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The Emerging World of TCR-T Cell Trials Against Cancer: A Systematic Review

Jianxiang Zhang[1] and Lingyu Wang, PhD[2]

Abstract

T-cell receptor–engineered T-cell therapy and chimeric antigen receptor T-cell therapy are 2 types of adoptive T-cell therapy that genetically modify natural T cells to treat cancers. Although chimeric antigen receptor T-cell therapy has yielded remarkable efficacy for hematological malignancies of the B-cell lineages, most solid tumors fail to respond significantly to chimeric antigen receptor T cells. T-cell receptor–engineered T-cell therapy, on the other hand, has shown unprecedented promise in treating solid tumors and has attracted growing interest. In order to create an unbiased, comprehensive, and scientific report for this fast-moving field, we carefully analyzed all 84 clinical trials using T-cell receptor–engineered T-cell therapy and downloaded from ClinicalTrials.gov updated by June 11, 2018. Informative features and trends were observed in these clinical trials. The number of trials initiated each year is increasing as expected, but an interesting pattern is observed. NY-ESO-1, as the most targeted antigen type, is the target of 31 clinical trials; melanoma is the most targeted cancer type and is the target of 33 clinical trials. Novel antigens and underrepresented cancers remain to be targeted in future studies and clinical trials. Unlike chimeric antigen receptor T-cell therapy, only about 16% of the 84 clinical trials target against hematological malignancies, consistent with T-cell receptor–engineered T-cell therapy's high potential for solid tumors. Six pharma/biotech companies with novel T-cell receptor–engineered T-cell ideas and products were examined in this review. Multiple approaches have been utilized in these companies to increase the T-cell receptor's affinity and efficiency and to minimize cross-reactivity. The major challenges in the development of the T-cell receptor–engineered T-cell therapy due to tumor microenvironment were also discussed here.

Keywords

adoptive T-cell therapy, TCR-T, tumor immunotherapy, tumor antigen, clinical trial

Abbreviations

AFP, α-fetoprotein; AML, acute myeloid leukemia; APC, antigen-presenting cell; CAR, chimeric antigen receptor; CD, cluster of differentiation; CID, chemically induced dimerization; CRISPR, clustered regularly interspaced short palindromic repeats; CT, cancer-testis; CTLA-4, cytotoxic T-lymphocyte antigen 4; CXCR3, CXC chemokine receptor type-3; gp100, glycoprotein 100; HLA, human leukocyte antigen; HPV, human papillomavirus; HTS, high-throughput screening; IL, interleukin; ITAM, immunoreceptor tyrosine-based activation motif; MAGE, melanoma-associated antigen; MART-1, melanoma antigen recognized by T cells; MHC, major histocompatibility complex; MM, multiple myeloma; NSCLC, non-small cell lung carcinoma; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand-1; pMHC, peptide-major histocompatibility complex; PRAME, preferentially expressed antigen in melanoma; scFv, single-chain fragment variant; SPEAR, specific peptide enhanced affinity receptor; TAA, tumor-associated antigen; TALEN, transcription activator-like effector nuclease; TCR, T-cell receptor; TCR-T, T-cell receptor-engineered T; TRuC, T-cell receptor fusion construct; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; WT1, Wilms tumor 1

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Introduction

Adoptive T-cell therapy is one potentially powerful treatment for cancer that genetically modifies natural T cells to make them tumor-specific and to improve their ability to destroy tumor cells.[1] The genetically modified T cells are able to express chimeric antigen receptors (CARs) or T-cell receptors

[1] The High School Affiliated to Renmin University, Beijing, People's Republic of China
[2] Department of Biology, Duke University, Durham, NC, USA

Corresponding Author:
Lingyu Wang, PhD, Department of Biology, Duke University, 130 Science Drive, Durham, NC 27708, USA.
Email: lingyu.wang@duke.edu

(TCRs), showing impressive results in multiple clinical trials.[1] Chimeric antigen receptor generally consists of 3 parts: an ectodomain containing a single-chain fragment variant (scFv) for recognition of specific antigen, a transmembrane domain, and an endodomain including a CD3ζ chain with 3 immunoreceptor tyrosine-based activation motifs (ITAMs).[2] Chimeric antigen receptor-T-cell therapy has shown excellent results against hematological malignancies, while its effect against solid tumors is unsatisfactory by comparison.[3,4] This review focuses on TCR-engineered T (TCR-T) cells, which have shown greater promises against solid tumor than CAR-T cells.[5] This review elucidates the basic mechanism of TCR-T-cell therapy and different types of tumor antigen targeted by TCRs, followed by a summary of information about TCR-T-cell therapy clinical trials gathered on ClinicalTrials.gov and about technologies developed and trials run by several major pharma/biotech companies. Finally, this review ends with a summary of major challenges and improvements made to this cancer therapy.

The potency of TCRs relies on their interaction with peptide-major histocompatibility complex (pMHC), complexes formed by peptide bound to MHC.[6] Intracellular antigens are cut up into peptide chains and displayed by MHC molecules to form pMHCs.[7] Cytoplasmic proteins to be expressed by class I MHC proteins, most of which are defective ribosomal translation products, are cleaved into peptide chains by proteolysis.[8] These peptides are then bound to class I MHC proteins, which are expressed on all nucleated cells' cell surface.[7] Some cells, called antigen-presenting cells (APCs), express class II MHC proteins.[9] They internalize foreign material proteins by endocytosis and cleave them into peptide chains to bind with class II MHC proteins.[10] T-cell receptors from T cells, which must be matched to human leukocyte antigen (HLA) alleles of patients,[5] recognize these pMHCs and cause the killing of cancer cells.[11] (Human class I MHC protein is expressed from 3 gene regions: HLA-A, HLA-B, and HLA-C, and human class II MHC protein is also expressed from 3 gene regions: HLA-DR, HLA-DP, and HLA-DQ.[12])

