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## REVIEW

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# TCR engineered T cells for solid tumor immunotherapy

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## Abstract

T cell immunotherapy remains an attractive approach for cancer immunotherapy. T cell immunotherapy mainly employs chimeric antigen receptor (CAR)- and T cell receptor (TCR)-engineered T cells. CAR-T cell therapy has been an essential breakthrough in treating hematological malignancies. TCR-T cells can recognize antigens expressed both on cell surfaces and in intracellular compartments. Although TCR-T cells have not been approved for clinical application, a number of clinical trials have been performed, particularly for solid tumors. In this article, we summarized current TCR-T cell advances and their potential advantages for solid tumor immunotherapy.

**Keywords:** T cell receptor, TCR-T cells, Cellular immunotherapy, Solid tumors

## Introduction

Cellular immunotherapy has shown great potential for cancer treatment. This method uses genetic engineering technology to modify T cells to endow them ability to recognize and kill tumor cells [1–4]. At present, there are two common methods for T cell immunotherapy: chimeric antigen receptor T (CAR-T) cells and T cell receptor (TCR) engineered T cells. Of these methods, CAR-T cell therapy has shown exciting results in clinical trials, and several products have been approved for the treatment of hematological malignant tumors [5–12]. Still its effects in solid tumor is unsatisfactory. Currently, TCR-T cell therapy has demonstrated encouraging potential for the treatment of solid tumors [13–16]. This review mainly summarizes the research status of anti-solid tumor immunotherapy using the tumor antigen-specific TCR-T cells.

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## TCR-T cell construction

The TCR is a molecule on the surface of T cells that specifically recognizes and mediates immune responses and consists of two highly variable heterogeneous peptide chains linked by disulfide bonds. TCRs include four peptide chains, α, β, γ, and δ. α and β peptide chains form αβ TCRs, while γ and δ peptide chains form γδ TCRs [17, 18]. αβ TCRs activate the TCR signaling pathway by binding to the major histocompatibility complex (MHC) on tumor cells or antigen presenting cells (APCs), which then activates a series of intracellular proteins including CD3ζ, 70-kD zeta-associated protein (ZAP70), and nuclear factor of activated T cells 2 (NFAT2), thereby mediating T cell immune function [18, 19]. TCR-T cells are constructed by transferring a TCR gene sequence that specifically recognizes tumor antigens into T cells through genetic engineering so that the T cells have the ability to specifically kill tumor cells [1, 20]. TCR-T cells can recognize not only specific antigens on the surface of tumor cells but also intracellular antigens, which allows TCR-T cells to recognize a wider spectrum of target antigens [21, 22].

