Gene-modified T cell therapy for cancer: Current challenges and potential solutions

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**Abstract**

Immunotherapy has achieved significant breakthroughs in patients with hematological diseases and various cancers. The adoptive transfer of T cells, especially gene-modified T cells such as chimeric antigen receptor T (CAR-T) cells and T cell receptor-engineered T (TCR-T) cells, is developing rapidly. Since 2017, eight CAR-T cell products have been approved for the treatment of hematological diseases, such as refractory or relapsed acute B lymphoblastic leukemia, specific subtypes of B cell lymphoma, and multiple myeloma. The first TCR-T cell product, Kimmtrak, was approved for the treatment of unresectable or metastatic uveal melanoma in March 2022. However, there are still many problems, including side effects, relapse, high demand for individualization, and expensive cost in hematological diseases, tumor heterogeneity, antigen escape, poor immune cell infiltration ability, immunosuppressive microenvironment, and low response in solid tumors. With the in-depth exploration of tumor immunology and the development of genetic engineering technology, many novel strategies for improving the anti-tumor effect and safety of gene-modified T cell immunotherapy have been attempted. This paper presents a systematic review of the literature on CAR-T cell and TCR-T cell therapy, focusing on applications, clinical trials, problems, and potential solutions.

**Keywords:** Immunotherapy; Chimeric antigen receptor T cell; T cell receptor-engineered T cell; Tumor microenvironment; Solid tumor

1. Introduction

In recent years, chimeric antigen receptor T (CAR-T) cell therapy has become one of the most promising therapeutic methods in cancer immunotherapy because of the remarkably effective and durable immune response it produces in hematological malignancies\textsuperscript{1,2}. CD19 and B cell maturation antigen (BCMA) CAR-T cell products have been approved for clinical applications worldwide\textsuperscript{3}. This has promoted further large-scale clinical trials of CAR-T cells in other malignant diseases. However, CAR-T cell therapy is afflicted with several problems, such as toxicity, resistance or relapse, and...
hypoergia in solid tumors. Optimization of CAR structures to reduce side effects and improve their efficacy in solid tumors has been widely studied to solve these problems.

Another gene-engineered T cell therapeutic pattern is T cell receptor-engineered T (TCR-T) cell therapy, which exhibits unique advantages in the treatment of solid tumors. TCR-T cells evoke a cytotoxic response and induce tumor regression in vivo. To date, only one TCR-T cell product (Kimmtrak) has been approved for the treatment of unresectable or metastatic uveal melanoma. More clinical trials of tumor-specific/associated antigen- or neoantigen-modified TCR-T cells in solid tumors are underway. While various challenges exist in the application of adoptive T cell therapy, basic research and clinical trials have been conducted to mitigate these limitations. As the technology develops, the accumulation of clinical trial data will support the approval of more engineered T cell products and benefit more patients with tumors. In this review, we discuss the current, challenges, and recent advances in the use of gene-engineered T-cells in cancer immunotherapy. Furthermore, we highlight potential strategies to conquer the disadvantages of extending adoptive T cell therapy.