T-cell receptor is a heterodimer composed of 2 different transmembrane polypeptide chains: an α chain and a β chain, each consisting of a constant region, which anchors the chain inside the T-cell surface membrane, and a variable region, which recognizes and binds to the antigen presented by MHCs.[11] The TCR complex is associated with 6 polypeptides forming 2 heterodimers, CD3γϵ and CD3δϵ, and 1 homodimer CD3 ζ, which together forms the CD3 complex.[13] In total, the CD3 complex contains 10 ITAMs, which take part in T-cell activation.[14] The different structures of CAR-T cells and TCR-T cells are illustrated in Figure 1. Additional costimulatory signals are also essential to the full execution of T-cell function, including CD8 on the surface of cytotoxic T cells, which binds to class I MHC complex, and CD4 on the surface of helper T cells, which binds to class II MHC complex.[12] There are also other well-studied costimulatory molecules including CD28 involved in CD28: B7 engagement on APCs and 4-1BB (CD137) that upregulate antiapoptotic factors to promote

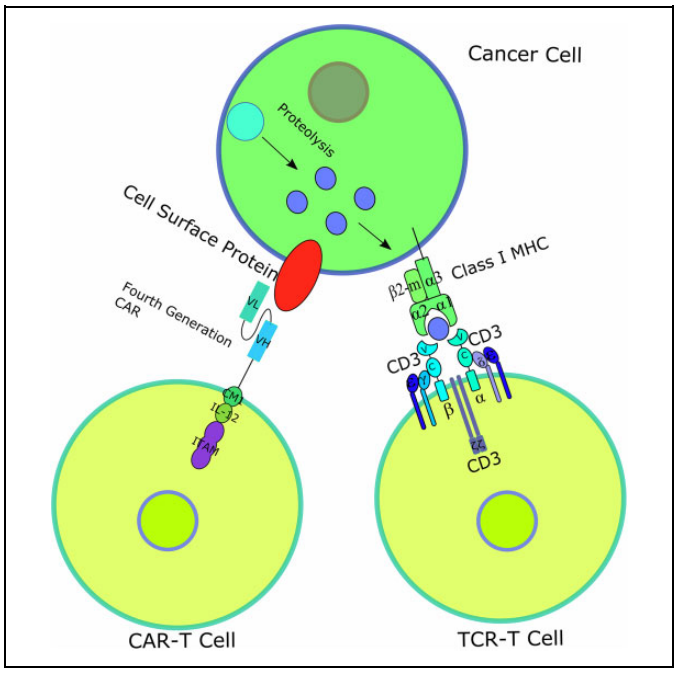

Figure 1. Diagrams of CAR-T and TCR-T-cell therapy. CAR-T cell, which normally targets only cell surface proteins, has had 4 generations and the latest generation is shown here. All generations of CAR-T cell contain an scFv consisting of an antibody's variable light (VL) and variable heavy (VH) chain, a transmembrane domain and a CD3ζ chain with ITAM for T-cell activation. The fourth-generation CAR further includes a costimulatory molecule (CM1), such as CD28 and B7, and an interleukin-12 (IL-12) domain for enhanced T-cell effects. T-cell receptor-engineered T cell has 1 TCR consisting of an α chain and a β chain, each containing a variable domain (v) and a constant domain (c), as well as an unlabeled transmembrane domain and 6 CD3 chains for T-cell activation. Class I MHC protein consists of an α chain with 3 domains α1, α2, α3, and a β2-microglobulin, presenting peptides derived from proteolysis of intracellular proteins. CAR indicates chimeric antigen receptor; ITAM, immunoreceptor tyrosine-based activation motif; MHC, major histocompatibility complex; scFv, single-chain fragment variant; TCR, T-cell receptor.

T-cell survival when binding with ligands on the surface of APCs.[14] Co-inhibitory molecules, such as cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1), are also part of the T-cell system in charge of extinguishing T-cell signaling.[15]

Chimeric antigen receptors, on the other hand, employ an antibody–antigen recognition machinery that consists of a scFv derived from an antibody in order to bind to antigens on the target cell's surface, which, along with transmembrane domains and costimulatory domains, activates immune responses.[16] Most proteins, however, are expressed inside cells instead of on the cell surface (only about 28% is expressed on the cell surface), making them unavailable to act as antigen for CARs.[17] As a result, the variety of antigens that can be targeted by CARs is often limited; moreover, the densities of cell surface antigens vary from cell to cell.[18] Traditionally, it is generally considered that CARs have higher affinity than TCRs. According to a recent study, however, by comparing between

the affinity of a single-chain TCR (Vβ-linker-Vα), an analog of scFv that serves as a CAR-like receptor, and that of a CAR with the same K_D, it has been shown that the full-length TCR has greater sensitivity than CAR even when CARs are expressed at higher densities and without the presence of CD8 coreceptor (which can lower the TCR affinity required by roughly 100 times and reduce the amount of pMHC required per target cell from over 30 molecules to just 1 molecule).[19] This higher sensitivity can enable more rapid destruction of tumor cells but also increases the risk of "on-target, off-tumor" toxicity, as observed in multiple clinical trials.[20-23] Interestingly, despite the higher sensitivity of TCRs than CARs, TCRs are found to mediate release of less amount of cytokines.[24,25] Thus, the risk of cytokine release syndrome is potentially lower with TCR-T-cell therapy compared to CAR-T-cell therapy. Although CAR-T cells have shown promising results against hematological cancers, their efficacy for solid tumor treatments is less so, which might be due to the immunosuppressive microenvironment of solid tumors, which will be discussed in the later sections, as well as the availability of cancer antigens that are present at sufficient level of density to be targeted without "on-target, off-tumor" toxicity.[18,26] T-cell receptor-engineered T cells, on the other hand, have shown some successes in treating both solid tumors, such as metastatic melanoma, and hematological cancers,[18,27] possibly due to the fact that the latter is conferred with higher penetrating power due to the low number of copies present on the surface of tumors, which is contrasted by the large number of copies of CAR-T antigens.[5]

T-cell receptor-engineered T-cell therapy utilizes the modification of T cells that retain these complexes to specifically target the antigens expressed by particular tumor cells.

