Strategies for Manipulating T Cells in Cancer Immunotherapy

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Abstract

T cells are attractive targets for the development of immunotherapy to treat cancer due to their biological features, capacity of cytotoxicity, and antigen-specific binding of receptors. Novel strategies that can modulate T cell functions or receptor reactivity provide effective therapies, including checkpoint inhibitor, bispecific antibody, and adoptive transfer of T cells transduced with tumor antigen-specific receptors. T cell-based therapies have presented successful pre-clinical/clinical outcomes despite their common immune-related adverse effects. Ongoing studies will allow us to advance current T cell therapies and develop innovative personalized T cell therapies. This review summarizes immunotherapeutic approaches with a focus on T cells. Anti-cancer T cell therapies are also discussed regarding their biological perspectives, efficacy, toxicity, challenges, and opportunities.

Key Words: Cancer immunotherapy, Checkpoint inhibitor, Bispecific antibody, Adoptive T cell transfer, Tumor-specific T cells

INTRODUCTION

The idea of utilizing immune system to treat cancer emerged in the nineteenth century. Since then, the field of cancer immunotherapy has enormously evolved. It currently provides efficacious and promising treatment strategies to fight against cancer. Correlation between immunity and cancer was initially observed in sarcoma patients. It was found that patients with skin wound infections had spontaneous regression of tumors. William B. Coley applied a mixture of bacterial extracts including Streptococcus pyogenes known to cause skin infection to patients with cancer for the induction of strong immune responses against tumor. The mixture, known as “Coley’s toxin”, exhibited favorable antitumor responses, resulting in regression of several types of malignancies such as sarcoma and lymphoma (Decker and Safdar, 2009). Despite the successful outcome of this treatment, a lack of scientific mechanism and the risk of potential toxicity by bacteria led radio/chemotherapy to become a standard treatment. Later, in appreciation of advances in research (Chow et al., 2012), the interaction of immune system with tumor cells was better understood by the concept of ‘cancer immuno-surveillance’, whereby immune cells can recognize antigens on tumor (neoantigen) and eliminate them to prevent carcinogenesis (Halliday et al., 1995). Cancer immunotherapy has been revolutionized in recent years by elucidating molecular mechanisms of anti-tumor immunity and providing strategies for manipulating immune system to overcome cancer.

In a cancer-immunity cycle, various immune cells are involved in development of immune response against tumor antigen at multiple steps, which include tumor antigen presentation and priming by antigen-presenting cells, immune cell infiltration into tumor, effector functions of T cells, and release of tumor antigen (Chen and Mellman, 2013). Recent therapeutic approaches target each step of anti-tumor responses and immune cell subsets for the intervention of cancer. For instance, cancer vaccine aims to elicit tumor antigen-specific immune response by introducing tumor antigen into antigen presenting cells such as dendritic cells and optimizing their antigen processing. Although this approach experiences lots of challenges, multiple clinical trials are currently in progress (Ott et al., 2017; Sahin et al., 2017). Moreover, advances in genomic DNA sequencing from tumors and algorithm software have enabled a comprehensive analysis of mutations in patient-derived tumor and identification of neoantigens, leading to the development of personalized cancer vaccines. Another modulation point is that stroma cells can hinder the infiltration of immune cells into tumor microenvironment. Based on investigations on the regulation of stroma cell function in murine tumor models, potential molecular targets have been suggested (Turley et al., 2015).

Due to their ability to kill target cells called cytotoxicity, T cells have been a main focus in the research of cancer immunology as well as drug development for immunotherapy to...
treat cancer. T cell-based cancer immunotherapy has rapidly evolved, providing various strategies to eradicate tumor cells. This review will emphasize T cells as a target of immunotherapy and discuss various approaches manipulating T cells to fight against cancer. Immune therapies targeting T cells are summarized in different categories (Fig. 1): 1) T cell-targeting antibody (Table 1), which includes immune checkpoint inhibitors and bispecific T cell engager, and 2) adoptive T cell transfer (Table 2), which utilizes processed or modified T cells in different way such as tumor-infiltrating lymphocytes, chimeric antigen receptor (CAR) T cells, and engineered T cell receptor (TCR) T cells.