To construct TCR-modified T cells, isolating and obtaining TCRs that specifically recognize tumor-specific antigen (TSA) or tumor-associated antigen (TAA)
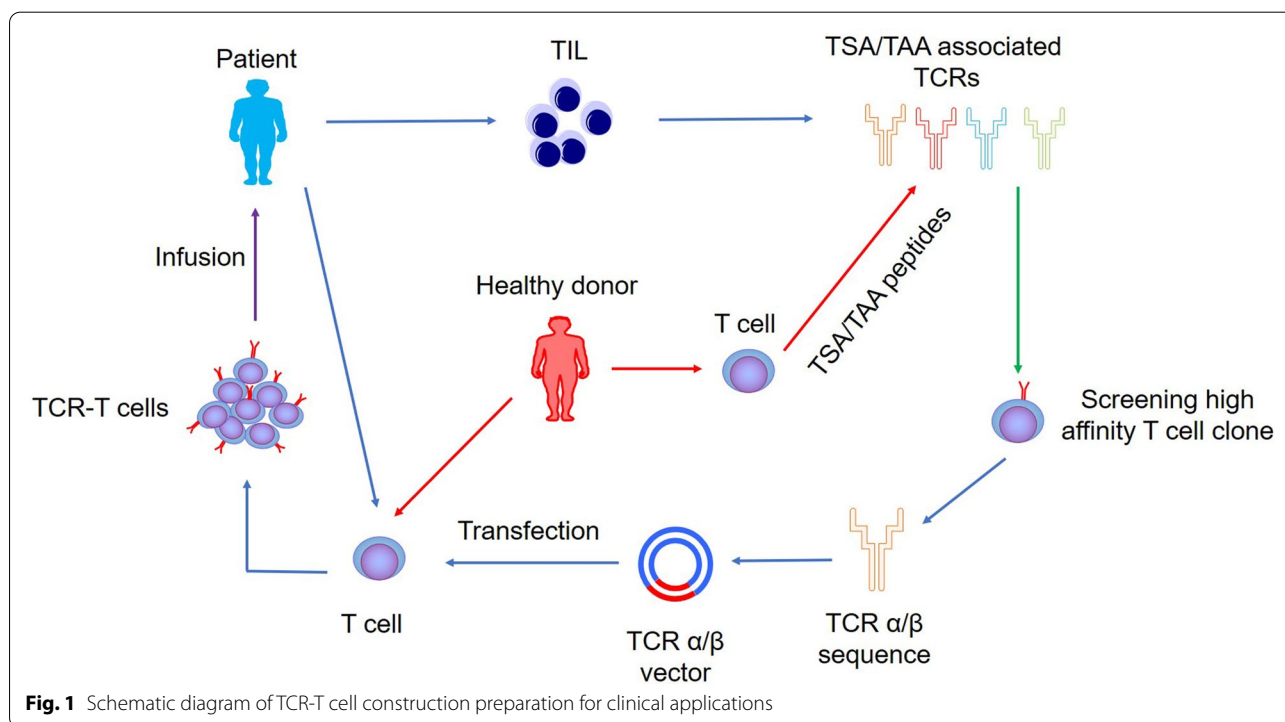
epitopes is the first step [13]. TCRs can be isolated from tumor-infiltrating T cells in the tumor tissues of patients or from healthy donor T cells induced by MHC I/II-restricted TSA or TAA peptides [23, 24]. Once a T cell clone with the highest affinity is obtained, TCR α and β chains can be cloned into the target T cell with the help of molecular cloning to form the capability to specifically recognize tumor antigens (Fig. 1) [25]. The method of obtaining tumor antigen-specific TCRs has also been improving. Our team and other researchers have found that specific TCR Vβ clones may recognize leukemia-associated antigens in leukemia patients by polymerase chain reaction (PCR) and GeneScan. We also discovered that our previously identified chronic myeloid leukemia (CML)-associated TCR Vβ clone specifically recognizes Wilm's tumor 1 (WT1) antigenic peptides by stimulating T cells in healthy donors with MHC-restricted antigenic peptides and TCR Vβ gene sequencing [26]. Recently, our team and partners have also found TCR Vβ clones that are closely related to the prognosis of lung cancer patients [27]. Our team recently analyzed the TCR Vα and Vβ chain genes of T cells from B cell-acute lymphoid leukemia (B-ALL) patients by single-cell sequencing and found that the patients' T cells had polyclonal subtypes, and most of them were effector subsets [28]. These results suggested that tumor antigen specifically paired TCR Vα and Vβ chain sequences can be obtained by single-cell sequencing, which can provide more accurate

information for the subsequent construction of specific high-affinity TCR-T cells. Kisielow et al. proposed a new technique for screening specific T cell epitopes. Through the construction of pMHC-TCR (MCR) engineered reporter cells, this group was able to screen for MHC II T cell epitopes that specifically recognize viral and tumor antigens. The researchers also demonstrated that the tumor neoantigen-associated lymphocytes screened by this platform could protect mice from tumor cells [29]. These findings suggested that the combined application of these techniques may be able to identify and help construct individual patient tumor antigen-specific, high-affinity TCR-T cells.

### The TCR-T cell development process

Since the 1990s, a number of studies have reported that specific TCR Vα/Vβ T cell clones are found in the tumor infiltrating lymphocytes (TILs) and peripheral blood of patients with melanoma [30–34]. These clonal T cells have specific anti-melanoma cytotoxicity, and specific single-chain TCR Vβ T cells expanded in vitro have specific killing effects [33]. Fujio et al. have demonstrated that the TCRαβ gene transduction system generated by retroviruses could reconstruct the antigen-specific immunity of peripheral T cells in mice [35]. Our team also confirmed that specific TCR α/β T cells constructed by genetic engineering could effectively kill CML cells in vitro [36]. These results also suggested that both



**Fig. 1** Schematic diagram of TCR-T cell construction preparation for clinical applications

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single-chain and double-chains TCR-T cells can effectively recognize and kill target cells.