Types of Antigens Used in TCR-T-Cell Therapy

In practice, there are different ways of categorizing tumor antigens targeted in TCR-T-cell therapy. Generally, human tumor antigens are classified into 2 main types: shared tumor-associated antigens (shared TAAs) and unique tumor-associated antigens (unique TAAs), or tumor-specific antigens. The former includes cancer-testis (CT) antigens, overexpressed antigens, and differentiation antigens, while the latter includes neoantigens and oncoviral antigens.[1,8,28] Human papillomavirus (HPV) E6 protein and HPV E7 protein belong to the category of oncoviral antigens. The diversity and complexity of tumor antigens often give rise to "on-target, off-tumor" toxicity.[1] Each type of antigen has its own characteristic advantages for TCR targeting but also its particular disadvantages.

Cancer-testis antigens are expressed in various tumor types as well as in testis, during times of spermatogenesis, and placenta.[8] Normally they are silent in adult tissues,[29] but they are active for transcription in different tumor types.[30] In theory, because many tumor types express CT antigens at a high level and normal tissues rarely express them, they are deemed attractive and safe immunotherapy targets,[5] but in practice, this is not always the case. Melanoma-associated antigen (MAGE)-A gene family is a group of CT antigens: MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A10, and MAGE-A12 are expressed at a frequency of more than 1/10 000.[22] In an NCI MAGE-A3 trial, TCR targeted at MAGE-A3 unexpectedly cross-reacted with the related peptide, MAGE-A12, which is expressed in the brain, resulting in the death of 2 of the first 9 patients and severe mental damage of the third.[23] It was found that the human brain might also express MAGE-A1, MAGE-A8, and MAGE-A9.[23] In another trial conducted by Adaptimmune, the TCR targeting MAGE-A3 bound to an unrelated protein, titin, in the heart.[5] These interactions between TCRs and normal tissues show the potential danger of targeting CT antigens.

Differentiation antigens are encoded by genes that express in a tissue-specific manner.[28] As a result, they are shared between tumor cells and healthy cells of corresponding tissues of origin,[8] unlike overexpressed antigens, which are expressed in certain, if not all, healthy tissue types.[28] Most such antigens, including glycoprotein (gp100), melanoma antigen recognized by T cells (MART-1), and tyrosinase, are mainly found in melanomas and normal melanocytes.[31-36] However, differentiation antigens do include carcinoembryonic antigen, which is often highly expressed in colon cancer,[37] and others are found in some epithelial tissues and tumors, including prostate cancers, in which case prostate-specific antigen is identified as one type of differentiation antigen.[38,39] Targeting such differentiation antigens will be likely to induce "on-target, off-tumor toxicity" on normal cells in critical organs.[40] The first 2 trials of TCR-T-cell therapy against MART-1 protein used 2 different proteins that were class I MHC-restricted but that were targeting similar epitopes, DMF4 and DMF5. Although DMF5-engineered T cells proved to be more avid than DMF4-engineered ones in functional assays, expanded trials with DMF5 T cells reported autoimmune toxicity on the eye, ear, and skin not reported with DMF4 T cells.[21] In one trial, such toxicity consisted of erythematous skin rash observed in 14 of 20 patients, anterior uveitis observed in 11 of 20 patients, and hearing loss observed in 10 of 20 patients.[41] Lethal cardiac toxicity was also observed in 3 patients undergoing trials against MART-1.[42] On one hand, to effectively eliminate solid tumors, TCR should be extremely potent in order to make any progress.[5] On the other hand, when a therapeutic is highly effective, it would have the same effect upon tissues with low expression level as well as upon those with high expression level.[5] Thus, the potency of TCR should be controlled in a precise range to not induce immune response up to a certain level but be potent enough to kill tumor cells after the threshold has been reached to reduce "on-target, off-tumor" toxicity.

Overexpressed antigens are antigens that are expressed in many normal tissues as well as different types of tumor.[8] They are expressed at a higher level in tumor cells to reach the threshold of T-cell recognition.[8] Because such antigens are also expressed, although, at a lower level, there are risks of "on-target, off-tumor" toxicity in normal tissues. For example, Wilms tumor 1 (WT1) is one kind of overexpressed antigen that is highly expressed in most acute myeloid leukemia (AML), acute lymphoid leukemia, and almost every type of