### T CELL-TARGETING ANTIBODY THERAPIES

#### Immune checkpoint inhibitors

Immune checkpoint blockade is an approach to treat cancer by unleashing T cell responses in the tumor microenvironment. Several molecules of co-stimulatory pathway are known to deliver negative signals to activated T cells to regulate the magnitude of immune response, thereby acting as a ‘checkpoint’ molecule. The most famous example of T cell checkpoint molecule includes cytotoxic T lymphocyte antigen 4 (CTLA4) and programmed cell death 1 (PD1). Accomplishments in research for these checkpoint molecules have enabled the development of T cell-targeting antibodies that exhibit good

Table 1. Features of T cell modulating antibodies

|                              | Immune checkpoint inhibitors                              | T cell bispecific antibody                          |
|------------------------------|------------------------------------------------------------|---------------------------------------------------|
| Structure                    | Monoclonal antibody                                        | Recombinant antibody                               |
| Generation                   | Hybridoma, “off-the shelf” protein                         | Mammalian cell lines, genetically engineered, “off-the shelf” protein |
| Tumor types                  | Mainly solid tumor                                          | Hematologic malignancies, several solid tumors     |
| Mechanism                    | Blocking inhibitory immune checkpoint proteins             | Inducing tumor cell lysis by recruiting T cell juxta position to tumor cells |
| Advantages                   | Application to broad spectrum of indications               | Tumor-infiltrating T cell-independent               |
| Disadvantages                | Tumor-infiltrating T cell-dependent                         | Antigen-dependent                                  |

Fig. 1. Schematic view of T cell-based cancer immunotherapy. (A) Negative regulators of T cell activation are highly expressed on T cells in tumor microenvironment and result in enrichment of exhausted T cells. Blockade of interaction between negative regulators and their ligands by ‘checkpoint modulator antibody’ can unleash non-functionality of T cells. (B) Bispecific T cell engager is the antibody with dual specificity to an antigen on tumor cell and a surface molecule of T cells such as CD3. The simultaneous binding of the antibody brings these cells close, enhancing lysis of tumor cells by T cells. (C) Tumor-infiltrating lymphocytes (TILs) isolated from tumor biopsy are expanded ex vivo, then infused to the patient. (D) Chimeric antigen receptors (CARs) are comprised of extracellular fragment of an antibody variable region and signaling domain of CD3 molecule. Transgenic CARs recognize surface antigen of tumor cells. (E) Expression of engineered T cell receptor (TCR) enables generation and expansion of tumor neoantigen-specific T cells ex vivo, which can recognize intracellular antigens presented by MHC molecule.
CTLA4: Naive T cells express CD28 that can bind to B7-1 and B7-2 on antigen presenting cells (APCs) to provide stimulatory signals for TCR while containing inhibitory CTLA4 in the cell. Upon T cell activation, CTLA4 is displayed on the cell surface to bind B7-1/B7-2 with higher affinity than CD28, competing out of CD28, which results in decrease of T cell activation. Besides conventional T cells, CTLA4 is constitutively expression on regulatory T (Treg) cells, contributing to immunosuppressive functions of these cells (Wing et al., 2008). Understanding the role of CTLA4 on T cell activation has led to the idea that blockade of this negative regulator could release T cells from suppression and reestablish anti-tumor responses of T cells. James Allison group first demonstrated the anti-tumor effect of neutralizing antibody against CTLA4 in a colon carcinoma model. Such effect was then demonstrated in various tumor models such as brain, bladder, and ovarian cancers (Leach et al., 1996; Yang et al., 1997; Fecci et al., 2007; Mangsbo et al., 2010). In addition, monoclonal antibody (mAb) for CTLA4 was shown to be effective in a clinical study of melanoma (Grosso and Jure-Kunkel, 2013). FDA approved Ipilimumab, a human IgG1 anti-CTLA4 mAb, for stage III/IV melanoma in 2011. It has been demonstrated that targeting CTLA4 with mAb can improve both short-term survival and long-term survival of patients with advanced melanoma (Hodi et al., 2003, 2010; Schadendorf et al., 2015). However, manipulating immune checkpoint machinery may cause an uncontrolled function of unleashed T cells, similar to an autoimmune phenotype observed in cta4-/- mice. A potential drawback of anti-CTLA4 therapy is an increased probability of severe immune-associated toxicity such as a loss of naive T cells and an inflammatory destruction of peripheral tissues by over-activated T cells (Kumar et al., 2017). On the other hand, in an effort to reduce side effects and improve efficacy of the therapy, one approach is to modify anti-CTLA4 mAbs. Engineered mAbs can prevent toxicity while improving clinical outcomes of the therapy. For example, given the observation that antibody-induced abnormal recycling and degradation of CTLA4 are associated with its adverse effects, antibodies can be modified to be sensitive to pH such that novel antibodies can show increased efficacy and reduced toxicity by maintaining normal recycling mechanism of CTLA4 (Zhang et al., 2019; Liu and Zheng, 2020).