2. The concept and generation of CAR-T cell therapy

CAR-T cells are prepared using gene-editing technology (i.e. viral or non-viral vector transfection) to endow common T cells with a single-chain variable fragment (ScFv) containing recognized tumor antigens and signals of T cell activation, producing specific recognition, and cytokine functions independent of major histocompatibility complex expression. Specifically, CAR-T cells rely on ScFv in the CAR structure, which recognizes tumor antigens and promotes T cell activation and expansion. Activated CAR-T cells secrete cytokines, such as interleukin (IL)-2 and interferon (IFN)-γ, which can induce apoptosis of target cells. In 1987, Kuwana et al. developed the first CAR prototype by fusing the V region of immunoglobulin with the constant region of the TCR. The first-generation CAR construct contained the ScFv and CD3 signaling regions, which endowed T cells with transient activation and cytotoxicity, but insufficient anti-tumor efficacy. In subsequent studies and clinical trials, second-generation CAR-T cells were developed, which integrated costimulatory signaling molecules CD28 or CD3 signaling regions, which endowed T cells with transient activation and cytotoxicity, but insufficient anti-tumor efficacy. With second-generation CAR-T cells targeting CD19 produces a high objective response rate (ORR) in relapsed/refractory (R/R) acute B cell lymphocytic leukemia (B-ALL), specific types of B cell lymphoma and chronic B cell lymphocytic leukemia, and several autologous CAR-T cell products targeting CD19 have been approved, providing novel therapeutic strategies for R/R B cell malignant diseases. Meanwhile, the products of CAR-T cells targeting BCMA have been approved by the U.S. Food and Drug Administration (FDA) in the treatment of R/R multiple myeloma. However, plenty of clinical trials have been conducted on second-generation CAR-T cells in the treatment of solid tumors without any breakthroughs. Scientists have extensively explored solutions to the current issues with second-generation CAR-T cells, such as reducing toxicity by altering CAR structure or expressing a “suicide gene” and improving the therapeutic effect with dual-target CAR-T cells. Expression of two costimulatory molecules enhances the amplification capacity and anti-tumor effect of CAR-T cells, which are regarded as third-generation CAR-T cells. Chemokine receptors, cytokines, or transcription factor-modified CAR-T cells are named fourth-generation CAR-T cells. Meanwhile, dual- or three-target modified CAR-T cells for preventing antigen escape, or other gene-editing strategies for improving the efficacy are defined as next-generation CAR-T cells. The clinical application and basic study of CAR-T cell therapy in hematological malignancies and solid tumors are described in detail below.

3. CAR-T cell therapy in hematological malignancies

Many clinical trials of differentially targeted CAR-T cells in various hematological malignancies are currently underway, including CD19, CD20, CD7, CD30, CD38, BCMA, CD22, CD70, CD79b, and dual targets. It was reported that 80 – 94% of R/R B-ALL patients who received CD19-targeted CAR-T cell infusion achieved complete remission (CR). Moreover, 80% of diffuse B-cell lymphoma patients had an objective response, and nearly 40% had a long progression-free survival. The result of ZUMA-2, a large clinical trial of CD19 CAR-T cell product TECARTUS, showed that 67% of patients with R/R mantle cell lymphoma achieved a CR. ZUMA-5 indicated that R/R indolent non-Hodgkin's lymphomatous (NHL) patients receiving axicabtagene ciloleucel therapy had a high overall response (92%) and CR (74%). More clinical trials have been conducted to evaluate the potential of CD19 CAR-T cells as a first- or second-line therapeutic method for large B-cell lymphoma. When 41 R/R Hodgkin's lymphoma patients received a CD30 CAR-T cell infusion, the total OR and CR rates were 72% and 59%, respectively. CAR-T cells (idecabtagene vicleucel) targeting BCMA have a high response rate in R/R multiple myeloma and have been approved by the U.S. FDA. In a
study of 33 R/R multiple myeloma patients who were treated by bb2121 CAR-T cells, with a total OR rate of 85%, 15 (45%) of patients achieved CR, with a median disease-free progression survival of 11.8 months\textsuperscript{[24]}. At present, approved CAR-T cell products are all indicated for R/R hematological malignancies targeting CD19 or BCMA.

4. Issues of CAR-T cell therapy in hematological malignancies

Although CAR-T cells produced a high ORR in hematological malignancies, it has certain limitations, including related toxicity in the treatment process, high demand for individualization, long production cycle, expensive production cost, no response and relapse after remission in some patients. Cytokine release syndrome (CRS) and on target, off-tumor toxicity are specific side reactions of CAR-T cell therapy\textsuperscript{[25]}. In addition to toxicity, resistance occurs during CAR-T cell therapy. With an increase in the number of patients receiving CD19 CAR-T cells and long-term follow-up data, 30 – 50% of B-ALL patients receiving CD19 CAR-T cells who achieved a CR would relapse within 1 year\textsuperscript{[26]}. Moreover, 10 – 20% of patients still show no response after treatment with CD19 CAR-T cells\textsuperscript{[27]}. Relapse in patients with multiple myeloma receiving BCMA CAR-T cell infusion is frequent as well\textsuperscript{[28]}. Thus, resistance after CAR-T cell therapy is a major problem in the field of cancer immunotherapy, and understanding the mechanism of resistance and non-responsiveness is critical for enhancing the anti-tumor effect of CAR-T cell therapies. Existing studies have confirmed that functional defects in CAR-T cells, the tumor cell mutations, and tumor microenvironment are implicated in the primary or secondary resistance of B cell malignant disease to CAR-T cells\textsuperscript{[29]}. The following sections describe these problems and their existing and potential solutions.