Mice remain the primary model organisms for studying tumor-specific TCR-T cell therapies, and the main T cell model used is αβ$^+$ CTL. In the clinical setting, dynamic analysis of the relationship between the persistence of clonal proliferative TCR Vβ subfamily T cells and disease status can provide more detailed information about specific cellular immune functions when designing immunotherapy for cancer patients. A study has shown that there was a persistent TCR Vα5 /Vβ7 CTL clone in a patient with large cell lung cancer, and this clone could still be detected 3 years after operation [37]. These data suggest that the persistence of this T cell clone may be related to the maintenance of continuous remission in this patient. Melanoma clinical trials have also shown that targeting Melan-A-specific CD8$^+$ CTL cells could effectively alleviate patient conditions [34, 38]. Because γδ$^+$ T cells are independent of the MHC, they also play an important role in tumor-specific immunotherapy. γδ$^+$ T cells induced by donor peripheral blood could effectively kill tumor cells, e.g., Vγ9Vδ2$^+$ T cells could dissolve liver cancer and rectal cancer cells but had no cytotoxic effect on normal tissues [39]. In addition, some studies have shown that a class of T cells expressing natural killer 1.1 (NK1.1) on the surface could play a cytotoxic role in tumor cells that lack expression of human leukocyte antigen-I (HLA-I) molecules. For example, the mucin 1 (MUC1) antigen is expressed in prostate cancer cells, but nearly all metastatic cancer cells lack HLA-I molecules; thus, metastatic cancer cells cannot be specifically recognized by T cells. However, this antigen may play an effective role in anti-tumor cells by stimulating NK cells [40]. Therefore γδ$^+$ T cells and NK T cells were also used to construct TCR-T and TCR-NK cells [41–45].

## Preclinical study

In vitro and animal experiments of TCR-T cells were performed very early. Dembić et al. successfully transduced MHC-restricted TCRα and TCRβ genes in mouse T cells in 1986 [25]. In 1999, Clay et al. successfully transferred melanoma-specific TCR Vα and Vβ chains into human peripheral blood primary T cells and confirmed that the TCR-T cells constructed by this method could effectively kill tumor cells in vitro [46]. With the continuous progression of identification methods and experimental techniques, there are increasing studies on TCR-T cells in vitro and in animal tumor models. Abad et al. constructed a premelanosome protein 1 (PMEL-1) TCR encoding the B16 melanoma antigen gp100 in peripheral blood T cells using a retroviral vector [47], and the TCR constructed in this manner could effectively slow the development of tumor cells in B16 tumor-bearing mice.

Frankel et al. demonstrated that TCR-T cells targeting tyrosinase, an enzyme involved in melanin synthesis, could also effectively kill B16/A2K(b) mouse melanoma cells in vivo [48]. This result further confirmed that the antigens recognized by TCR-T cells were not only located on the surface of cells but also in the cells. Kerkar et al. constructed MHC-I restricted melanocyte differentiation antigen100 (peml-1) TCRs in mouse CD8$^+$ T cells and MHC-II restricted tyrosinase related protein 1 (TRP-1) TCR in mouse CD4$^+$ T cells by a γ-retrovirus (MSGV1). This group confirmed that both MSGV1-peml-1 TCR-CD8$^+$ T cells and MSGV1-TRP-1 TCR-CD4$^+$ T cells could effectively kill tumor cells in B16 melanoma mice [49]. This result further clarified the function and interaction of CD8$^+$ and CD4$^+$ T cells during the anti-tumor process. Heemskerk et al. transduced the ovalbumin (OVA)-specific OT-1 TCRαβ gene into γδ T cells in a mouse model and confirmed that the TCR-T cells constructed in this manner could effectively kill tumor cells and avoid internal dimerization between derived TCRαβ chains [42]. These data indicate that using different types of T cells to construct TCR-T cells can maintain anti-tumor effects and avoid adverse reactions. These results also broadened the range of cell selection for the construction of TCR-T cells. Recently, Wei et al. constructed an HLA-A*02:01-restricted neoantigen library, which was transferred into HLA-matched APCs to stimulate T cells from the peripheral blood of patients. This group then screened and constructed a neoantigen-specific TCR targeting the KIAA1429D1258E mutation, and these TCR-T cells showed efficiency in killing human head and neck squamous cell carcinoma in mice [50]. This result provided a new strategy for screening mutant neoantigen-specific TCRs under conditions where it is difficult to obtain tumor tissue. Moreover, this finding indicates that the construction of TCR-T cells targeting mutant antigens and the specific killing of precise individualized immunotherapy-carrying mutant antigens may be a strategy for clinical trials.

## Clinical study

Clinical trials of TCR-T cells in cancer patients were relatively early. In 1999, Clay et al. constructed melanoma antigen recognized by T cells 1 (MART-1)-targeting TCR-T cells from human primary T cells and successfully transfused them into melanoma patients [46]. In 2006, Morgan et al. reported that they transfused TCR-T cells specifically recognizing MART-1 into 15 melanoma patients and successfully achieved remission in two patients [51]. These results further confirmed the potential of TCR-T cells that specifically recognize tumor antigens to treat tumors. The information on clinical trials of

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TCR-T cell therapy in solid tumors are summarized in Table 1.

### Melanoma

Clinical trials of TCR-T cells in the treatment of solid tumors were first performed in patients with melanoma. A variety of melanoma-associated antigens have also been confirmed to have specific TCR therapeutic potential, and the two most attractive tumor-associated antigens were MART-1 and NY-ESO-1 [52–54].

