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Review

Gene editing in T cell therapy

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ABSTRACT

The adoptive transfer of engineered T cells for the treatment of cancer, autoimmunity, and infectious disease is a rapidly growing field that has shown great promise. Gene editing holds tremendous potential for further improvements of T cell therapy. Here we review the applications of gene editing in various T cell therapies, focusing on antiviral strategies and cancer immunotherapies, and discuss the challenges and future prospects.

1. Introduction

Adoptive cell transfer (ACT) using autologous or allogeneic lymphocytes has shown remarkable responses in patients with various diseases. As an early success of ACT-based immunotherapy, tumor-infiltrating lymphocytes (TIL) were extracted from tumor, expanded *ex vivo*, and infused back to patients to treat metastatic melanoma (Rosenberg et al., 1988). In combination with host lymphodepletion, TIL therapy achieved a 50% objective response (OR) rate (Dudley et al., 2002, 2008; Rosenberg et al., 2011). However, TIL can only be generated from a subset of patients, and the process of TIL preparation is long and technically challenging. More importantly, it is difficult to generate TILs from many types of tumor, limiting its broad applications. To generate T cells with specificity against shared cancer antigens, genes encoding T-cell receptors (TCRs) recognizing tumor peptide presented by the major histocompatibility complex (MHC) have been identified from TILs (Cole et al., 1995). TCR engineered lymphocytes (TCR-T) were generated by transduction of viral vector encoding *TCR* gene, and first successfully used to treat metastatic melanoma in clinical study in 2006 (Morgan et al., 2006). TCR-T therapy is limited to a subset of patients due to the MHC restriction, and many tumors reduce antigen presentation by down regulating MHC expression and defective antigen processing. Chimeric antigen receptor (CAR) engineered T cell technology was developed as an ACT method that is MHC-independent. The concept of CAR was originally proposed and demonstrated by Zelig Eshhar (Gross et al., 1989). The structure of CAR is consisted of three parts: the extracellular region usually containing an antibody-derived single-chain variable fragment (scFv), the transmembrane region and the intracellular region which contains the signal transduction domain of CD3ζ and one or more co-stimulatory molecules such as CD28, 4-1BB, CD27 and OX40 (Lee et al., 2002; Imai et al., 2004; Finney et al., 2004; Kowolik et al., 2006; Milone et al., 2009; Savoldo et al., 2011). Upon scFv binding to the target antigen, CAR-T cells are activated through CD3ζ domain. While CAR-T cell therapy treating B-cell acute lymphoblastic leukemia (B-ALL) has been remarkably successful in early phase clinical studies with a complete response rate of 70%–90% (Kalos et al., 2011; Brentjens et al., 2013; Grupp et al., 2013; Maude et al., 2014; Lee et al., 2015), treating solid tumor appears to be more challenging (Kershaw et al., 2006; Park et al., 2007; Morgan et al., 2010; Louis et al., 2011; Lamers et al., 2013; Ahmed et al., 2015; Brown et al., 2015; Katz et al., 2015).

With the development of gene editing technologies, it is now possible to make various genetic modifications in primary human T cells to develop safer and more effective T cell therapies. Zinc-finger nucleases (ZFNs) (Urnov et al., 2010), transcription activator-like effector nucleases (TALENs) (Bogdanove and Voytas, 2011) and the CRISPR-Cas9 system (Doudna and Charpentier, 2014; Hsu et al., 2014) are the most commonly used gene editing tools. They can be designed to bind to specific genomic loci and create DNA double

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strand breaks (DSBs), repair of which leads to various genetic modifications.