5. Strategies to overcome issues in CAR-T cells for hematological malignancies

5.1. Toxic reactions of CAR-T cell therapy

CRS is considered a specific side effect related to CAR-T cell infusion, which mainly manifests as fever, hypotension, hypoxia, organ dysfunction, hemocytopenia, coagulation dysfunction, and hemaphagocytic lymphohistiocytosis\textsuperscript{[30]}. CRS is an inflammatory syndrome caused by a variety of cytokines produced by activated CAR-T cells and other immune cells. If CAR-T cells recognized target cells, they secrete large amounts of the cytokines IFN-γ and tumor necrosis factor alpha (TNF-α), which activate monocytes and macrophages to produce large amounts of pro-inflammatory cytokines, predominantly IL-6\textsuperscript{[23,31]}. IL-6 can activate other immune cells through classical signaling pathways, thus further activating T cells in a “positive feedback” manner. It can act on other cells such as endothelial cells to cause systemic inflammatory reactions, mainly involving monocyte Chemotactrant Protein (MCP)-1, IL-8, and IL-6, increased secretion of endothelial permeability factors such as vascular hemophilia factor, and reduced levels of endothelial stabilizer angiotensin 1, which causes loss of vascular integrity, hemodynamic instability, capillary leakage syndrome, and coagulation dysfunction\textsuperscript{[32]}. Moreover, nitric oxide produced by macrophage-derived nitric oxide synthase can further enhance hemodynamic instability and vasodilatation; therefore, macrophage activation plays a key role in CRS development\textsuperscript{[33]}. The risk factors of CD19 CAR-T cell therapy induced-CRS mainly include high tumor burden, high-doses of CAR-T cells, expansion of CAR-T cell in vivo, thrombocytopenia and endothelial cell activation, the status of myelosuppression after pretreatment, and costimulatory molecules of CD28\textsuperscript{[34,35]}. At present, scientists have found that IL-1, catecholamine activation, and pyroptosis of target cells can activate macrophages and participate in the pathogenesis of CRS related to CAR-T cell therapy\textsuperscript{[36-38]}. Thus, a dissection of the mechanisms of CRS production caused by CAR-T cell therapy will be beneficial for exploring therapeutic strategies to control CRS and elevate the safety during CAR-T cell therapy. The cytokines closely related to CRS mainly include IL-6, IL-1, IL-10, TNF-α, and IFN-γ\textsuperscript{[39]}. The IL-6 receptor blocker tocilizumab has been approved for the treatment of CRS produced by CAR-T cell therapy. Glucocorticoids (dexamethasone or methylprednisolone) can be used to treat suboptimal CRS after treatment with tocilizumab injection\textsuperscript{[35]}. In addition, plasma purification (hemofiltration or plasmapheresis) is an effective therapeutic method for handling CAR-T cell infusion-related cytokine storm\textsuperscript{[36,40]}. JAK and IL-1 pathway inhibitors have also been shown to control CRS\textsuperscript{[36,41]}. Activation of the catecholamine system participates in the CRS process, and whether β-receptor blockers can control CRS or reduce grades should be further explored\textsuperscript{[37]}. One of the means to improve safety is altering the CAR structure expressing suicide gene\textsuperscript{[42]}, which can selectively remove CAR-T cells, but increase the risk of disease recurrence. Altering the affinities of the ScFv structure of CAR did not affect its efficacy, but the associated side effects were reduced\textsuperscript{[43]}. Twenty patients with B-cell lymphoma who were treated with Hu19-CD828Z T cells had lower cytokine levels compared to those treated with FMC63-28Z T cells, and the severe neurotoxicity incidence rate was only 5%\textsuperscript{[44]}. In short, the control of CRS should follow the principles of prediction, close monitoring, timely intervention, and prevention (Figure 1).
5.2. Potential strategies to improve the efficacy of CAR-T cell therapy