The results of a clinical trial of targeting MART-1 TCR-T cells combined with a dendritic cell vaccine demonstrated that 9 of 13 patients (69%) had tumor regression after treatment. Among these patients, three who received non-cryopreserved cell therapy had longer persistence of MART-1 TCR-T cells, but two patients developed serious adverse acute respiratory distress events and required intervention [55]. This result suggested that fresh cells might be the ideal cell model for constructing TCR-T cells with a longer-lasting antitumor response, while determining how to reduce its side effects needs further investigation. Another clinical trial with targeted MART-1 TCR-T cell infusion demonstrated that 20% of 20 patients who received MART-1

**Table 1** Major clinical trials of TCR-T cell therapies for solid tumor patients

| Disease | Antigen | HLA | TCR-T cell source | Clinical trial number | Phase | Status |
|---|---|---|---|---|---|---|
| Melanoma | MART-1 | HLA-A*02:01 | PB (patient) | NCT00910650 | II | Completed |
| | MART-1 | HLA-A*02:01 | PB (patient) | NCT00509288 | II | Completed |
| | MAGE-A3 | HLA-DP*04:01/04:02 | PB (patient) | NCT02111850 | I/II | Completed |
| | MAGE-C2 | HLA-A*0201 | PB (patient) | NCT04729543 | I/II | Recruiting |
| | PRAME | HLA-A*02 | PB (patient) | NCT03686124 | I | Recruiting |
| | NY-ESO-1 | HLA-A*0201 | PB (patient) | NCT03638206 | I/II | Recruiting |
| | MAGE-A3/12 | HLA-A*02:01 | PB (patient) | NCT01273181 | I/II | Terminated |
| | NY-ESO-1 | HLA-A*02:01 | PB (patient) | NCT00670748 | II | Terminated (A more highly selected protocol with ESO TCR opened for pts with melanoma) |
| | gp 100 | HLA-A*02:01 | PB (patient) | NCT00509496 | II | Terminated (Low accrual) |
| | PRAME | HLA-A*02:01 | PB (patient) | NCT02743611 | I/II | Unknown |
| Lung cancer | NY-ESO-1 | HLA-A*02 | PB (patient) | NCT02457650 | I | Unknown |
| | NY-ESO-1/LAGE-1a | HLA-A*02:01 HLA-A*02:05 HLA-A*02 | PB (patient) | NCT03709706 | Ia/IIb | Active not recruiting |
| Sarcoma | NY-ESO-1 | HLA-A*02 | PB (patient) | NCT01343043 | I | Completed |
| | MAGE-A4[c1032] | HLA-A*02 | PB (patient) | NCT03132922 | I | Active not recruiting |
| Renal cell carcinoma | HERV-E | HLA-A*11:01 | CD8^{+}/CD34^{+} T cell (patient) | NCT03354390 | I | Recruiting |
| | TRAIL | / | PB (patient) | NCT00923390 | I/II | Terminated |
| Bladder cancer | MAGE-A10[c796] | HLA-A*02:01 HLA-A*02:06 | T cells (patient) | NCT02989064 | I | Completed |
| | MAGE-A3 | HLA-A*01 | PB (patient) | NCT02153905 | I/II | Terminated (Slow, insufficient accrual) |
| Esophageal cancer | MAGE-A4 | HLA-A*24:02 | Lymphocytes (patient) | UMIN000002395 | I | Completed |
| Metastatic cancer | CEA | HLA-A*02:01 | PB (patient) | NCT00923806 | I | Terminated (Study was terminated due to poor accrual) |
| Pancreatic cancer | KRAS G12D | HLA-C*08:02 | TIL (patient) | NCT03935893 | II | Recruiting |
| Hepatocellular carcinoma | HBV | HLA-A*02:01 HLA-Cw*08:01 | PBMC (patient) | NCT03899415 | I | Recruiting |
| | AFP[c332] | HLA-A*02 | T cells (patient) | NCT03132792 | I | Recruiting |
| HPV[+] cancer | HPV-16 E6 | HLA-A*02 | PB (patient) | NCT02280811 | I/II | Completed |
| | HPV-16 E7 | HLA-A*02 | PB (patient) | NCT02858310 | I/II | Recruiting |

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TCR-T cells responded, while 19% of 16 patients who received targeted gp100 TCR-T cells responded [56]. Similarly, clinical trials also demonstrated encouraging results with 11 of 20 melanoma patients responding to targeted NY-ESO-1 TCR-T cells therapy [57]. There were also two clinical trials of targeting melanoma antigen gene A3 (MAGE-A3) TCR-T cells in the treatment of melanoma. One of them (NCT01273181) was terminated due to the varying degrees of neuronal damage, and the other one (NCT02111850) has been completed, but the results have not yet been released [58, 59]. Currently, there are a number of clinical trials of TCR-T cells for the treatment of patients with melanoma (trial registry nos. NCT02743611, NCT03686124, NCT04729543, and NCT03638206).