2. The application of gene editing in T cells against HIV infection

$CD4^+$ T cells are the primary targets of human immunodeficiency virus (HIV) infection (Douek et al., 2002). Through virus-mediated apoptosis, induced autoimmune response (Kalams et al., 1999; McCune, 2001) and other mechanisms, the number of $CD4^+$ T cells is greatly reduced, leading to the loss of cellular immunity, humoral immune response and the development of acquired immunodeficiency syndrome (AIDS) (Doitsh et al., 2014). The majority of HIV strains use C-C motif chemokine co-receptor type 5 (CCR5) to enter T cells (Deng et al., 1996; Dragic et al., 1996). The naturally occurring *CCR5* mutant allele (*CCR5Δ32*) exists in Caucasian population at high frequency (approximately 10%) (Liu et al., 1996; Samson et al., 1996). T cells with this mutation do not have CCR5 molecule on cell surface and therefore are resistant to HIV infection.

The first cured HIV-infected patient is the famous “Berlin patient”. In addition to AIDS, this patient was also suffering from acute myeloid leukemia and received allogeneic hematopoietic stem cell transplantation from a *CCR5Δ32* mutant donor. He has remained HIV free even after discontinuation of anti-HIV drug therapy (Hutter et al., 2009). The *CCR5Δ32* mutant genotype was considered to be the key factor for the cure of “Berlin patient”, although the intensive chemotherapy pre-transplantation also reduced HIV reservoir. Subsequently, HIV patients with *CCR5* wild-type allogeneic hematopoietic stem cell transplantation did not reach the status of a cure (Cannon et al., 2014). Since it is very difficult to find human leukocyte antigen (HLA) matched donor with homozygous *CCR5Δ32* mutation, an alternative strategy of knocking out *CCR5* gene in the autologous uninfected $CD4^+$ T cells or $CD34^+$ hematopoietic stem cells using gene editing to treat HIV infection is highly attractive.

Early studies have shown that ZFNs can mediate *CCR5* gene knockout in human primary $CD4^+$ T cells efficiently (40%–60%). *CCR5* knockout T cells showed good proliferation and anti-HIV effect both *in vitro* and in engrafted immunodeficient mice. Compared with infusion of unmodified T cells, treatment with gene-modified $CD4^+$ T cells significantly reduced the viral load (more than 7-fold) and increased the number of $CD4^+$ T cells (more than 5-fold) in peripheral blood from HIV-infected mice. This study suggests that transfusion of HIV resistant $CD4^+$ T cells may reconstruct the patient's immune system, resulting in a lasting and effective anti-HIV effect, providing an important basis for using gene editing to treat HIV infection (Perez et al., 2008).

Subsequently, two groups used ZFN to knock out *CCR5* gene in human $CD34^+$ hematopoietic stem cells and infused edited cells into irradiated immunodeficient mice. *CCR5* knockout hematopoietic stem cells can successfully engraft and produce multiple lineages of *CCR5*-negtive cells *in vivo*.The mice received *CCR5* gene knockout stem cell transplantation maintained a normal ratio of $CD4^+/CD8^+$ and showed a good anti-virus ability after being challenged with HIV infection (Holt et al., 2010; Li et al., 2013).

A series of clinical trials (Table 1) were initiated to evaluate the safety and efficacy of infusing autologous $CD4^+$ T cells with *CCR5* gene knockout. A group at the University of Pennsylvania reported the results of an open-label, non-randomized, non-controlled, phase I clinical trial for the treatment of HIV infection firstly. Twelve patients with chronic HIV infection who received highly effective anti-HIV drug therapy were divided into two groups based on whether the number of $CD4^+$ T cells were greater than $450/mm^3$. Each group had 6 patients and each patient was infused with 10^{10} ZFN treated $CD4^+$ T cells. The *CCR5* gene knockout efficiency was 11%–28%. Except for mild transfusion related side effects observed in one patient, the gene-modified T cells were well tolerated and engrafted normally in the remaining patients. These T cells have an average half-life of 48 weeks and can persist in the body for more than 42 months and further migrate to the intestinal mucosa-associated lymphoid tissue. One group of patients received a 12-week discontinuation assessment after transfusion. Normally, the rapid increase in viral load occurs at 2–4 weeks after discontinuation, reaching the level before antiviral therapy and leading to viremia. Four patients completed the discontinuation assessment, and all rebounded to reach a viremia with decreased $CD4^+$ T cells. However, the levels of HIV DNA in the peripheral blood were reduced and the speed of gene-modified T cell descending (-1.81 cells/mm^3/day) was significantly lower than that in the unmodified T cells (-7.25 cells/mm^3/day) (Tebas et al., 2014).