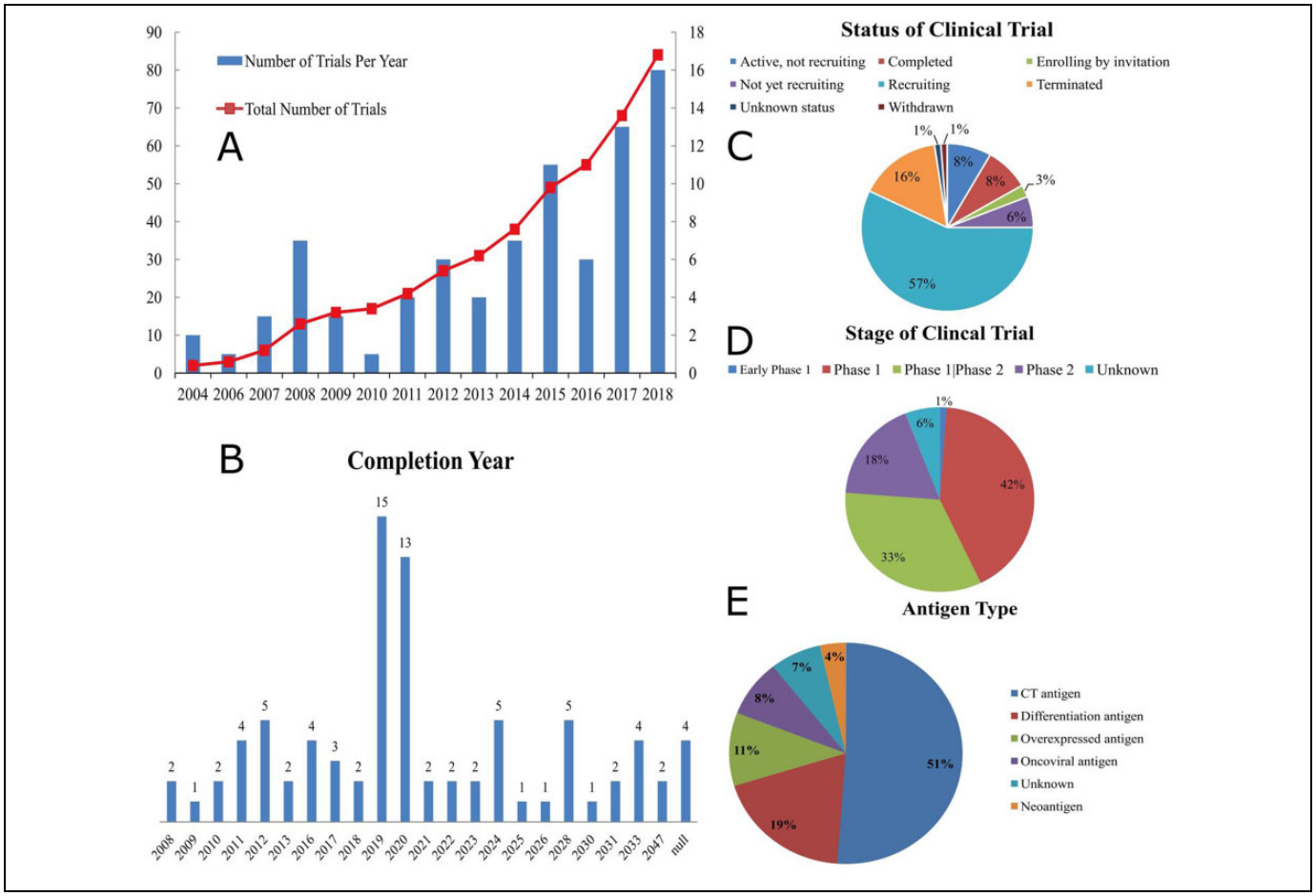

Figure 2. Major information and trend of 84 TCR-T clinical trials. (A) Figure 2A is the number of clinical trials started each year (bar chart, based on the right-hand side y-coordinate) and the number of clinical trials that have been completed since 2004 (line graph, based on the left-hand side y-coordinate). (B) Figure 2B is the number of clinical trials that was completed or will be completed each year. (C) Figure 2C is the respective proportions of trials not yet recruiting, active but not recruiting, recruiting, enrolling by invitation, withdrawn, terminated, completed and of unknown status. (D) Figure 2D is the respective proportions of different clinical stages in the 84 trials. (E) Figure 2E is the proportion of each type of antigen in the 84 trials.

solid tumor.[43] It is also present in several critical tissues, such as heart tissues.[44] Mesothelin, another kind of overexpressed antigen, is highly expressed in mesothelioma but is also present on mesothelial cells of several tissues, including trachea.[45-47] Despite common apprehension of the "on-target, off-tumor" toxicity by targeting such antigens, reports have suggested that WT1-targeting T cells are able to differentiate between tumor cells and normal tissues.[48] Using murine models, it was also suggested that enhanced-affinity TCRs targeting WT1 antigens, though surpassing the threshold for thymic selection, showed no autoimmune toxicity when transferred into wild-type mice.[48] It thus could be deducted that WT1, and other overexpressed antigens, might have the ability to attack tumor cells but largely spare normal cells, making them ideal targets for TCR-T therapy.[5]

Neoantigens are formed by random somatic mutations specific to individual tumors and also vary when tumor cells are isolated from patients at different sites or different times.[49] Targeting such antigens thus can reduce the risk of "on-target, off-tumor" toxicity but are expensive in practice. This is because the identification of neoantigens sometimes requires the sequencing of each individual tumor's whole genome so as to identify mutated genes and to choose peptides comprising motifs predicted to be presented by HLA alleles of the patient.[8] Moreover, several neoantigens have to be sequenced and targeted for the same type of tumor due to tumor heterogeneity.[5,50-54] Tumor heterogeneity is divided into certain types, including interpatient tumor heterogeneity, intratumor heterogeneity, and intermetastatic heterogeneity.[55] It results in the target for immunotherapies being specific to only one portion of all tumor cells, which reduces the efficacy of such therapies as well as increases the risk of metastasis.[56] The nature of tumor heterogeneity means that targeting neoantigen is necessary if complete eradication of tumor is desired.

Because of the different characteristics of each individual type of tumor antigen, products from different pharma/biotech companies and research institutes have different choices regarding which category to focus on.