### Non-small cell lung cancer

With the development of targeted drugs and immunotherapy, treatment of non-small cell lung cancer (NSCLC) has been significantly improved and has benefited many patients. Some progress has been made in TCR-T cell therapy in NSCLC, and targeting NY-ESO-1 TCR-T cells has shown potential. The result of a clinical trial demonstrated that two of four NSCLC patients who received targeted NY-ESO-1 TCR-T cell infusion had a response and no severe toxicity [60]. Another ongoing clinical trial (NCT03709706) is evaluating the safety and tolerance of TCR-T cells in advanced/recurrent NSCLC patients who were treated with NY-ESO-1/LAGE-1a TCR-T cells alone or in combination with pembrolizumab, an anti-PD-1 antibody. The result of this study contributes to a better understanding of the role of TCR-T cell therapy in NSCLC. In the clinical trial CTONG 1104, our team and partners found that TCR Vβ5–6 Jβ2−1, TCR Vβ20−1 Jβ2−1, and TCR Vβ24−1 Jβ2−1 in NSCLC patients with an EGFR mutation were associated with good overall survival (OS) in the gefitinib group, while TCR Vβ29−1 Jβ2–7 was related to good OS in the conventional chemotherapy group [27]. These results suggested that TCR-T cells, based on these specific TCRs and combined with existing targeted therapies, may benefit more NSCLC patients. Moreover, the recognition, identification, and construction of TCRs that recognize novel antigens derived from tumor mutations is also an attractive research direction.

### Sarcoma

Because NY-ESO-1 and MAGE-A4 are expressed in approximately 80% of synovial sarcoma cells [61, 62], clinical trials of TCR-T cells directed against sarcoma mainly target these two antigens. A total of 42 patients were enrolled in a phase I/II clinical trial of NY-ESO-1 TCR-T cell therapy. The results demonstrated that one patient achieved a complete response (CR), 14 patients had a partial response (PR), 24 had stable disease (SD), and 3 had PD [63]. It was demonstrated that the expansion response of the TCR-T cells infused was related to the expression of NY-ESO-1 on patient tumor cells. Another phase I clinical trial involving NY-ESO-1 TCR-T cells in the treatment of 45 patients with advanced sarcomas reported that one patient achieved CR, 14 patients achieved PR, and 25 patients had SD [64]. In the ongoing phase I clinical trial NCT03132922, preliminary results of 16 patients with advanced synovial sarcoma targeted by MAGE-A4$^{c1032}$ TCR-T cells demonstrated that 44% had a PR, while 50% had SD [65]. The preliminary results of these clinical trials apparently suggest that TCR-T cell immunotherapy may be a potential strategy for the treatment of sarcoma. However, more studies are needed to prove the effectiveness and safety of TCR-T cells to promote their clinical application.

### Renal cell carcinoma

Two TCR-T cell therapy clinical trials for renal cell carcinoma (RCC) have been performed. One is a phase I/II clinical trial targeting TNF-related apoptosis-inducing ligand (TRAIL) TCR-T cells, NCT00923390, which enrolled five patients with metastatic RCC. Unfortunately, this trial was terminated 10 years after its inception, and the results were not released. Another phase I clinical trial of metastatic RCC is currently ongoing to evaluate the efficacy and safety of human endogenous retroviruses-E (HERV-E) TCR-T cells. It was reported that TCR-T cells targeting trophoblast glycoprotein (5T4) have tumor killing effects in vitro, and greater than 90% of RCC cells express the 5T4 antigen [66]. This finding indicates that 5T4 may be a potential target antigen for TCR-T cell therapy in RCC. Similarly, more in vitro and in vivo studies are needed to evaluate the efficacy and safety of 5T4-specific TCR-T cells in the treatment of RCC.

### Bladder cancer

Some clinical trials have been conducted on TCR-T cell therapy for bladder cancer, but there were no striking results. For example, a clinical trial of MAGE-A3 HLA-A*01-restricted TCR-T cells in the treatment of metastatic bladder cancer had to be terminated because of inadequate design. In another phase I clinical trial to evaluate the efficacy of MAGE-A10 TCR-T cells in three patients with bladder cancer, the anti-tumor effects of TCR-T cells were weak despite being well tolerated [67]. In general, TCR-T cell therapy in bladder cancer needs further research.