5.2.1. Improving the persistence of CAR-T cells

The existence of CAR-T cells is associated with a durable anti-tumor ability in vivo. T cell exhaustion is a crucial factor participating in the resistance to CAR-T cell therapy. In the process of target cell activation, some immunosuppressive molecules will upregulate their expression, such as programmed cell death protein 1 (PD-1), and the expression of the ligand of immunosuppressive molecules (i.e. PD-L1) is increased on the surface of tumor cells or other immune cells. Activation of the axis of PD-1/PD-L1 pathway negatively regulates the anti-tumor function of CAR-T cells. CAR-T cells combined with PD-1 blockers were able to produce synergistic anti-tumor immune response. CRISPR/Cas9-edited PD-1 negative CAR-T cells exhibited enhanced anti-tumor ability in vitro and vivo. Some patients with B-cell lymphoma could benefit from the treatment with pembrolizumab after the failure of CD19 CAR-T cell therapy. It was reported that Bruton's tyrosine kinase (BTK) inhibitors could improve the immune response of CD19 CAR-T cells in vitro and in vivo. Patients with B-cell lymphoma resistant to the first CD19 CAR-T cell infusion could benefit from BTK inhibitor salvage therapy and re-treatment with the same CAR-T cell product. Exploring the mechanism of CAR-T cell exhaustion could guide novel strategies for improving anti-tumor effect. DNMT3A-mediated T cell exhaustion has been reported to be regulated by epigenetics. When exposed to a low dose of decitabine, CAR-T cells exhibit enhanced anti-tumor immune responses, cytokine expression and proliferation. Overexpression of c-Jun regulated by Ap-1 could inhibit the exhaustion of CAR-T cells. Basic leucine zipper ATF-like transcription factor (BATF) was confirmed to be participating in the regulation of T cell function through inhibiting the exhaustion-associated marker expression and enhancing the production of effector cytokines. Combining CAR-T cells with an immunopotentiator or altered CAR structure to prevent exhaustion will enhance their anti-tumor ability.

5.2.2. Antigen escape: Dual/three-target modified CAR-T cells

Depending on the analyzed data of the molecular characteristics of relapsed patients infused with CD19 CAR-T cells, single-target CAR-T cells are prone to
immune escape, and 50% of patients experienced target-antigen negative relapse. It has been confirmed that dual- or three-target CAR-T cells could boost the anti-tumor effects of CAR-T cells and reduce immune escape in vitro and in vivo[57]. Moreover, the CR rate of patients with B-cell lymphoma treated with CD19 CAR-T cells is lower than that with R/R B-ALL. Increasing the CR rate of CAR-T cell treatment in B-cell lymphoma is the focus of current research trend. Han et al. reported that CD19/CD20 dual-target CAR-T cells have been displayed to increase the CR rate of patients with B-cell lymphoma. In a phase I/IIa clinical trial, 33 patients with R/R B-cell lymphoma were recruited, 28 of whom received pretreatment and infused with CAR-T cells, the total response and CR rates was 79% and 71%, respectively, and 14% of patients experienced grade 3 CRS[57]. In a trial containing 16 patients with R/R B-NHL treated by CD19/CD22 dual-target CAR-T cells, the overall response rate was 87.5%, the CR rate was 62.5%, and the 2-year overall survival and disease progression-free survival rates were 77.3% and 40.2%, respectively[58]. Similarly, treatment with CD19/CD22 CAR-T cells similarly showed a higher CR rate in patients with R/R B-ALL[59]. In one patient with primary B-cell lymphoma of the central nervous system, CD19/CD70 CAR-T cells induced a CR acquisition, and no significant side effects were observed[60]. CD19/BCMA CAR-T cells had enhanced anti-tumor effect in multiple myeloma[61]. About 87% of R/R multiple myeloma patients who received CD38/BCMA CAR-T cells attained a clinical response, with 52% achieving a stringent CR at a median 9.0 months follow-up[62]. Therefore, the use of dual- or multi-target CAR-T cells for the treatment of hematological malignant disease will improve the CR rate in lymphoma and reduce the recurrence rate of B-ALL. This provides a theoretical and practical basis for the exploration of this treatment in solid tumors[63] (Figure 3).