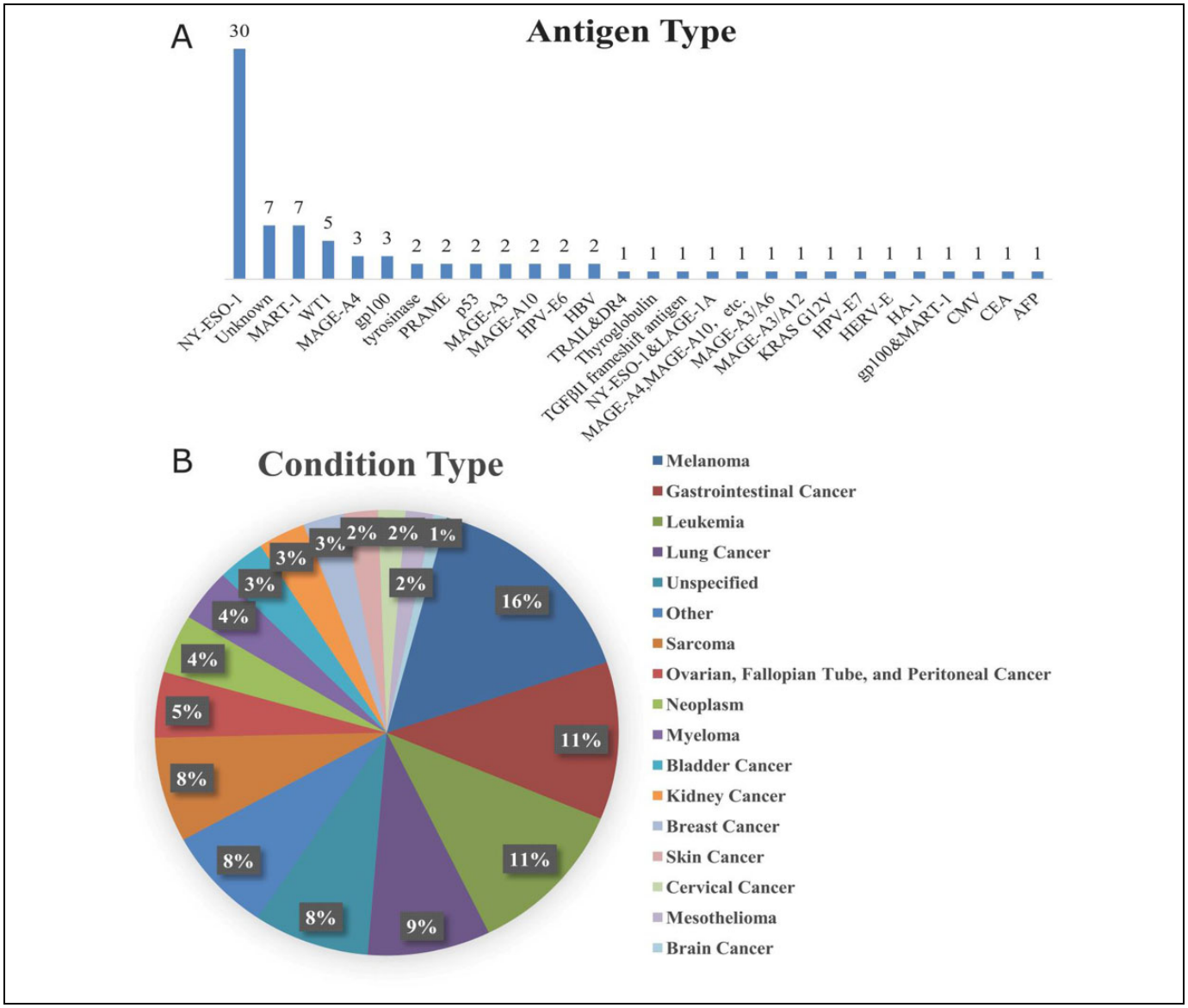

Figure 3. Proportion of each type of antigen and cancer targeted by 84 TCR-T clinical trials. (A) Figure 3A is the proportion of each particular antigen targeted in the 84 trials. (B) A total of 210 cancers are targeted in the 84 trials collected (many trials target more than one cancer, and many types of cancer are targeted by more than one trial). Figure 3B is the proportion of each type of cancer targeted in the 84 trials.

Statistics for Clinical Trials of TCR-T-Cell Therapy

Among the 376 clinical trials containing the keyword "TCR" published on ClinicalTrials.gov updated by June 11, 2018, 84 of these trials have been identified to employ TCR-T-cell therapy technique by manual curation. Certain patterns can be revealed from these 84 clinical trials, which are presented in Figures 2 and 3. Since June 11, when the data were gathered, to July 28, 13 more trials were included in ClinicalTrials.gov, where only 1 was related to TCR-T-cell therapy (with trial number NCT03578406) and was targeting HPV E6 antigen. The information on this new trial was not included in the figures and supplementary table.

First, as shown in Figure 2A, the start years of these clinical trials have shown a peculiar pattern. Three earliest clinical trials for TCR-T-cell therapy began in 2004. Since then, starting in 2006, the number of clinical trials started each year has shown a periodic pattern of fluctuation for every 3 or 4 years. Take the 8 years from 2006 to 2013 as an example, from 2006 to 2008, the number of clinical trials showed a steady increase and reached a peak in 2008, but the number then experienced a decrease in 2009, followed by a steady increase from 2010 to 2012 and then a decrease in 2013, and so on, but demonstrating an overall trend of increase in number of clinical trials from 2004 to 2018. This may be due to the fact that funds are periodically injected in this field, and it may require a period of 3 or 4 years to

Table 1. Clinical Trials by Major Pharma/Biotech Companies.

Sponsors/Collaborators	NCT Trial Number	Target Antigen	Agent	Targeted Tumor Type	Stage
Medigene	NCT03503968	PRAME	MDG1011	Unknown	Phase I/II
Bellicum Pharmaceuticals	NCT02743611	PRAME	BPX-701	AML, myelodysplastic syndrome	Phase I
Kite Pharma	NCT03139370	MAGE-A3/A6	KITE-718	Unknown	Phase I
Immatics	NCT03247309	Unknown	IMA-201	Head and neck squamous cell carcinoma, squamous cell NSCLC	Phase I
Adaptimmune	NCT02592577	MAGE-A10	MAGE-A10c796T	NSCLC	Phase I
Adaptimmune	NCT01343043	NY-ESO-1	NY-ESO-1c259T	Synovial sarcoma	Phase I/II
Adaptimmune	NCT01892293	NY-ESO-1	NY-ESO-1c259T	Multiple myeloma	Phase I/II
Adaptimmune	NCT01352286	NY-ESO-1	NY-ESO-1c259T	Multiple myeloma	Phase I/II
Adaptimmune	NCT01567891	NY-ESO-1	NY-ESO-1c259T	Ovarian cancer	Phase I/II
Adaptimmune	NCT01350401	NY-ESO-1	NY-ESO-1c259T	Melanoma	Phase I/II
Adaptimmune	NCT02588612	NY-ESO-1	NY-ESO-1c259T	NSCLC	Phase I/II
Adaptimmune	NCT03132792	AFP	AFPc332T	Hepatocellular cancer	Phase I
Adaptimmune	NCT03168438	NY-ESO-1	NY-ESO-1c259T	Refractory multiple myeloma	Phase I
Adaptimmune	NCT02989064	MAGE-A10	MAGE-A10c796T	Urinary bladder cancer, head and neck cancer, melanoma	Phase I

Abbreviations: AFP, α-fetoprotein; AML, acute myeloid leukemia; MAGE, melanoma-associated antigen; NSCLC, non-small cell lung carcinoma; PRAME, preferentially expressed antigen in melanoma.

reach another peak in the number of clinical trials, whereas the demand and interest for TCR-T-cell therapy have increased in the long run due to its huge potential. As shown in Figure 2B, 28 of these 84 clinical trials are expected to be completed in 2019 and 2020. It will be the best time to evaluate the results of the TCR-T application over other immunotherapy approaches.

