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Review
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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

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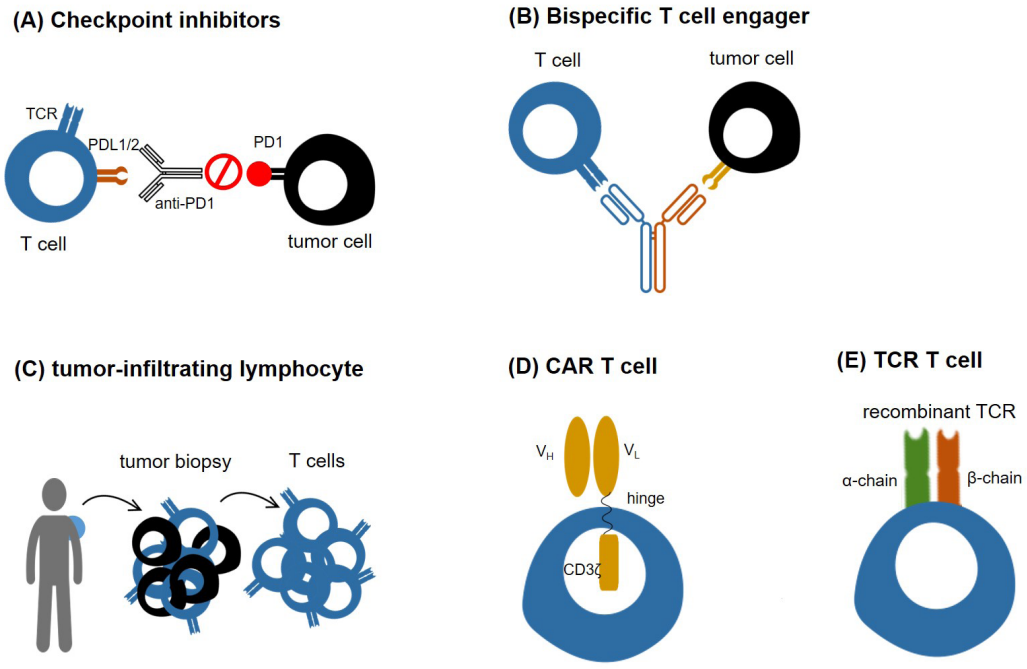

Fig. 1. Schematic view of T cell-based cancer immunotherapy. (A) Negative regulators of T cell activation are highly expressed on T cells in tumor microenvironment and result in enrichment of exhausted T cells. Blockade of interaction between negative regulators and their ligands by 'checkpoint modulator 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.

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 juxtaposition to tumor cells
Advantages	Application to broad spectrum of indications	Tumor-infiltrating T cell-independent
Disadvantages	Tumor-infiltrating T cell-dependent	Antigen-dependent

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 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

efficacy for a broad array of cancers.

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 *ctla4*$^{-/-}$ mice. A potential drawback of anti-CTLA4 therapy is an increased probability of severe immune-associated toxicity such as a loss of naïve 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 PDL1/PDL2 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.

T cell-bispecific antibody

One major problem that limits the efficacy of T cell-based immunotherapy is an insufficient infiltration of T cells into the

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 Ravetch, 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 (Blincyto) 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 cytotoxicity 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-

gen (HLA) of the patient. To bypass this complexity, the initial engineering of T cells took an advantage of antibody that could recognize surface molecules of cells. A synthetic chimeric antigen receptor (CAR), comprised of antigen-binding domain from heavy and light chains of antibody variable regions and signaling domains of TCR, can interact with surface antigen of tumor cells and confer cytotoxic function of the engineered T cell, respectively. The signaling domain of the first-generation CAR T cells was derived from the CD3 ζ-chain (Kuwana *et al*., 1987). However, it was insufficient to deliver signals for T cell activation or effector function (Brocker, 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). Recently, it has been 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 vicleducel (Abecma), a CAR T-cell therapy targeting B cell maturation antigen (BCMA), for patients with nonresponding or recurrent multiple myeloma (Mullard, 2021). Recently, CAR T cells binding to B7-H3 (CD276), a novel target for solid tumor, has demonstrated beneficial effects in a preclinical study using a pediatric solid tumor and brain tumor model (Majzner *et al*., 2019). Given the aforementioned features of antibody-derived extracellular domain of CAR, a benefit of CAR is that it is applicable to cancers with downregulated MHC molecules (Garrido *et al*., 2016). However, CAR is limited to recognize tissue-specific targets expressed on tumor cell surface. Thus far, CAR T-cell therapy has been mostly successful for hematologic malignancies. Research studies are proceeding to improve the efficacy of CAR T-cell therapy for solid tumors.

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).

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).

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 mutations (Gilboa, 1999). It is often interpreted that tumor mutations can predict the degree of anti-tumor immune responses induced by tumor –specific neoantigens. However, presentation of neoantigen on MHC to T cells is essential for efficient T cell responses. Downregulation of MHC class I has been observed in a large number of human cancers, leaving MHC-low tumor cells evading immune responses by T cells (Bubeník, 2003; Garrido *et al*., 2016). On the other hand, TCR is needed to be highly selective with optimal TCR affinity and minimal recognition of other peptides (Chandran and Klebanoff, 2019). Affinity/avidity of TCR can largely influence the safety and efficacy of T cell therapy (D'Ippolito *et al*., 2019). It has been demonstrated that high affinity of TCR is required for proper T cell activation and that the affinity determines the function of tumor-specific CD8 T cells (Tan *et al*., 2015). Moreover, low affinity TCR can activate T cells, although high affinity binding is necessary for sustained T cell activation (Zehn *et al*., 2009).

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 T 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 (Rapoport *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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