5.3. Reducing costs with universal CAR-T (U-CAR-T) cells

At present, CAR-T cell products that have been approved for clinical application are individualized therapies with
Figure 3. Reducing antigen immune escape with dual- or multiple-target CAR-T cells. Multiple CAR and tandem CAR structures are designed to improve the ability of CAR-T cells to recognize different antigens on tumor cell surface for preventing antigen immune escape.

many limitations. First, due to their specific matching as well as long production preparation and quality control period, patients with rapid disease progression may miss the treatment opportunity for CAR-T cell infusion. Second, in infants, severe patients, and some patients with immune system damage, their own T-cells cannot be used for CAR-T cell preparation or will undergo culture failure, and they will not be able to receive autologous CAR-T cell infusions. Moreover, the consistency of individual products is difficult to guarantee and the production cost is high, thereby limiting the large-scale production of CAR-T cells and their wide application. Therefore, exploring U-CAR-T cells has become a focus in the field of immunotherapy. TALEN and CRISPR/Cas9 are the two most commonly used gene-editing methods. The main targets of U-CAR-T cell clinical trials include CD19, BCMA, CD7, CD22, CD123, CS1, and mesothelin. Allogeneic CD19 CAR-T cell by silencing T-cell receptor α constant (TRAC) was first reported in 2012. TALEN-edited CD19 CAR-T cells of CD52/TCR killed target cells with high specificity. CRISPR/Cas9 editing of allogeneic CAR-T cells with multiple genes has been reported, and studies have shown that the product of pure CAR expression is safe and efficient, and can direct CAR to the TRAC loci of TCR in T-cells. Simultaneous CD52 knockdown in TCR gene-edited CAR-T cells, combined with CD52 monoclonal antibody, inhibited the immune cell activity in the host and enhanced the expansion of allogeneic CD19 CAR-T cells, and produced an anti-leukemic effect.

Twenty-one patients with R/R B-ALL received U-CAR-T cell infusion, and CR or non-hematologic CR was achieved in 14 patients within 28 days. CRS occurred in 91% of these patients, three patients had Grade 3 – 4 CRS, two patients experienced Grade 1 skin rejection, and eight patients manifested Grade 1 – 2 neurotoxicity. It has been reported that CD19/CD22 dual-target U-CAR-T cells created by CRISPR/Cas9-edited TRAC and CD52 produced a CR rate of 83.3% without dose-limiting toxicity, graft-versus-host disease, neurotoxicity, or other adverse events associated with genome editing in six R/R B-ALL patients. Clinical trials of allogeneic CAR-T cells in other malignancies are in progress.

6. CAR-T cell therapy in solid tumors

CAR-T cell therapy was first intended for use in treating solid tumors, but due to the specificity of the target, the complexity of the tumor microenvironment and tumor heterogeneity, T cell recruitment, and other factors caused poor efficacy in solid tumors. With the development of efficient gene-editing technology, an endless stream of CAR-T therapies has emerged. The selection of CAR-T cell-targeted antigen has important effects on both therapeutic efficacy and safety. Unlike hematological tumors, the therapeutic targets in solid tumors are mostly over-expressed antigens. Thus, those tumor-associated antigens (TAAs) with relatively specific expression and higher individual positivity rates may represent optimal targets for CAR-T cell therapy. At present, the main targets of relevant clinical trials in solid tumors are GPC3, mesothelin, EGFR, PSPA, CEA, and GD2. Although the development of CAR-T cell therapy is rapid, there are still many limitations and challenges in solid tumors. These major challenges include tumor heterogeneity and antigen escape, insufficient immune cell infiltration, tumor immunosuppressive microenvironment, T cell exhaustion, and dysfunction. The clinical trial status of different targets in different tumors is detailed below (Table 1). Thirteen patients with hepatocellular carcinoma infused with a median dose of 19.9 × 10⁶ GPC3 CAR-T cells, eight patients experienced Grade 1 – 2 CRS without Grade 3 – 4 neurotoxicity, two patients achieved partial responses (PR), and one patient maintained a stable disease (SD) and was alive after 44.2 months. Six patients with metastatic pancreatic ductal adenocarcinoma received autologous mesothelin-specific CAR-T cell infusion; none of them experienced CRS and neurotoxicity, two patients achieved SD with progression-free survival times of 3.8 and 5.4 months. Sixteen patients with metastatic pancreatic carcinoma were treated with 1 – 3 cycles of the epidermal growth factor receptor (EGFR)-CAR-T cells within half a year, 4 of 14 evaluated patients achieved PR for 2