Second, as presented by Figure 2C, about 57% of these clinical trials (48 clinical trials) are still recruiting, whereas only less than one-quarter of these clinical trials (20 clinical trials) are either terminated or completed. This proves that TCR-T-cell therapy is still a relatively new target for research, compared to CAR-T-cell therapy, indicating that TCR-T-cell therapy may have huge potential in the future.[5]

Third, according to Figure 2D, about 42% of these trials (35 trials) are for phase 1 clinical trials, whereas approximately 33% of these trials (28 trials) are for both phase 1 and phase 2 clinical trials, again showing that most of the TCR-T clinical trials are at the start of development, and the application of TCR-T-cell therapy in industries or hospitals is still a long way to go.

Fourth, as illustrated by Figures 2E and 3A, more than half of the antigens targeted by the 84 clinical trials (43 trials) are CT antigens, and 31 of these 43 trials target NY-ESO-1 (30 trials target NY-ESO-1, 1 trial targets NY-ESO-1&LAGE-1A), probably because the NY-ESO-1-specific TCR-T cells have been most thoroughly examined and tested in terms of therapeutic potentials in synovial cell sarcoma, melanoma, and myeloma.[40] Only 3 trials are targeting neoantigens, probably due to the fact that it requires a great deal of time and costs to perform the sequencing as well as to identify the effective TCRs for each patient and the same process needs to be repeated for each individual patient. On the other hand, if the method of sequencing and good manufacturing process can be made more efficient and cost-effective, such as applying robotics system to make sequencing faster and using the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system or sleeping beauty transposons,[5,57] neoantigens can be of huge potential without much competition for TCR-T industries.

Finally, as indicated by Figure 3B, the most frequently targeted cancer type is melanoma, being the target of 33 trials. This is probably because that the frequency of melanoma cases is high. Melanoma accounts for almost 92% of new cases of skin cancer and about 69% of skin cancer-related death in the United States in 2018,[58] and the incidence of melanoma has been increasing at a faster rate than the majority of other cancers.[59] Another reason might be that melanoma lesions are relatively accessible and that the nature of melanoma to easily adapt to tissue culture means that tissue samples and cell lines can be readily available for research purposes, making melanoma being preferred by clinical trials.[59] Leukemia and gastrointestinal cancers fall as the second, being the target of 24 trials. Of the 210 cancers mentioned in 84 clinical trials, only 34 trials (approximately 16%) focuses on hematological malignancies only (leukemia: 24 trials; myeloma: 8 trials; lymphoma: 1 trial; [unspecified] hematological malignancies: 1 trial), so 84% of cancer types targeted by these 84 clinical trials belong to the category of solid tumor, possibly due to the fact that CAR-T has already well proved efficiency in treating hematological malignancies, whereas, as proven by multiple studies, its effects toward solid tumor has been proven unsuccessful as compared to TCR-T-cell therapy. Nevertheless, there have indeed been attempts to improve CAR-T cells' performance against solid tumor because of such unsatisfactory results.[60-65] Therefore, it is possible that CAR-T cells might be widely available for solid tumor treatment in the future.[66,67]

Major Pharma/Biotech Companies Developing TCR-T-Cell Therapy

Table 1 has presented clinical trials of several major pharma/biotech companies discussed later in this section.

Adaptimmune (Oxfordshire, United Kingdom) modifies a patient's own CD4+ and CD8+ cells' TCRs to express specific peptide enhanced affinity receptors (SPEARs) to increase the binding affinity of natural TCRs, overcoming their low affinities as a result of negative thymic selection during maturation of T cells in the thymus.[68-70] Specific peptide enhanced affinity receptors are made by modifying the hypervariable complementarity-determining regions of native TCRs and selecting for TCRs with high affinity and lack of alloreactivity.[71-73] The main targets for Adaptimmune's SPEARs are CT antigens, including MAGE-A4, MAGE-A10, and NY-ESO-1.[74] It is also collaborating with GlaxoSmithKline (Brentford, United Kingdom) to target preferentially expressed antigen in melanoma (PRAME), another CT antigen.[75] These antigens are either not processed into peptides or processed minimally on normal adult tissues.[5] Adaptimmune is also developing SPEAR for α-fetoprotein (AFP), a glycoprotein highly expressed in fetal serum but is of low content in adult blood, mainly due to mature hepatocytes' inability to produce AFP.[74,76] α-fetoprotein is expressed in high levels in hepatocellular carcinoma, which Adaptimmune is trying to cure with AFP TCRs. Targeting these antigens should reduce the risk of "on-target, off-tumor" toxicity. In addition, it has developed a proprietary preclinical screening program consisting of molecular analysis to systematically identify peptides similar to the target cancer peptide inside human bodies to eliminate further cross-reactivity. It has developed human cell testing to determine whether the SPEARs bind to samples of normal cells and whether they are effective in whole blood samples as well.[70]

Kite Pharma is targeting MAGE-A3/MAGE-A6, using KITE-718 (NCT trial number: NCT03139370), as well as neoantigens.[5,77] It has strengthened its TCR product platform in 2015 through its acquisition of T-cell Factory B.V. (TCF), a privately held Dutch company, and renamed it as Kite Pharma EU, thus acquiring its proprietary TCR-GENErator discovery platform in order for rapid, high-throughput identification of TCRs, including those that target neoantigens.[78] It mainly incorporates T cells that induce apoptosis of cancer cells when the engineered TCRs bind to tumor antigens.[79]

