T-cell adoptive immunotherapy using tumor-infiltrating T cells and genetically engineered TCR-T cells

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Abstract

Immunotherapy has received the expectation that it should contribute to the therapy of cancer patients for >100 years. At long last, recent clinical trials of immunotherapy with immune checkpoint inhibitors and adoptive cell therapy with genetically engineered T cells have reported their remarkable efficacies. Nowadays, it is expected that T-cell adoptive immunotherapy can not only control tumor progression but even cure cancer in some patients. Conversely, severe adverse events associated with efficacy have frequently been reported in clinical trials, suggesting that the assessment and control of safety will be indispensable in the future development of the therapy. New approaches in T-cell adoptive immunotherapy such as reduction of adverse events, targeting of new antigens or utilization of allogeneic cells will open a new gate for less harmful and more effective immunological treatment of cancer patients.

Keywords: adoptive T-cell therapy, gene engineering, neoantigen, T-cell receptor, tumor-infiltrating lymphocytes

Introduction

After the proposal of the cancer immunosurveillance concept by Burnet and Thomas in the late 1950s (1), people became enthusiastic about the potential of the immune system to recognize and eradicate cancer and expected the application of the power of immunity to the therapy of cancer patients. At last, recent clinical trials of immune checkpoint inhibitor therapy have shown its efficacy in the treatment of patients with various types of malignancy including melanoma, non-small cell lung carcinoma, ovarian cancer or renal cell carcinoma (2). Simultaneously, chimeric antigen-receptor (CAR)-T cell therapy—which involves infusion of in vitro-expanded T cells with tumor-reactivity by transducing an artificial receptor gene CAR that contains the antigen-binding region of an antibody fused with the signal-transduction domains of CD3ζ and co-stimulatory molecules such as CD28 or 4-1BB—has been reported to be significantly effective in the treatment of progressive B cell malignancy (3). A related type of genetic engineering that generates T-cell receptor (TCR)-T cells—which express tumor antigen-specific TCRs that consists of α and β chains of TCR genes derived from a tumor-reactive T cell clone—has also been shown to induce tumor regression in many types of tumors (4, 5).

Adoptive transfer of tumor-infiltrating lymphocytes (TILs) has been also successful in the treatment of patients with melanoma. Recent advances in the next-generation sequencing technology is starting to define the target molecules of the TIL therapy, suggesting the importance of neoantigens derived from individual mutations in the tumor cells. A recent report on adoptive immunotherapy with marrow-infiltrating lymphocytes for the treatment of patients with multiple myeloma indicated a significant reduction in disease burden and increased in progression-free survival (6), suggesting the extension of the concept to hematological malignancy.

Reports of successful treatments of cancer patients with adoptive T-cell therapy encouraged academia and industry to rapidly introduce such treatments into clinical use. However, it is becoming increasingly evident that this approach holds promise, but not without severe adverse events. Particularly, the use of artificially engineered TCRs needs high caution because of possible unexpected cross-reactivity to normal tissue because such TCRs have never gone through the thymic checking system that excludes self-reactive T cells.

In this review, I discuss the promising future and challenges in the development of adoptive cell therapy with TILs and genetically engineered TCR-T cells. CAR-T cells will be discussed in the review by Frigault and Maus in this issue.
Adoptive immunotherapy using TILs

Long before the development of genetically engineered T-cell therapy, there were attempts to isolate TILs from surgically removed tumor samples followed by the stimulation by tumor cells or tumor antigens in vitro to generate large numbers of tumor-reactive T cells to be infused into cancer patients (4, 5, 7). This approach has been tested almost exclusively in the treatment of melanoma patients with very few exceptions because of the extraordinary high success rate in generating primary tumor cell lines and TIL lines in melanoma patients compared with the patients with other tumor types. Rosenberg et al. treated metastatic melanoma patients with patients’ TILs and reported a 49–72% response [complete remissions (CRs) plus partial responses (PRs)] ratio according to the Response Evaluation Criteria in Solid Tumors (RECIST) measurements (4, 5, 7). Recently, successful treatment was also reported in a patient with cholangiocarcinoma (8). Importantly, many patients who experienced CR after TIL therapy were reported to sustain CR status.