Volume 1 Issue 1 (2022)

https://doi.org/10.36922/gpd.v1i1.114
Gene & Protein in Disease

Genetically engineered T cells in cancer immunotherapy

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Table 1. Major published trials of engineered T cell therapy in solid tumors.

<table>
<thead>
<tr>
<th>Type</th>
<th>Target</th>
<th>Disease</th>
<th>Pre-chemotherapy</th>
<th>Cell dose</th>
<th>Number of patients</th>
<th>CR</th>
<th>PR</th>
<th>SD</th>
<th>PD</th>
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<td>GPC3</td>
<td>HCC</td>
<td>Cyclophosphamide</td>
<td>1 - 3×10^6/kg</td>
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<td>/</td>
<td>2</td>
<td>1</td>
<td>/</td>
<td>2</td>
</tr>
<tr>
<td>CAR-T</td>
<td>mesothelin</td>
<td>PDAC</td>
<td>/</td>
<td>1 - 3×10^6/m^2</td>
<td>6</td>
<td>/</td>
<td>/</td>
<td>2</td>
<td>4</td>
<td>/</td>
</tr>
<tr>
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<td>EGFR</td>
<td>MPM</td>
<td>Cyclophosphamide</td>
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<td>8</td>
<td>2</td>
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<td>/</td>
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<tr>
<td>CAR-T</td>
<td>CEA</td>
<td>PC</td>
<td>Nab-paclitaxel</td>
<td>1×10^6 – 1×10^9/kg</td>
<td>16</td>
<td>4</td>
<td>8</td>
<td>4</td>
<td>/</td>
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<tr>
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<td>CRC</td>
<td>Cyclophosphamide</td>
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<td>/</td>
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<tr>
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<td>NY-ESO-1</td>
<td>MM</td>
<td>Cyclophosphamide</td>
<td>/</td>
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<td>2</td>
<td>/</td>
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</tr>
<tr>
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<td>Cyclophosphamide</td>
<td>/</td>
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</tbody>
</table>

BTC: Biliary tract cancers; CR: Complete remission; CRC: Colorectal cancer; GC: Gastric cancer; HCC: Hepatocellular carcinoma; HPV: Human papillomavirus; MM: Multiple myeloma; MPM: Malignant pleural mesothelioma; ORR: Objective response rate; PC: Pancreatic cancer; PD: Progressive disease; PDAC: Pancreatic ductal adenocarcinoma; PR: Partial response; SD: Stable disease; SSC: Synovial sarcoma.

- 4 months, and eight patients had SD for 2 – 4 months. Downregulation of EGFR expression on surface of tumor cells has been found in patients with SD[79]. Twenty-eight patients with gastric cancer received CLDN18.2-targeted CAR T cells, the ORR and disease control rates were 57.1% and 75.0%, respectively, and the percent of overall survival at six months was 81.2%[78]. Two of ten patients with colorectal cancer achieved SD after CEA-CAR-T cell infusion without any severe adverse events[80]. In general, the safety and efficacy of different antigens targeted by second-generation CAR-T cells have been widely evaluated in various cancers. Low incidences of CRS and low responsiveness were observed in solid tumors, indicating that the process of CAR-T cell therapy has been promising but tortuous.

6.2. CAR-T cell trafficking into tumor site

γδ T cells have a natural tumor microenvironment chemotaxis, and GD2-γδ CAR-T cells have a strong anti-tumor immune response, suggesting that γδ CAR-T cells might be more dominant in solid tumors[78]. The chemokine-chemokine receptor axis is a key factor influencing the accumulation of T cells in the tumor fraction, and the combination of chemokines released by tumor cells and chemokine receptors on T cells participates in the resistance to CAR-T cell therapy[77]. To overcome antigen escape, dual- or multiple-target CAR-T cells have been explored in several cancer types[83].