It is noteworthy that after treatment of genetically modified T cells, the virus load in one patient with congenital *CCR5Δ32* gene mutation was significantly reduced and plasma HIV detection remained negative. These data suggested that bi-allelic knockout of *CCR5* is essential for HIV resistance and gene editing efficiency is an important factor for the success of treatment. Up to now, the results of other completed clinical trials (NCT01252641, NTC01044654) have not yet been released (Patel et al., 2016). Current studies have demonstrated the safety and a certain degree of effectiveness of T cell gene editing for the treatment of HIV infection.

With further study of HIV infection, it was found that *CCR5* was not the only co-receptor for HIV virus to enter target cells. As *CCR5* plays a main role in the early stages of infection, C-X-C motif

Table 1
ZFN-mediated T cells or hematopoietic stem cell gene editing and treatment of HIV infection clinical trials.

Clinical trial	Title	Status (2017.6)
NCT00842634 Phase 1	Autologous T-cells genetically modified at the *CCR5* gene by zinc finger nucleases SB-728 for HIV	2009 to 2013 Completed
NCT01044654 Phase 1	Phase 1 dose escalation study of autologous T-cells genetically modified at the *CCR5* gene by zinc finger nucleases in HIV-infected patients	2009 to 2014 Completed
NCT01252641 Phase 1/2	Study of autologous T-cells genetically modified at the *CCR5* gene by zinc finger nucleases in HIV-infected subjects	2010 to 2015 Completed
NCT02225665 Phase 1/2	Repeat doses of SB-728mR-T after cyclophosphamide conditioning in HIV-infected subjects on HAART	2014 to 2018 Active, not recruiting
NCT01543152 Phase 1/2	Dose escalation study of cyclophosphamide in HIV-infected subjects on HAART receiving SB-728-T	2011 to 2016 Active, not recruiting
NCT02388594 phase 1	A phase I study of T-cells genetically modified at the *CCR5* gene by zinc finger nucleases SB-728mR in HIV-infected patients	2015 to 2017 Recruiting
NCT02500849 Phase 1	Safety study of zinc finger nuclease *CCR5*-modified hematopoietic stem/progenitor cells in HIV-1 infected patients	2015 to 2018 Recruiting

chemokine receptor 4 (*CXCR4*) is the other important entry co-receptor in the late stages of HIV infection (Hoffmann, 2007). Considering that *CXCR4* is important to hematopoietic stem cell homing, knockout of *CXCR4* gene in T cell is a more promising strategy to gain resistance to CXCR4-utilizing strain infection (Peled et al., 1999). *CXCR4* knockout $CD4^+$ T cells had normal growth and function, and showed resistance to CXCR4-utilizing strain (Wilen et al., 2011; Yuan et al., 2012), while *CCR5* and *CXCR4* double knockout $CD4^+$ T cells were resistant to all HIV strains (Didigu et al., 2014).

One of the important issues should be considered in the clinical applications of gene editing is the potential off-target effects. The disruption rate at the top predicted off-target site of *CCR5* ZFN pair in clinical trial is about 5%, which occurs mainly in the C-C motif chemokine co-receptor type 2 (*CCR2*) gene (Perez et al., 2008). Upon optimizing the design, it is possible to reduce the off-target activity of ZFNs while maintaining a high on-target efficiency (Miller et al., 2007; Szczepek et al., 2007). Based on these pioneering studies of ZFNs, TALENs and CRISPR-Cas9 system have also been applied to eliminate *CCR5* or *CXCR4* in T cells and hematopoietic stem cells (Cho et al., 2013; Yang et al., 2013; Mandal et al., 2014; Mussolino et al., 2014; Hendel et al., 2015; Li et al., 2015; Hultquist et al., 2016; Xu et al., 2017).