There have been two major technical improvements that increased efficacy of TIL therapy. One is the pretreatment of patients with lympho-depleting chemotherapy or radiotherapy before the infusion of TILs (4, 5, 7). This strategy was shown to improve the proliferation and in vivo survival of the transferred lymphocytes resulting in increased antitumor efficacy of TIL therapy. Another is the shortened period of in vitro culture of TILs before the infusion into the patients (4, 5, 7). Long-term in vitro culture has been recognized to endow lymphocytes with senescent phenotypes with a lowered capacity of survival in vivo. Attempts to culture lymphocytes with cytokines such as IL-21 or IL-15 were also reported to enhance the in vivo survival and function of the lymphocytes after the infusion into patients (9, 10).

Adoptive immunotherapy with TCR-T cells

As mentioned above, adoptive immunotherapy with tumor-reactive T cells derived from TIL has been used almost exclusively to treat patients with malignant melanoma, with very few exceptions, because of the difficulty of isolating and expanding pre-existing tumor-reacting T cells from patients with tumor types other than melanoma. To overcome this limitation, patients’ lymphocytes have been genetically engineered to express tumor antigen-specific TCRs that consists of α and β chains of TCR genes derived from a tumor-reactive allogeneic T-cell clone, generating genetically engineered cells, TCR-T cells.

In 2006, Rosenberg et al. reported that metastatic melanoma patients were treated with lymphocytes genetically engineered to express a TCR specific for a melanocyte-differentiating antigen (MART-1), resulting in long-term persistence of infused cells and tumor regression in 2 of 17 patients (11). A subsequent study with a higher-affinity TCR reported tumor regression in 6 (30%) of 20 patients (12). In addition, a study using a modified high-avidity TCR recognizing NY-ESO-1 antigen demonstrated objective clinical responses in 4 (60%) of 6 patients with synovial cell sarcomas and 5 (45%) of 11 patients with melanoma, according to RECIST criteria (13), and the many patients who experienced CR showed sustained CR status (14). Table 1 summarizes the reports of clinical trials of TCR-T gene therapy, some of which have reported highly promising results.

The existence of endogenous TCRs in lymphocytes has been reported to reduce the expression of transduced TCRs and cause the assembly of mispaired TCRs between endogenous and transduced TCR α and β subunits that produces TCRs with unexpected specificity including self-reactive TCRs (15). To solve this issue, introduction of an additional disulfate bond in TCR constant (C) regions (16), replacement of human αα and ββ domains with corresponding murine C domains (17) or introduction of short inhibitory RNA (siRNA) molecules specific for endogenous TCRs in retrovirus vectors has been proposed (18). Genome-editing technology such as Zinc finger nucleases was reported to work for the same purpose (19). So far, no severe adverse events related to mispaired TCR formation have been observed in clinical trials.

Future challenges of T-cell adoptive immunotherapy

Prediction and minimization of adverse events

Although remarkable clinical responses have been observed in therapies using adoptive immunotherapy with genetically engineered T cells, adverse events have occurred with a high frequency in many trials as shown in Table 1.

Specifically, patients treated with lymphocytes with a MART-1-specific or a gp100-specific high-affinity TCR exhibited severe histological destruction in normal tissues where melanocytic cells were present, including skin, eyes and inner ears (12). Patients with metastatic colorectal carcinoma treated with a CEA-specific high-affinity TCR exhibited severe inflammatory colitis possibly because of the reactivity to CEA expressed in normal mucosa in colon (20). Three of nine patients treated with MAGE-A3-specific high-affinity TCR (HLA-A2 restricted) exhibited mental disturbance, and two of them died of leukoencephalopathy (21). In these cases, cross-reactivity to MAGE-A12, which contains the epitope sequence, was considered to be responsible. In a trial using another MAGE-A3-specific high-affinity TCR (HLA-A1 restricted), two patients died from cardiac toxicity. In those cases, a peptide derived from cardiac muscle-expressing protein, titin, was demonstrated to cross-react with the TCR even though the titin-derived peptide possessed limited sequence similarity (five out of nine amino acids) to the MAGE-A3-derived epitope peptide (22, 23).