6.1. Tumor heterogeneity and antigen escape

Antigen recognition is a prerequisite for an anti-tumor immune response. Antigen-specific TCR-T or CAR-T cells exert potent anti-tumor effects by targeting TAA. An important reason for the success of immunotherapy in hematological tumors is the existence of clear, uniformly expressed tumor antigens and controllable toxicity. However, due to the heterogeneous expression of solid tumor antigens in terms of intensity and distribution, the TAA currently used for solid tumor treatment is highly expressed in tumor tissues. Downregulation of antigens participates in the resistance to CAR-T cell therapy[77]. To overcome antigen escape, dual- or multiple-target CAR-T cells have been explored in several cancer types[83].

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https://doi.org/10.36922/gpd.v1i1.114
CAR-T cells could increase the infiltration and existence of CAR-T cells\(^{[40]}\). GPC3 and mesothelin CAR-T cells expressing IL-7 and CCL19 produce efficient anti-tumor response in patients with hepatoma and pancreatic cancer, respectively.\(^{[43]}\) In these studies, chemokine receptor- or cytokine-modified CAR-T cells showed significant tumor localization and anti-tumor activity in vivo (Figure 4).

### 6.3. Immunosuppressive microenvironment

In the tumor microenvironment, immunosuppressor cells such as myeloid-derived suppressor cells, regulatory T cells (Tregs), tumor-associated macrophages (TAMs), and various cytokines contribute to tumor progression dependent on promoting tumor invasion and metastasis and inhibiting the T cell immune response.\(^{[84]}\) Overcoming the immunosuppressive tumor microenvironment would elevate the anti-tumor capacity of adoptively transferred T cells in solid tumors.\(^{[46-47]}\) Arming CAR-T cells through expressing pro-inflammatory cytokines such as IL-12, IL-18, or IL-23 can remodel the tumor microenvironment and improve anti-tumor capability.\(^{[46-50]}\) Transforming growth factor beta (TGF-β) is a vital factor for tumor initiation and progression, which negatively regulates antitumor immunity of T cells. It has been reported that TGF-β could promote T cell exhaustion through up-regulating PD-1 expression in cytotoxic CD8^+ T cells\(^{[84,87]}\). CRISPR/Cas9-edited TGF-β R2 enhances the anti-tumor capability of CAR-T cells by reducing the transformation of Tregs and T cell exhaustion\(^{[83]}\). Thirteen patients with metastatic castration-resistant prostate cancer received prostate specific antigen-directed CAR-T cells armored with a dominant-negative TGF-βR; among the patients, five patients experienced grade ≥r CRS, and 4 patients experienced a reduction in prostate-specific antigen level.\(^{[84]}\) The density of TAMs is negatively correlated with the prognosis in various types of solid tumors. Regulating the polarization of macrophages would reshape the tumor microenvironment. For example, folate receptor β (FR β) is highly expressed on the surface of macrophages, and FR β^+TAM is related to poor outcome in pancreatic cancer. FRβ-CAR-T cells could delay tumor progression through eliminating TAM, increasing pro-inflammatory monocytes and promoting endogenous tumor-specific CD8^+ T cell infiltration\(^{[95]}\).