PD1: PD1 shares about 20% of amino acid sequence homology with CTLA4. PD1 expression is induced after activation of T cells. It is further increased after activation of T cells in peripheral tissues (Carreno and Collins, 2002). Binding of PD1 with its ligands PD-L1/PD-L2 plays a critical role in causing an exhaustion of T cells in peripheral regions of immune responses such as tumor microenvironment and infection sites. With a notion that PD1 axis is involved in negative regulation of T cells, the effect of PD1 inhibition has been explored in preclinical studies for cancer treatment. Blockade of PD1 by either PDL1 expression or mAbs can result in reduced tumor growth in a syngeneic melanoma model or enhanced T cell cytotoxicity, respectively (Iwai et al., 2002; Strome et al., 2003; Hirano et al., 2005). Moreover, anti-PD1 mAbs have demonstrated their efficacy in clinical trials, leading to FDA approval of pembrolizumab and nivolumab for refractory melanoma in 2014 (Weber et al., 2017; Gong et al., 2018; Hargadon et al., 2018). Since pembrolizumab has also been approved for the treatment of non-small cell lung carcinoma (Herbst et al., 2016; Reck et al., 2016), the application of pembrolizumab and nivolumab has been expanded to various indications including head and neck squamous cell carcinoma and Hodgkin’s lymphoma (Ansell et al., 2015; Ferris et al., 2016; Chen et al., 2019; Cohen et al., 2019). In addition to PD1, PDL1 is targeted for the development of mAbs that currently include several approved antibodies such as atezolizumab, the first anti-PDL1 humanized antibody,avelumab, and durvalumab (Hargadon et al., 2018). Inhibiting the PD1/PDL1 axis also exhibits an increased risk of adverse effect on thyroid and liver cells (Kumar et al., 2017) despite its less severe toxicity compared to CTLA4 blockade. Recent research studies are focused on targeted delivery of anti-PD1 mAbs, such as transdermal delivery in a mouse melanoma model, for an advanced efficacy of checkpoint inhibitors (Riley et al., 2019).

Beyond a successful usage of single checkpoint inhibitor, combination therapies, either combination of checkpoint inhibitors or coupling of radiotherapy with checkpoint inhibitor, have shown promising results in various indications. Analysis of clinical studies has demonstrated that combination therapy of ipilimumab and nivolumab can augment long term survival of patients with advanced melanoma in comparison with ipilimumab alone, indicating a synergistic effect from blockade of both CTLA-4 and PD-1 (Wolchok et al., 2017; Motzer et al., 2018). Combining radiation with inhibition of PD1 pathway also shows successful complementarity of dual therapy in a tumor model (Twyman-Saint Victor et al., 2015). Despite increased toxicity of combination therapy, collaboration of therapies with different targets can lead to augmented benefit in the treatment of cancer.