A major limitation of the above approaches is that they can only prevent the spread of infection while do not eliminate HIV provirus integrated in the genome. Gene editing can also be used to disrupt or excise integrated HIV provirus, reducing the size of the HIV reservoir. ZFN, TALEN, and CRISPR-Cas9 have all been applied to target different regions of the HIV genome to reduce HIV-1 content in various cell lines (Ebina et al., 2013, 2015; Qu et al., 2013; Hu et al., 2014; Zhu et al., 2015). However, the lack of an efficient method to enable *in vivo* delivery of nucleases to HIV infected cells makes it challenging to translate into clinical application.

At present, a variety of studies using gene editing to prevent HIV infection and replication are in full swing and bring the hope to the cure of HIV (Fig. 1).

3. The application of gene editing in T cells for cancer treatment

3.1. Immune checkpoint

ACT in cancer immunotherapy has shown great promise in preclinical and clinical studies in recent years (Couzin-Frankel, 2013). ACT such as TILs, TCR-T and CAR-T have achieved encouraging results in the treatment of a series of tumors, and a large number of clinical trials have been carried out (June et al., 2015). However, the current success of CAR-T therapy is largely confined to B-cell-derived hematologic malignances (Kalos et al., 2011; Brentjens et al., 2013; Grupp et al., 2013; Maude et al., 2014; Lee et al., 2015) and the therapeutic effect on solid tumors is very limited (Kershaw et al., 2006; Park et al., 2007; Morgan et al., 2010; Louis et al., 2011; Lamers et al., 2013; Ahmed et al., 2015; Brown et al., 2015; Katz et al., 2015). ACT therapy for solid tumors is currently facing three major obstacles. First, tumor-specific antigens are limited. Second, T cells cannot effectively homing and penetrate into the tumor tissues. Third, exogenous infused T cells are inhibited by tumor immunosuppressive microenvironment leading to exhaustion and loss of function (Khalil et al., 2016).

In particular, the immunosuppressive signals mediated by immune checkpoints, such as programmed cell death 1 (PD1), cytotoxic T-lymphocyte antigen 4 (CTLA4), lymphocyte-activated gene-3 (LAG3) and T cell immunoglobulin and mucin domain 3 (TIM3) in the tumor microenvironment, play important roles in promoting tumor immune escape (Khalil et al., 2016). Early studies have established that the use of monoclonal antibodies blocking immune checkpoints can rescue T cell exhaustion and restore T cell function (Nishimura et al., 1999; Hirano et al., 2005; Barber et al., 2006; Schietinger and Greenberg, 2014; Nguyen and Ohashi, 2015; Wherry and Kurachi, 2015). Based on these findings, immune checkpoint blockade therapy has been used in clinical trials and achieved exciting results with partial or complete regression in patients with advanced tumors to which standard treatment is