Medigene employs viral vector–mediated transfer to infuse genes that code for specific TCRs into both CD4+ and CD8+ T cells.[80] Its TCR-T platform delivers TCR-T cells recognizing various tumor antigens, including common antigens shared by tumors and neoantigens specific to each individual patient.[80] Since March 2018, the company has been engaging in its first clinical trial study: a phase I/II clinical trial with MDG1011, its TCR-T therapy targeting PRAME, for the treatment of various types of hematological malignancies, including multiple myeloma (MM), AML, and myelodysplastic syndrome[80] (NCT trial number: NCT03503968). Meanwhile, it is collaborating with Charité Hospital and the Max-Delbrück-Center for an investigator-initiated TCR study that equips patients suffering from relapsed or refractory MM with TCR-T cells recognizing MAGE-A1 antigen in Germany.[80]

TCR2 Therapeutics has a unique TRuC (which stands for "T-cell receptor fusion construct", Cambridge, Massachusetts) platform for recruiting TCRs without the need of HLA matching.[81] This platform conjugates tumor antigen binder, such as scFv, single-domain antibody, and antigen-binding fragment, to the CD3 γ chain of TCR.[81] This construct enables engineered TCRs to target surface antigens, which do not require HLA matching, overcoming the major limitation of TCR-T-cell therapies, but retains the complete TCR system consisting of TCR and CD3 complex.[81] Another advantage of the TRuC platform is that it is flexible and can take variable forms, one of which is the dual-target TruC platform, where 2 tumor antigen binders are conjugated to a single TRuC-T cell product to overcome antigen escape mechanisms common in some tumor types.[81] Finally, each TRuC-T cell product can be engineered to carry built-in accessories or modulators including T-cell enhancers, up/down control genes, and stroma remodelers, to activate T cells or inactivate tumor cells[81] (tissue-resident stromal cells are known to be actively participating in tumor onset and/or evolution[82]). This innovation, therefore, provides a more intricate machinery for T cells to more effectively kill tumor cells but at the same time evade "on-target, off-tumor" toxicity in theory, while its practical application still requires clinical trials to assess.

Immatics company has developed its ACTengine approach that genetically engineers a patient's autologous T cells to express exogenous TCR upon lentiviral transduction.[83] Highly specific exogenous TCRs with optimal affinity are identified from natural, human T-cell repertoire through proprietary high-throughput screening (HTS), similar to what Kite Pharma and Medigene are doing.[83] By this approach, it has developed 3 products: IMA-201, IMA-202, which have entered the clinical phase I/II stage, and IMA-203, in collaboration with MD Anderson Cancer Center.[84] It has also invented bispecific TCR molecules that could be easily synthesized in mammalian host cells and that contain 2 domains: a T-cell recruiting antibody domain that targets immunomodulating T-cell surface proteins, such as CD3, and a specific TCR domain that targets and binds to tumor antigens presented by class I MHC complexes.[85] This design has the benefit of activating T cells to attack tumor cells regardless of their intrinsic specificity.[85] Thus, it is like an indirect recruitment of T cells against tumor cells, which can also prevent "on-target, off-tumor" toxicity because no enhanced exogenous TCRs are required. Nevertheless, it also requires several clinical trials to determine whether this design is actually effective in actual human bodies.

Bellicum Pharmaceutical, on the other hand, does not provide another alternative for enhancing the affinity or potency of TCRs against cancer cells. Instead, its novel technology is to prevent occasions when engineered T cells demonstrate side effects in patients' body. It develops a switch technology for TCR-T cell therapy called CaspaCIDe. This technology involves transforming T cells with genes that code for caspase-9 and chemically induced dimerization (CID) protein

with specially designed domain allowing for binding of rimiducid.[86] When a patient is experiencing severe side effect due to "on-target, off-tumor" toxicity, rimiducid is introduced and dimerizes CID proteins. The dimerization of CID proteins initiates a signaling cascade that activates caspase-9 and then caspase-3, which eventually leads to apoptosis of T cells to alleviate side effects.[86]

Obstacles and Breakthroughs

One everlasting challenge for TCRs is their binding affinity to tumor antigens. Several methods have been proposed, including those platforms applied by companies mentioned above. Medigene, in collaboration with other institutes, has proposed a humanized mouse model for identification of affinity-enhanced TCRs with higher affinity and more substantial therapeutic effect compared to human-derived TCRs.[87] As a matter of fact, the affinity of TCRs should be controlled as well, not just because of cross-reactivity but also because of self-reactivity among transduced T cells via fratricide, making such enhanced T cells not viable in culture, which is the case when the affinity of TCRs for NY-ESO-1 is enhanced to $K_D = 26$ pM.[88] The affinity of TCRs is also directly related to their "on-target, off-tumor" toxicity toward normal body cells. Intratumoral injections of TCR-T cells, a potential solution to this problem, enables high concentrations and bioavailability of T cells to be reached locally while the actual systemic exposure of normal body cells to these infused T cells can remain (very) low.[89] Another approach would be to add an inhibitory CAR molecule to the TCR-T cell, in which a separate CAR containing an scFv targeting an antigen on normal tissue but not on tumor tissue is fused to an inhibitory cytoplasmic domain, for example, the immune checkpoint molecule PD-1.[18] This approach can enable TCR-T cells to distinguish between tumor cells and normal cells with the same antigen. Moreover, there have been proposals of inducing the apoptosis of engineered T cells: iCas9, where *caspase 9* gene is modified to be inducible upon the addition of a small molecule is a potential ideal suicide switch for T cells.[90] Finally, another approach is to transduce engineered T cells with a gene for modified human CYP4B1 enzyme, which leads to bioactivation of the protoxin 4-ipomeanol and induces T-cell killing.[91]