### Table 2. Comparison of adoptive T-cell transfer therapies

| Tumor-infiltrating lymphocytes | CAR T cells | TCR T cells |
|-------------------------------|------------|------------|
| Targets | Multiple neoantigens | Surface antigen | Defined neoantigens (Intracellular) |
| MHC involvement | MHC-dependent | MHC-independent | MHC-dependent |
| Tumor types | Inflamed tumors | Currently limited to hematologic malignancies | All tumors |
| Origin | Autologous tumor infiltrated lymphocytes | Autologous PBMCs | Autologous PBMCs |
| Constructs | No requirement | Artificial receptor complex in vector | Naïve or engineered TCR in vector |
| Manufacturing | No receptor engineering | Receptor engineering | Receptor engineering |
tumor microenvironment. To redirect T cells to tumor cells, T cell-engaging bispecific antibodies (bsAbs) have been designed to simultaneously bind an antigen on tumor cells and a surface molecule on T cells, combining the specificity of two antibodies in a single molecule (Kontermann and Brinkmann, 2015). Crosslinking of cytotoxic T cells with tumor cells can lead to T cell activation and lysis of target cells by cytotoxic granules secreted from activated T cells. The bsAbs can be categorized into two forms depending on the existence of Fc domain. Advantage of having the Fc domain includes increased stability and half-life of bsAbs, whereas a potential risk of having the Fc domain could be antibody-dependent cell-mediated cytotoxicity (ADCC) induced by an interaction between Fc domain and its receptors on immune cells such as natural killer (NK) cells (Thakur et al., 2018; Wang and Ravech, 2019). The bsAb without Fc domain, named as bispecific T cell engager (BiTE), is a small molecule comprised of variable regions from an anti-tumor cell antigen and an anti-CD3 antibody with a short linker connecting these two fragments (Loffler et al., 2000; Klinger et al., 2016).

T cell surface target for bsAbs is generally CD3 chain of TCR due to its invariant characteristics, while the other arm of bsAb binds to tumor cell surface antigen. Abnormal and specific expression of CD19 on hematologic malignancies makes it as an attractive target of bsAb, leading to the development of blinatumomab, a CD19/CD3 bsAb designed in the BiTE format (Wu et al., 2015). FDA granted an accelerated approval for blinatumomab (Blinksyto) for treating adult and pediatric patients with B-cell precursor acute lymphoblastic leukemia (B-ALL) in 2018 (Hilal and Prasad, 2018). Other anti-CD19/CD3 bsAbs include TNB-486, which was reported to reduce cytokine secretion (Malik-Chaudhry et al., 2021). Regarding its specific-detection on malignant myeloid cells in acute myeloid leukemia (AML), CD33 is also considered as an effective target of CD3 bsAbs (Nair-Gupta et al., 2020). Multiple clinical trials are recently being conducted for CD33/CD3 bsAbs in refractory AML (Daver et al., 2021). In addition, B-cell maturation antigen (BCMA), which is essential for the survival of plasma cells, is highly expressed on plasma cells in multiple myeloma (MM) patients (Carpenter et al., 2013). Clinical trials for anti-BCMA BiTEs, including AMG 420 and AMG 701 have proven their beneficial efficacy in treating refractory MM (Harrison et al., 2020; Topp et al., 2020). These favorable results from clinical studies of bsAbs in hematologic malignancies have led to the expansion of bsAb application to solid tumors. Multiple panels of bsAbs are in early clinical trials. An example of bsAb target on solid tumor is prostate-specific membrane antigen (PSMA) with expression restricted in prostate cancer (Mhawech-Fauceglia et al., 2007). Clinical trials for PSMA-targeting T-cell engagers such as pasotuxizumab and HPN424 have shown anti-tumor activities in patients with metastatic castration-resistant prostate cancer (mCRPC) (Bendell et al., 2020; Hummel et al., 2021). The epidermal growth factor receptor variant III (EGFRVIII) is an additional target on solid tumor. It is frequently overexpressed in glioblastoma. An early clinical study for panel of EGFRVIII-specific BiTE including AMG 596 has been performed for patients with recurrent glioblastoma (Rosenthal et al., 2019).