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### Esophageal cancer

Miyahara et al. constructed TCR-T cells targeting the MAGE-A4 antigen using retroviral vectors [68]. In a clinical trial involving ten patients with esophageal cancer, MAGE-A4 TCR-T cells persisted in 5 patients for greater than 5 months, and three patients with the lowest tumor burden had an OS of greater than 27 months [69]. In this clinical trial, it was also observed that although the infused TCR-T cells persisted in the patient, there was no clinical response. This outcome may be related to the absence of lymphocyte clearance of interleukin-2 (IL-2) administration before the reinfusion of TCR-T cells. These results suggested that lymphocyte clearance pretreatment before the reinfusion of TCR-T cells may improve the efficacy of TCR-T cell therapy.

### Colorectal cancer

Carcinoembryonic antigen (CEA) is an antigen highly expressed in metastatic colorectal cancer cells [70, 71]. In 2011, Parkhurst et al. constructed TCR-T cells targeting CEA (691–699) that proved to be effective in recognizing and targeting HLA-A*0201-restricted human CEA+ colon cancer cells in mouse models [72]. Although this TCR-T cell demonstrated anti-tumor capability in a clinical trial of three patients with high CEA expression, two patients had PD 5–6 months after treatment, while the other did not respond. In addition, all three patients developed severe colitis 1 week after receiving a targeted CEA TCR-T cell transfusion [72]. This type of colitis may be autoimmune colitis caused by targeting CEA TCR-T cells. These results suggest that screening antigens specifically expressed on colorectal cancer cells will be an important key step in developing the idea of using TCR-T cells for colorectal cancer immunotherapy.

### Pancreatic cancer

More recently, Leidner et al. reported that a patient with progressive metastatic pancreatic cancer was treated with TCR-T cells and had regression of visceral metastases; the response was ongoing at 6 months (NCT03935893). These TCR-T cells were original from the autologous T cells that had been genetically engineered to clonally express two allogeneic HLA-C*08:02–restricted TCRs targeting mutant KRAS (Kirsten rat sarcoma viral oncogene homolog) G12D [73]. As we know, KRAS mutation is frequent in tumors. G12D, a single amino acid mutation of KRAS, is the most frequent mutation and is seen in diverse cancers such as pancreatic ductal adenocarcinomas [74]. The result provides the promising novel cellular immunotherapy approach for pancreatic cancer, which is the deadliest of all common cancers and lacking effective specific targeted therapy. Moreover, the KRAS G12D-TCR-T cells may be further tried to treat cancer patients who carry KRAS G12D mutation, such as NSCLC and colorectal cancer [75].

### Hepatocellular carcinoma

Several clinical trials of TCR-T cells in hepatocellular carcinoma (HCC) are underway. These trials mainly selected NY-ESO-1 and MAGE-A1 as target antigens for TCR-T cells [76]. As TCR-T cells can recognize not only antigens on the cell surface but also intracellular antigens, alpha fetoprotein (AFP) and hepatitis B (HBV) have also become attractive target antigens for TCR-T cells in clinical trials.

The first patient who received targeted HBV TCR-T cell therapy had a transplanted liver and was hepatitis B virus surface antigen (HBsAg) negative but had an extrahepatic HCC metastasis that was HBsAg positive. The serum HBsAg level decreased significantly within 30 days after receiving an HBV TCR-T cell transfusion. Unfortunately, there was no clinical reaction, and the patient died 8 months later [77].

In another phase I clinical trial of HBV-positive HCC patients, eight patients received targeting HBV TCR-T cell (LioCyx-M) reinfusion therapy [78, 79]. In this trial, one patient had PR for 30 months, while two patients had SD, and all three patients had increased serum chemokine levels after receiving a TCR-T cell transfusion. This TCR-T cell therapy is currently in a phase II clinical trial to further evaluate its effects. These results suggest that targeting HBV TCR-T cell therapy may be effective for the treatment of HCC, but more trial results are needed to support it.

In the first ADP-A2AFP TCR-T cell clinical trial targeting HCC patients, nine were treated with TCR-T cells, and one patient achieved CR for 6 months, while six patients had SD, and two patients had PD [80]. The results of this trial suggested that targeting AFP TCR-T cells may be another effective strategy for treating HCC.

### Human papillomavirus positive cancer

The research of TCR-T cells targeting human papillomavirus (HPV) E6 and E7 antigens in the treatment of HPV+ cancer has been the focus of many studies [81, 82]. At present, clinical trials have been performed on TCR-T cell therapy directed against HPV E6 and E7 antigens. These clinical trials enroll patients with head and neck tumors, cervical cancer, anal cancer, vaginal cancer, and vulvar cancer.