### 7. TCR-T cell therapy

Tumor-specific lysis of T cells is mediated by specific TCR-recognition peptides and HLA complex.\(^{[96]}\) TCR-engineered T cells refers to the genetic editing of T cells in vitro by transducing TCR gene sequences (α and β chains) to T cells, resulting in enhanced ability of T cells to specifically recognize tumor antigens on the tumor cell surface and effectively induce specific lysis of target tumor cells. The TCR expressed by the exogenous gene binds CD3 molecules in a non-covalent bond, forming a TCR-CD3 complex expressed on the cell membrane, which further recognizes the HLA-antigen peptide complex. Unlike CAR-T cells, TCR-T cells can recognize HLA peptides derived from intracellular proteins and kill target cells in HLA-restricted dependent manner.\(^{[96-98]}\) The expression of cancer/testis antigens is silent in normal tissues, except in reproductive system, but is significantly increased in tumor tissues, including melanoma-associated antigen (MAGEA)-3, MAGEA-4, MART-1, gp100, and New York esophageal squamous cell carcinoma (NY-ESO-1), which are considered attractive targets for TCR-T cell therapy.\(^{[99]}\) Approximately 37% of published clinical trials are related to TCR-T cell therapy targeting NY-ESO-1.\(^{[100]}\) In 2011, the first clinical trial of autologous NY-ESO-1-specific TCR-T cells on advanced malignant melanoma and synovial cell sarcoma was conducted; 4 of 6 synovial cell sarcoma patients and 5 of 11 melanoma patients achieved ORR, and with a CR after one year was observed in two melanoma patients.\(^{[5]}\) MAGE-A4 TCR-T cells produced significant anti-tumor ability in patients with esophageal cancer.\(^{[101]}\) However, off-target toxic effects were observed in several patients who received MAGE-A3-specific TCR-T cell infusion.\(^{[102]}\) Fatal neurotoxicity and lethal cardiac toxicity occurred due to cross-reactivity of MAGE-A-specific TCRs in the brain and myocardium with a...
high affinity\textsuperscript{[102,103]}. Although TCR-T cell immunotherapy has shown some clinical efficacy in most treated patients, some obstacles remain to be overcome to realize the real promise of TCR-T cells therapy. Some nonresponsive patients lack in vivo persistence of T cell infusion in clinical trials, suggesting that TCR-T cells require additional support to improve their persistence in vivo\textsuperscript{[104]}. T cell infiltration into the tumor is not observed in some patients with late recurrence, and the infused TCR-T cells confront with an unfavorable immunosuppressive tumor microenvironment. In general, the application of TCR-T cells is still a challenge in many areas, including targeted immunotoxicity caused by normal tissues, low transient efficiency of TCR in engineered T cells, exhaustion and dysfunction of T cells, tumor immune escape and the lack of effective tumor-specific antigens in most patients with tumors\textsuperscript{[105]}. Therefore, overcoming these challenges is key to achieving greater clinical success in the future.

8. Improving the safety and efficacy of TCR-T cell therapy

The persistence and expansion of antigen specific T cells in vivo are related to the response to TCR-T cell therapy\textsuperscript{[100,104]}. The function of tumor antigen-specific T cells expressing exhaustion marker in the tumor is impaired\textsuperscript{[107]}. Exhaustion limits the anti-tumor immune response of T cells, and combination of immune checkpoint inhibitors with TCR-T cells could produce synergistic anti-tumor effect. Like in CAR-T cells, IL-7 and CCL19 were constructed into TCR-T cells to generate potent and durable anti-tumor immunity when synergizing with PD-1 blockade therapy\textsuperscript{[108]}. Co-expressing PD1-41BB-modified PRAME-specific TCR-T cells produced strong anti-tumor reactivity in an animal model\textsuperscript{[109]}. After identifying TCR sequences that specifically targeting neoantigens, TCR-T cells were reconstructed so that they are equipped with the potential anti-tumor ability\textsuperscript{[110]}.

9. Outlooks

The remarkable success of adoptive T cell therapy for hematological tumors (B-ALL, B-cell lymphoma, and MM) makes CAR-T cells a promising therapeutic method for tumors, and promotes the rapid development of gene-modified T cell therapy. However, there are significant differences in anti-tumor mechanisms between CAR-T cells and TCR-T cells, such as structure, target, affinity, costimulatory molecule, and HLA limitation. They have the same issues, including toxicity, poor efficacy, poor infiltration, and immunosuppressive microenvironment in the treatment of solid tumors. The optimization of CAR or TCR structure will further improve its efficacy and safety, and facilitate the development of solid tumor treatment.

We believe the accumulation of findings from basic research and clinical translation will hasten and support the approval of more gene-engineered T cell products for clinical applications.

Acknowledgments
None.

Funding
This study was supported by grants from Healthy Talents Project of Henan Province (YXKC2020052) and the National Natural Science Foundation of China (No.8210286, No. 81771781, No. 81872333).

Conflict of interest
The authors declare that they have no competing interests.

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Writing – review & editing: Xinfeng Chen, Shasha Liu, Zhen Zhang

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