Adverse effects of bsAbs are mostly characterized for blinatumomab, including cytokine release syndrome and neurotoxicity as main concerns. Cytokine release syndrome is a systemic inflammatory response caused by increased pro-inflammatory cytokines derived from activated T cells. Its symptom varies from mild flu-like symptoms to severe multi-organ failure (Shimabukuro-Vornhagen et al., 2018). Cytokine release syndrome is often proportional to drug dose and tumor burden (Viardot et al., 2016). Neurotoxicity generally appears as symptoms of dizziness, state of confusion, and encephalopathy. It is also related to the dose of blinatumomab (Stein et al., 2019). One study regarding mechanisms of neurotoxicity has proven that blinatumomab can recruit peripheral T cells to the brain by inducing adhesion of T cells to endothelial cells of blood-brain barrier, resulting in uncontrolled release of cytokines and immune responses (Klinger et al., 2020). In addition to their adverse effects, T cell-engaging antibodies also have limitations such as tumor evasion of the therapy and narrow range of applications for solid tumors. To overcome these limitations, various types of advanced antibodies are being generated. One example of the modification is by adding Fc domain to BiTE molecule to extend the half-life of BiTE, leading to reduced frequency of administration of the therapy for patients (Lorenczewski et al., 2017). Alternatively, BiTE can be designed to have Fc domain with point mutation to avoid unwanted immune responses by interaction between Fc domain and Fc receptors while maintaining enhanced half-life of BiTE by Fc domains (Schlothauer et al., 2016; Ishiguro et al., 2017). To improve cytokotoxicity of T cells by providing co-stimulatory signaling, two BiTE antibodies targeting separate tumor antigens (for example, with each BiTE for binding to CD3 or CD28) can be simultaneously administrated (Correnti et al., 2018). A canonical bispecific BiTE can be further engineered to be a multivalent T cell-engaging antibody to recognize more tumor antigens and molecules for T cell activation to overcome tumor evasion by antigen loss (Costa et al., 2019).

ADOPTIVE T CELL TRANSFER THERAPIES

Tumor-infiltrating lymphocytes

In the 1980s, transfer of tumor infiltrating lymphocytes (TIL) was initially explored for the treatment of melanoma using autologous lymphocytes isolated from interleukin-2 (IL-2)-expanded tumor biopsy for infusion into patients (Rosenberg et al., 1988). To reduce the objective response rate and to improve response, following studies have included lymphodepletion in TIL-based therapy, resulting in an increased frequency of complete tumor regression among metastatic melanoma patients (Rosenberg et al., 2011). A fundamental pitfall of this approach is that it is only applicable to 'inflamed' tumors where TIL contains effector T cells. In addition, TIL is comprised of heterogeneous population of T cells with unidentified TCR specificity and varying activities. Recent research studies have incorporated a high-throughput screening to enrich tumor neoantigen-specific T cells with a genetic modification of negative regulator of TCR signaling to promote T cell activation (Palmer et al., 2015; Zacharakis et al., 2018).

CAR T cells

A promiscuous nature of TCR specificity of TIL has led researchers to seek for T cells bearing receptors with defined specificity to tumor antigen. However, a challenge of using TCR is that TCR is restricted to major histocompatibility complex (MHC) for recognition of tumor antigen, which requires identification of neoantigen as well as human leukocyte anti-
T-cell therapy are continuing, including further modification.

Adverse effects of CAR T cells also involve neuroleukemia may occur after CD19 CAR T-cell therapy (Grupp et al., 2013). However, it was insufficient to deliver signals for T cell activation or effector function (Brocke, 2000). The following generations of CARs have adopted intracellular domains from co-stimulatory molecules such as CD28 and 4-1BB to provide positive signals to support for T cell activation (Maher et al., 2002; Imai et al., 2004). Furthermore, CAR T cells of subsequent generations have incorporated additional modifications to stimulate cytotoxic function of CAR T cells. For example, IL-12-expressing CAR T cells have been designed to enhance cytotoxic activity of T cells by overcoming the suppressive tumor microenvironment (Zhao et al., 2012). CAR T cells can also be engineered to express chimeric cytokine receptor for IL-4, a cytokine abundantly present in the tumor microenvironment. Such modification with IL-4 proved its ability to promote expansion of CAR T cells bearing tumor-associated antigen (TAA) in a preclinical study (Wilkie et al., 2010). Recent work has demonstrated that overexpression of c-Jun is able to induce CAR T cells to acquire resistance to T cell exhaustion (Lynn et al., 2019).