Doran et al. performed phase I/II clinical trials targeting HPV16 E6 TCR-T cells. Of 12 patients in this trial, two patients with anal cancer had a PR for 6 months and 3 months, respectively. One patient with vaginal cancer, one with head and neck tumors, and two with cervix uteri had SD [82]. These results indicate that targeting

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HPV16 E6 TCR-T cells had anti-HPV$^+$ tumor cell effects. In this clinical trial, the researchers also found an IFNGR1 mutation associated with the T cell response in patients with cervical cancer. The loss of HLA-A*0201, a limiting element required by HPV16 E6 TCR-T cells, was also found in patients with PD after treatment [82]. This finding indicates that TCR-T cells may miss or fail in the treatment of HPV$^+$ tumors, and further understanding of the factors and mechanisms causing these phenomena will help to improve the efficacy of HPV16 E6 TCR-T cells in the treatment of HPV$^+$ tumors.

In another study targeting HPV E7 antigen, high affinity TCR-T cells with an HLA-A*020-restricted E7 (11–19) epitope was successfully constructed and proven to be effective in killing tumor cells in vitro [81]. In the clinical trial of this TCR-T cell infusion (NCT02858310), a total of 12 patients were enrolled, six patients had a PR, and four patients had SD. Among these patients, those with multiple metastasis maintained PR for 9 months after receiving TCR-T cell treatment, and most of the metastatic lesions in the body were wholly eliminated [83]. At present, phase II branch clinical trials of NCT02858310 have been opened to further evaluate the efficacy and safety of HPV E7 TCR-T cells at the maximum tolerated doses, which signals that targeted HPV E7 TCR-T cell therapy demonstrated its power in treating HPV$^+$ cancer patients. We expect this therapy to benefit more patients.

## Barriers to TCR-T cell therapy
Although TCR-T cell therapy is an ideal cellular immunotherapy for cancer, it still faces obstacles that limit its application.

### TCR mismatch
TCR mismatch is an obstacle to TCR-T cell therapy. There may be some mismatching between the exogenous TCR αβ gene sequence introduced by genetically engineered T cells and the endogenous TCR αβ gene sequence of T cells [84]. The genetically engineered T cells resulting from this mismatching may recognize and attack patients' own tissue. This phenomenon was found in a mouse model of genetically engineered T cells that were produced by a mismatched TCR, leading to graft-versus-host disease (GVHD) [85]. There are two common methods to reduce the occurrence of TCR mismatching. One is to knock out endogenous TCRs using siRNA [86, 87], zinc-finger nuclease [88], transcription activator-like effector nuclease (TALEN) [89, 90], and clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR/Cas9) technologies [91, 92]. Another is to select γδ$^+$ T cells or NK cells as a source cells to construct TCR-T cells, which can avoid the mismatching of αβTCRs to some extent [41–45].

### Nonspecific cytotoxicity
Nonspecific cytotoxicity of TCR-T cells mainly means that so-called antigen-specific TCR-T cells also attack healthy tissues expressing an antigen or epitope similar to the antigen. The teams of both Van Den Beng and Linette found fatal cardiotoxicity when they used TCR-T cells targeting MART-1 and MAGE-A3 to treat melanoma, which may be related to the high expression of MART-1 and MAGE-A3 in heart tissue [93, 94]. Parkhurst et al. also found severe colitis in three patients with metastatic colorectal cancer treated with TCR-T cells targeting CEA (691–699) [72]. Thus, the principle of selecting tumor-specific antigens as targets for constructing TCR-T cells is to avoid selecting antigens expressed in healthy tissues, particularly in important organs. Targeting neoantigens produced by tumor gene mutations may be an effective way to reduce the nonspecific cytotoxicity of TCR-T cells. In the clinical trial CTONG 1104 (the 1st generation EGFR-TKI adjuvant gefitinib improves disease-free survival (DFS) for resected EGFR-mutant NSCLC with N1/N2 metastasis), we found that significant TCR rearrangements (Vβ5-6-Jβ2−1, Vβ20-1-Jβ2−1, Vβ24-1-Jβ2−1, and Vβ29-1-Jβ2−7) in NSCLC patients are associated with favorable overall survival (OS) and may have a specific response to tumor gene mutations [27]. Thus, with the comprehensive application of immunome library sequencing, single-cell sequencing, and MCR engineering report cell technology, obtaining and constructing TCR-T cells targeting neoantigens may be a potential strategy for cancer immunotherapy.