The native TCRs for self-antigens such as cancer-testis antigens (e.g. NY-ESO-1 or the MAGE-A family), developmental antigens (e.g. MART-1 or gp100) or over-expressed antigens (e.g. CEA or HER2) showed low to intermediate affinity. It is unclear whether these TCRs can induce effective tumor eradication or not. In contrast, high-affinity TCRs against these antigens were established by immunizing HLA-transgenic mice or by genetic modification of TCR sequences (4, 5, 7). When these methods are employed, the resultant TCRs have never experienced thymic negative selection in human bodies, and therefore have not gone through ‘inspection’ for cross-reactivity to normal tissues.

To reduce the risk in utilizing these receptors, it is critical to develop a preclinical study strategy for predicting the adverse effects. To predict cross-reactivity, it would be informative to combine database searches, use core
# Table 1. TCR-T cell gene-therapy clinical trials

<table>
<thead>
<tr>
<th>Antigen/TCR type</th>
<th>Disease/pts</th>
<th>Pretreatment</th>
<th>Adverse events</th>
<th>Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MART-1/wild type</td>
<td>Melanoma/17 pts</td>
<td>Cyclophosphamide (60mg kg⁻¹ x 2 days) + fludarabine (25mg m⁻² x 5 days)</td>
<td>No related toxicities</td>
<td>PR 2/17</td>
<td>(11)</td>
</tr>
<tr>
<td>MART-1/high affinity Melanoma/20 pts (MART-1) gp100/mouse derived Melanoma/16 pts (gp100) p53/mouse derived Breast cancer/4 pts Melanoma/2 pts Esophageal cancer/1 pt Others/2 pts</td>
<td>Cyclophosphamide (60mg kg⁻¹ x 2 days) + fludarabine (25mg m⁻² x 5 days)</td>
<td>G2 skin, eye, G3 ear</td>
<td>PR 6/20</td>
<td>(12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CR 1/16, PR 2/16</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PR 1/9</td>
<td>(27)</td>
</tr>
<tr>
<td>CEA/mouse derived Colorectal cancer/3 pts</td>
<td>Cyclophosphamide (60mg kg⁻¹ x 2 days) + fludarabine (25mg m⁻² x 5 days)</td>
<td>G3 diarrhea (inflammatory colitis)</td>
<td>PR 1/3</td>
<td>(20)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Decrease of CEA 3/3</td>
<td></td>
</tr>
<tr>
<td>NY-ESO-1/high affinity Melanoma/11 pts Synovial cell cancer/6 pts</td>
<td>Cyclophosphamide (60mg kg⁻¹ x 2 days) + fludarabine (25mg m⁻² x 5 days)</td>
<td>No related toxicities</td>
<td>CR 2/11, PR 3/11</td>
<td>(13)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PR 4/6</td>
<td></td>
</tr>
<tr>
<td>MAGE-A3/mouse derived Melanoma/7 pts Synovial cell cancer/1 pt Esophageal cancer/1 pt</td>
<td>Cyclophosphamide (60mg kg⁻¹ x 2 days) + fludarabine (25mg m⁻² x 5 days)</td>
<td>3 mental disturbance (2 died of necrotizing leukencephalopathy)</td>
<td>Regression 5/9</td>
<td>(21)</td>
<td></td>
</tr>
<tr>
<td>MAGE-A3/high affinity Melanoma/1 pt Myeloma/1 pt</td>
<td>Cyclophosphamide (60mg kg⁻¹ x 2 days) or melphalan, auto-SCT</td>
<td>2 cardiogenic shock, died (off-target effect)</td>
<td>NE</td>
<td>(23)</td>
<td></td>
</tr>
<tr>
<td>MART-1/high affinity Melanoma/14 pts</td>
<td>Cyclophosphamide (60mg kg⁻¹ x 2 days) + fludarabine (25mg m⁻² x 5 days)</td>
<td>2 respiratory distress (in pts with fresh cell transfer)</td>
<td>Regression 9/14</td>
<td>(28)</td>
<td></td>
</tr>
<tr>
<td>MAGE-A4/wild type Esophageal cancer/10 pts NY-ESO-1/high affinity Multiple myeloma/20 pts</td>
<td>None</td>
<td>Autologous-SCT with high-dose melphalan</td>
<td>No related toxicities</td>
<td>3 long survivors nCR or CR 14/20</td>
<td>(29)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 GvHD</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 skin rash</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 diarrhea</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1 hypotension etc.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CR, complete response; GvHD, graft-versus-host disease; nCR, near complete response; NE, not evaluated; PD, progressive disease; pts, patients; SCT, stem-cell transplantation; SD, stable disease; VGPR, very good partial response.
sequence analysis based on amino-acid substitutions, screen for reactivity against a panel of normal cells and use complex tissue–organ cultures. It is also important to select appropriate antigens. A strategy for monitoring patients, early detection and treatment, such as administration of anti-IL-6R antibody because IL-6 plays one of the major roles in cytokine release syndrome, should be established. Development of the technology that controls the fate of the infused cells including the incorporation of a suicide gene will be useful (24).