A well-known successful target of CAR T cells is CD19 that is exclusively expressed on B cells and certain B cell lymphoma. Indeed, CARs targeting CD19 have demonstrated benefit in treating B cell malignancies including acute or chronic leukemia and large B cell lymphoma, leading to their FDA approval for hematologic malignancies in 2017 (Porter et al., 2011a, 2011b; Brentjens et al., 2013). Moreover, targets other than CD19 have been investigated to treat hematologic as well as solid tumors. FDA has approved idecabtagene vilodecel (Abecma), a CAR T-cell therapy targeting B cell leukemia/lymphoma (Lee et al., 2019). Additionally, targets to CD3ζ and surface tumor antigens can predict the degree of anti-tumor immune responses (Gilboa, 1999). It is often interpreted that tumor mutability is crucial for the development of effective and safe TCR T cell therapy. Theoretically, target tumor antigens should be exclusively expressed on tumor cells but not in normal tissues. Tumor neoantigens are defined as peptides absent from normal tissues. They can be derived from tumor cell-specific mutated proteins. A selected TCR may be able to bind multiple peptide-MHC complex, leading to off-target effects due to a potential recognition of normal tissues. To reduce this risk, in vitro screening of peptide diversity can be performed for candidate TCRs (Green et al., 2019; Karapetyan et al., 2019).

Given specificity of interaction between TCR and peptide-MHC complex, selecting antigen and corresponding TCR is crucial for the development of effective and safe TCR T cell therapy. Theoretically, target tumor antigens should be exclusively expressed on tumor cells but not in normal tissues. Tumor neoantigens are defined as peptides absent from normal tissues. They can be derived from tumor cell-specific mutated proteins. A selected TCR may be able to bind multiple peptide-MHC complex, leading to off-target effects due to a potential recognition of normal tissues. To reduce this risk, in vitro screening of peptide diversity can be performed for candidate TCRs (Green et al., 2019; Karapetyan et al., 2019).

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Engineered TCR T cells

In adoptive cell transfer therapy, modified T cells such as CARs and TCRs have been intensively investigated and successfully used for hematologic malignancies and solid tumors. TCR-based adoptive cell transfer can supply an effective pool of effector T cells with anti-tumor activity and identified specificity by ex vivo manufacturing. Both CAR and TCR T cell therapies employ engineered receptors on effector T cells. However, CAR recognizes tumor surface antigens without MHC whereas engineered TCRs have ability to bind to intracellular and surface tumor antigens in the form of peptide-MHC, which endows TCR-based therapy a potentially broader application with better efficacy for solid tumors than CAR therapy (Table 2). The majority of TCRs are comprised of α- and β-chains and CD3 molecules with ab TCR transmitting initial activation signaling for T cells. Thus, TCRs have advantages in their structures compared to CARs. For example, TCRs possess more subunits including immunoreceptor tyrosine-based activation motifs (ITAMs), and co-stimulatory receptors such as CD4 and CD28 than CARs. Additionally advantageous features of TCR for the therapy are that TCR can elicit T cell response to a single peptide-MHC complex and that the repertoire of TCR specific to tumor-associated antigens is diverse (Huang et al., 2013; Hoffmann and Slansky, 2020).

A canonical pathway of antigen presentation by MHC class I involves peptides generated from intracellular proteins such as normal proteins, tumor associated differentiation antigens, and tumor cell-specific mutated proteins. A selected TCR may be able to bind multiple peptide-MHC complex, leading to off-target effects due to a potential recognition of normal tissues. To reduce this risk, in vitro screening of peptide diversity can be performed for candidate TCRs (Green et al., 2019; Karapetyan et al., 2019).