### Cytokine storm
Cytokine storm is T cell immunotherapy's most common adverse reaction [95]. Significantly elevated cytokines were detected in patients with a cytokine storm, including IL-6, interferon-γ (IFN-γ), IL-10, IL-2 receptor (IL-2R), monocyte chemotactic protein-1 (MCP-1), and macrophage inflammatory protein-1β (MIP-1β). These patients mainly had adverse reactions such as high fever, myalgia, hypotension, and dyspnea [96–98]. The severity of cytokine storms is related to tumor load [99], while reducing the tumor load before cellular immunotherapy could somewhat reduce the risk of a cytokine storm. Therefore, preventing and controlling the risk of cytokine storms can increase the safety and effectiveness of cellular immunotherapies such as TCR-T cell therapy.

### Tumor microenvironment
The tumor microenvironment (TME) is an important factor affecting T cell function. Decreased expression of chemokines, such as C-X-C motif chemokine ligand 9 (CXCL9), CXCL10, CXCL11, and intercellular cell adhesion molecule-1 (ICAM-1) adhesion molecules related

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to T cell infiltration in the TME can inhibit T cell infiltration to tumor sites by affecting T cell migration and adhesion [100–103]. Secondly, hypoxia in the TME could promote high expression of programmed cell death ligand 1 (PD-L1) in tumor cells, tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs). PD-L1 binds to the programmed cell death protein 1 (PD-1) on T cells and then mediates T cell exhaustion [104]. Hypoxia can also lead to high potassium levels and an acidic environment, thus affecting the ability and activity of T cells to secrete cytokines [105]. Moreover, other immunosuppressive cells in the TME, such as regulatory T cells (Treg), MDSCs, and TAMs, can inhibit the ability of CD8$^+$ T cells to recognize and kill tumor cells by secreting immunosuppressive factors such as IL-10 and transforming growth factor-β (TGF-β) [106]. Thus, the combination of targeted TME therapies may be a potential strategy for improving the efficacy of TCR-T cell immunotherapy.

## Conclusions

In summary, TCR-T cell therapy has been shown to be an attractive prospect in both in vitro studies and clinical trials for solid tumors. The advantage of such genetically engineered T cells is that they can recognize and target intracellular tumor neoantigens in solid tumors that lack specific surface tumor markers. With the development of tumor immunology and the application of new technologies, e.g., immunome library sequencing and single-cell transcriptional sequencing, TCR-T cells may have more potential for solid tumor immunotherapy.

## Abbreviations

AFP: Alpha fetoprotein; APCs: Antigen presenting cells; B-ALL: B cell-acute lymphoid leukemia; CAR: Chimeric antigen receptor; CEA: Carcinoembryonic antigen; CML: Chronic myeloid leukemia; CR: Complete response; CRISPR/Cas9: Clustered regularly interspaced short palindromic repeats-associated protein 9; CXCL9: C-X-C motif chemokine ligand 9; DFS: Disease-free survival; EGFR: Epidermal growth factor receptor; GVHD: Graft-versus-host disease; HBsAg: Hepatitis B virus surface antigen; HBV: Hepatitis B; HCC: Hepatocellular carcinoma; HERV-E: Human endogenous retroviruses-E; HLA-I: Human leukocyte antigen-I; HPV: Human papilloma virus; ICAM-1: Intercellular cell adhesion molecule-1; IFN-γ: Interferon-γ; IL-2: Interleukin-2; IL-2R: Interleukin-2 receptor; KRAS: Kirsten rat sarcoma viral oncogene homolog; MAGE-A3: Melanoma antigen gene A3; MART-1: Melanoma antigen recognized by T cells 1; MCR: pMHC-TCR; MCP-1: Monocyte chemotactic protein-1; MDSC: Myeloid-derived suppressor cell; MHC: Major histocompatibility complex; MIP-1β: Macrophage inflammatory protein-1β; MSGV1: γ-retrovirus; MUC1: Mucins1; NFAT2: Nuclear factor of activated T cell 2; NK 1.1: Natural killer 1.1; NSCLC: Non-small cell lung cancer; OS: Overall survival; OVA: Ovalbumin; PCR: polymerase chain reaction; PD: Progression of disease; PD-1: Programmed cell death protein 1; PD-L1: Programmed cell death ligand 1; PMEL-1: Premelanosome protein 1; PR: Partial response; RCC: Renal cell carcinoma; SD: Stable disease; TAA: Tumor-associated antigen; TAM: Tumor-associated macrophage; TALENs: Transcription activator-like effector nucleases; TCR: T cell receptor; TGF-β: Transforming growth factor-β; TILs: Tumor infiltrating lymphocytes; TME: Tumor microenvironment; TRAIL: TNF-related apoptosis-inducing ligand; Treg: Regulatory T cell; TRP-1: Tyrosinase related protein 1; TSA: Tumor-specific antigen; WT1: Wilm's tumor 1; ZAP70: 70-kD zeta-associated protein; 5T4: Trophoblast glycoprotein.

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