Identification of new targets
The second challenge is the identification of new targets for effective immunotherapy. There has been a very limited list of targets for effective and safe T-cell adoptive immunotherapy. It was suggested that the effectiveness of the TIL therapy performed for melanoma patients might be attributed to the T cells specific to neoantigens created from specific mutation of genes in individual cancers (4, 8, 25). Targeting neoantigens would be advantageous for the development of a 'tailor-made-option' for immunotherapy of cancer. Supported by the recent advances in the next-generation sequencing technology, it is becoming possible to detect mutations unique to each cancer in individual patients in a short period with relatively reasonable costs. It is possible that the immunotherapy targeting neoantigens will be a realistic choice for the treatment of cancer patients in the near future.

Utilization of allogeneic T cells
The third challenge in the development of effective TCR gene therapy is the use of allogeneic cells or cell lines instead of autologous cells. This approach has the potential to provide ‘off-the-shelf’ cell-therapy products supplied in a timely way to the patients who need the therapy. It may also overcome the low availability and low quality of lymphocytes obtained from patients with malignancies. To practically use allogeneic T cells, it is necessary to control issues of graft-versus-host disease caused by transduced alloreactive lymphocytes and rejection of transferred lymphocytes by the host immune system. Attempts to solve these issues with technologies such as siRNA, genome editing or induced pluripotent stem-cell technology have been suggested (18, 19, 26). Donor-lymphocyte infusion after allogeneic stem-cell transplantation might be a first step for the usage of allogeneic T cells for cancer immunotherapy because it does not require the control of rejection of transferred T cells. Future development of the technology controlling the rejection of transferred cells will be required to use allogeneic T cells in the broad settings of immunotherapy.

The clinical characteristics of T-cell adoptive immunotherapy
One major clinical characteristic of T-cell adoptive immunotherapy is that this approach often shows tumor response that can be evaluated by RECIST (4, 5, 7). Similar to the trials for solid tumors, hematological tumor responses such as the decrease or clearance of blast cells were frequently observed in the trials targeting hematopoietic malignancy (3). This is a characteristic that makes clear contrast to many tumor vaccine trials. However, it is possible that the appearance of effectiveness may need a relatively long time resulting in a delayed response after progression.

Another major clinical characteristic is the sustained antitumor effect for a long period (4, 5, 7). The sustained of not only CRs but even PRs in some patients was reported to continue without tumor progression, although the precise mechanism is not clear yet. The sustainment of response can contribute to the observation of a plateau of the patient survival curve resulting in the improved overall survival.

Conclusion
T-cell adoptive immunotherapy has shown the clear potential to be a remarkably effective treatment of patients with cancer. Conversely, there are issues that we need to manage such as the prediction and control of adverse events associated with efficacy, search for new targets including neoantigens and the development of off-the-shelf cell products including utilization of allogeneic lymphocytes. We envisage that T-cell adoptive immunotherapy will provide an effective and safe clinical treatment for patients with many malignancies by overcoming these issues in the near future.

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