity, the suicide gene is activated 
*in vivo*, which results in the transduced lymphocyte lysis (92, 93).

**Concluding Remarks**

Treatment of MRCC represents a great dilemma in clinical prac-
tice. Although there are currently number of therapeutic options
available such as immunomodulating cytokines and the anti-angiogenic 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 developments. 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.

References

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