T-cell receptors also have trouble eradicating metastatic tumors because of the immunosuppressive microenvironment of tumors. Tumor tissue inhibits T-cell trafficking toward tissues by limiting expression in tumor endothelial cells of T cell-specific adhesion molecules, such as intercellular adhesion molecule 1, costimulatory ligands, or shutting down T-cell-specific chemoattractants.[92,93] Tumor cells hinder T-cell migration by cancer-associated fibroblasts and extracellular matrix components.[94] Certain molecules derived from tumor cells, including vascular endothelial growth factor (VEGF), interleukin 10 (IL-10), and prostaglandin E2, which cooperate to induce expression of FAS-ligand and thus can mediate the apoptosis of FAS-positive CD8 effector T cells.[95] The second barrier to T-cell-mediated killing of tumor cells is suppressed T-cell activation. T cell will generally encounter hypoxia, which, when sustained, often leads to T-cell evasion as well as tumor progression: all mammalian cells that divide rapidly require high glucose uptake to sustain their proliferation.[96] As a result, tumor cells, stromal cells, and immune cells must undergo fierce competition against the limited glucose in the natural environment.[96] However, tumor cells can drive higher expression of the glucose transporter GLUT1 under situations of hypoxia, maintaining a high metabolic rate and proliferation, and outcompete T cells, reducing their antitumor activity.[96] Moreover, tumor cells often increase the expression of co-inhibitory ligands (checkpoint inhibitors), including PD-1 ligand 1 (PD-L1) and PD-1 ligand 2 (PD-L2), as well as reduce the expression of B7 proteins that produces costimulatory signals when bind to CD28 on T cells.[94] Cytotoxic T-lymphocyte antigen-4, a homolog of CD28 but have greater binding affinities than CD28 and is expressed mainly by activated T cells, prevents further activation of T cells when binding to ligand B7 on APCs.[40,97,98] The PD-1, another inhibitory molecule belonging to the immunoglobulin superfamily, induces apoptosis of antigen-specific T cells and reduces apoptosis of regulatory T cells when binding to PD-L1.[98-100] Moreover, engagement of PD-1 by PD-L2 can drastically inhibit TCR-mediated proliferation and cytokine production by helper T cells.[101] There might also be an insufficient amount of chemokine receptors, such as CXC chemokine receptor type-3 (CXCR3), in tumor cells to attract T cells, and tumors may induce enhanced necrosis.[96,102,103]

Fortunately, TCRs could be modified to improve T-cell trafficking and activation. One method is to engineer T cells with genes coding for receptors for chemokines expressed by tumors to improve T-cell trafficking. One study demonstrated the effective induction of interferon-γ secretion by T cells transduced with genes that encoded CXCR2, receptors for growth-regulated oncogene α (CXCL1), which is expressed by a range of tumor cell lines.[92] Another method, which has been proved successful on 5 different types of vascularized tumors using CAR-T cells, is to engineer T cells with CARs targeted against VEGFR-2 protein, which is overexpressed in tumor endothelial cells.[104] As a result, TCRs could be engineered to express such receptors as well as to improve T-cell trafficking. For T-cell activation, one approach is to incorporate a signal switch to the T cells that reverses the suppressive signal when binding to tumor chemokines into an activation signal that increases T-cell proliferation, such as a chimeric chemokine receptor by fusing a IL-7 receptor endodomain to a IL-4 receptor exodomain, which showed striking antitumor benefits against EBV-transformed B-cell tumors.[105]

Multiple immune checkpoint blockade inhibitors were developed to enhance the efficacy of immunotherapy against poorly responding tumors. Cytotoxic T-lymphocyte antigen-4 blockade and PD-1 blockade were proved to be effective in enhancing T-cell activation.[97,106-108] Cytotoxic T-lymphocyte antigen-4 blocking antibody MDX-CTLA-4, which is now called ipilimumab, is commonly used as immune checkpoint blockade and is used in 2 of the 84 identified clinical trials.[98]

Programmed cell death protein-1 inhibitors and PD-L1 inhibitors are also common among clinical trials aiming at the treatment of cancers; moreover, 2 PD-1 inhibitors, nivolumab (which is used in 1 of the 84 trials) and pembrolizumab (which is used in 2 of the 84 trials), and 1 PD-L1 inhibitor, atezolizumab, has been approved by Food and Drug Administration in the treatment of certain cancer types.[109] Another method involves the removal of genes in T cells coding for co-inhibitory-ligand-binding molecules using gene-editing technologies. For example, transcription activator-like effector nuclease (TALEN)-mediated *PD-1* gene inactivation in tumor-reactive CD4+ and CD8+ T cells (which was transferred adoptively) has shown to increase T cells' resistance to PD-1-mediated cell death in tumor tissues.[110]

Another challenge, especially for neoantigen-specific TCRs, is to efficiently identify such special antigens. As applied by companies like Kite Pharma, HTS-IR technology at both bulk and single-cell levels, including computational methods like TraCeR and single-cell TCRseq for reconstructing TCRs and identifying immunogenic neoantigens have provided useful tools for analyses of the diversity, dynamics, and clonality of T cells.[111] Although its cost might be lowered by future advancement in gene-sequencing and bioinformatics analysis, it is still a relatively expensive method for now.[111,112]

Other improvements have also been attempted, including the knockdown of endogenous TCRs by nuclease genome editing using zinc finger, TALEN, or CRISPR/Cas9 system, for more efficient recognition and higher level of expression of exogenous TCRs. Targeting multiple antigens simultaneously, such as the adoptive transfer of 2 populations of CD8+ T cells targeting gp100 and ovalbumin, in one trial resulted in delayed recurrence of B16-OVA melanoma.[42,113] With such improvements, TCR-T-cell therapy is likely to be more effective in eradicating tumors, but the lowering of cost is likely to be another issue for patients with cancer.[1,49,114]

Overall, TCR-T-cell therapy has been evolving rapidly and has become a promising strategy against various types of cancer, especially against solid tumors. Our study did not require an ethical board approval, because it did not contain human or animal trials.

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ORCID iD

Jianxiang Zhang https://orcid.org/0000-0001-5119-4157
Lingyu Wang, PhD https://orcid.org/0000-0002-2736-7588

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