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The most common toxicity caused by CAR T-cell therapy involves cytokine release syndrome due to strong activation and proliferation of CAR T cells upon infusion into patients. Cytokine release syndrome can induce serious symptoms such as fever, hypotension, and multiorgan failure (Neelapu et al., 2018). Moreover, lymphopenia or hypogammaglobulinemia may occur after CD19 CAR T-cell therapy (Grupp et al., 2013). Adverse effects of CAR T cells also involve neurological disorders such as encephalopathy syndrome (Brudno and Kochenderfer, 2016). Efforts to reduce toxicity of CAR T-cell therapy are continuing, including further modification of affinity of CD19 CAR, utilization of CAR T cells with multivalent receptors, and application of a transient expression of the receptor (Bielamowicz et al., 2018; Hung et al., 2018; Ghorashian et al., 2019).
There are practical obstacles in TCR engineering and application of the therapy. Introduction of tumor-specific TCR may result in paring of a-chain and b-chain of transduced TCR with endogenous TCR chains. Resulted expression of receptors with mixed TCR chains can generate a novel TCR specificity which causes off-target effects by recognition of self-peptides. To enhance the expression of homogenous tumor-specific TCR, various approaches in genetic modification are underway, including simultaneous knock-out of endogenous TCR during knock-in of novel TCR. Large efforts are being made to improve transduction efficiency in TCR engineering. In addition to viral transduction of novel TCR, CRISPR/Cas9 system is also introduced to facilitate efficient genetic modification with lowered off-targeting. Regarding the screening of patients for HLA typing, a limitation of current studies is that engineered tumor-specific TCRs are only available for HLA-A*02:01 positive patients as this is the most prevalent HLA haplotype, especially in Caucasian (Song et al., 2013). To broaden TCR therapy to patients with various HLA genotypes, novel TCRs restricted to multiple HLA types are being investigated.

Current TCR-based therapies are mostly targeting tumor-specific peptides overexpressed in certain types of cancers and shared among individuals. For instance, several TCR-T cell products are developed targeting MAGE (melanoma-associated antigen) peptides which are enriched in various solid tumors including melanoma, non-small cell lung carcinoma (NSCLC), and urothelial cancer (Huang, et al., 1999; Schultz-Thater et al., 2011). NY-ESO-1 (The CTA New York Esophageal Squamous Cell Carcinoma-1) expressed in melanoma, NSCLC, or breast cancer is another tumor marker selected for TCR-based therapy (Lee et al., 1999; Sugita et al., 2004; Aung et al., 2014). Programs for TCR-T development are also focused on WT1 (Wilms tumor 1) antigen, which is specifically presented on kidney or breast cancer cells (Campbell et al., 1998; Miyoshi et al., 2002). Promising results have been reported for TCR-engineered T cell therapy targeting aforementioned antigens from multiple pilot and clinical trials. NY-ESO-1 has shown anti-tumor immune responses in myeloma (Rapport et al., 2015), melanoma (Robbins et al., 2015), and synovial sarcoma (D’Angelo et al., 2018; Ramachandran et al., 2019). T cells bearing MAGE-A3-reactive TCR has been subjected to a phase I study for patients with solid malignancies, showing a potential efficacy of TCR-based therapy for metastatic cancers (Lu et al., 2015).

The entire procedure to deliver TCR-T adoptive transfer therapy is complex, which relies on technologies for genetic engineering and manufacturing immune cells. Briefly, this process starts with patient screening with HLA typing and targeted tumor antigen expression from tumor biopsy. For TCR engineering, patients-derived T cells are activated and transduced with TCR for target antigen, followed by subsequent expansion and tests before infusion to patients. Each step is optimized to reduce processing time and provide successful therapy. Furthermore, advances in technologies will enable the identification of patient-specific tumor antigens and administration of corresponding TCR, thus facilitating the development of personalized treatment for a wide range of cancers.

**CONCLUSION**

T cell-based immunotherapy has been greatly advanced to provide successful tools for fighting cancer. The ability of T cells to attack tumor cells has led to a development of molecules that can modulate functional status or recruitment of T cells. The adaptive feature of T cells through antigen-specific recognition of TCR has further advanced the therapy by enabling transfer of T cells with defined tumor antigen specificity. Next-generation technical innovations are facilitating the evolution of T cell therapy toward customized treatment tailored for individual patients. Further studies will allow us to overcome immune-associated adverse effects while improving efficacy and safety for applicability of the therapy to extended indications.

**CONFLICT OF INTEREST**

The authors have no conflicts of interest related to this work to disclose.

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