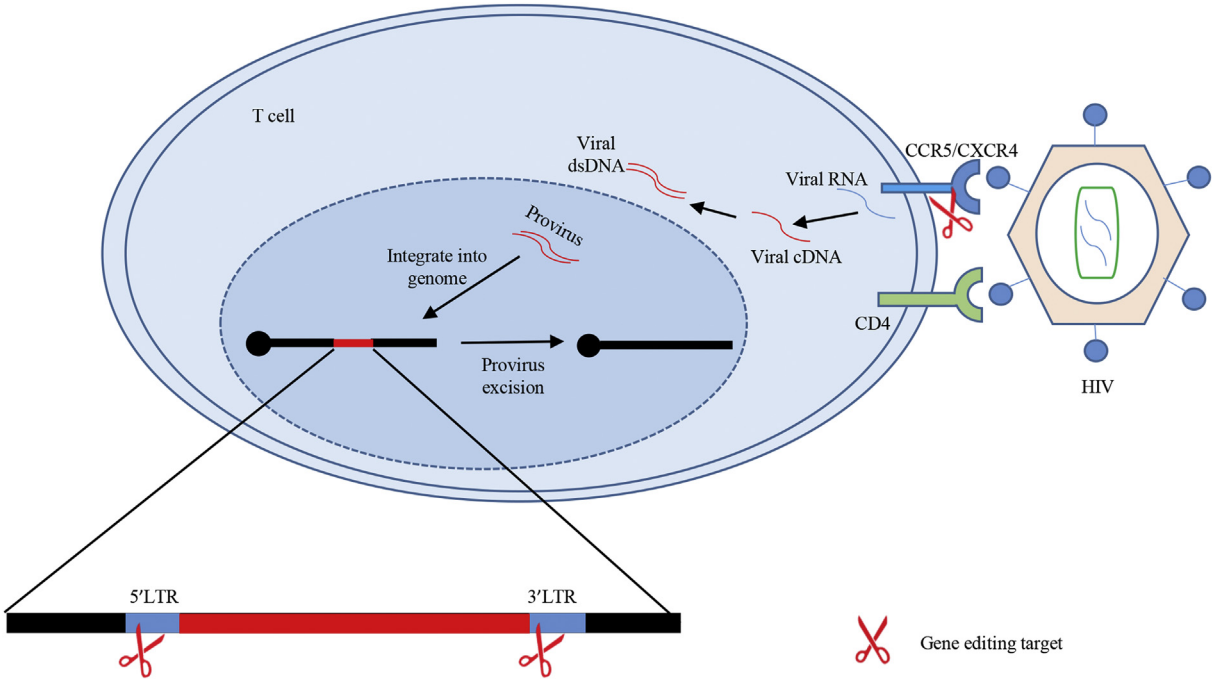

Fig. 1. Gene editing in T cells against HIV infection. HIV infects host cells through the recognition of CD4 receptor and CCR5/CXCR4 co-receptors. Upon entering cytoplasm, its RNA genome is reverse-transcribed into DNA that is then integrated into the host genome. Gene editing has been applied to knockout *CCR5* and/or *CXCR4* gene, eliminating the co-receptors necessary for the entrance of the virus. Gene editing has also been used to target HIV provirus to remove it from the host genome.

ineffective (Hodi et al., 2010; Hamid et al., 2013; Ribas et al., 2016). Unfortunately, sustained tumor regression is not common, and in many patients there were no effects. To break the immunosuppressive microenvironment, ACT and monoclonal antibody combined therapy is an attractive strategy (Khalil et al., 2016). However, immune checkpoints play a critical role in both regulating the physiological immune response and maintaining autoimmune tolerance, and long-term systemic use of immune checkpoint inhibitors may break the immune tolerance, leading to serious side effects (Pardoll, 2012). With the development of gene editing technologies, it is possible to knock out immune checkpoint genes prior to ACT, therefore generating more powerful T cells that are nonresponsive to the immunosuppressive microenvironment of solid tumors, while avoiding the long-term use of immune checkpoint inhibitors.

PD-1 is the most promising gene editing target based on the clinical results of anti-PD-1 antibodies. *PD-1* is expressed after T cell activation and binds to the corresponding ligand PD-Ll/PD-L2, which inhibits the transmission of T cell activation signals (Freeman et al., 2000; Karwacz et al., 2011). In chronic infection or tumor microenvironment, due to long-term stimulation of antigens, *PD-1* is highly expressed on T cells and impairs their function, which is an important feature of T cell exhaustion (Wherry, 2011). As a variety of tumor cells express PD-1 ligands (Topalian et al., 2012; Zitvogel and Kroemer, 2012), using gene editing technology to eliminate *PD-1* in T cells provides a rational strategy to resist immunosuppression. Using ZFN to knock out *PD-1* in TILs isolated from melanoma patients, Beane et al. (2015) revealed a knockout efficiency of 75% and the bi-allelic mutation incidence in the range of 40%–48%, leading to 76% decrease of PD-1 expression on T cell surface. While *PD-1* knockout did not affect the T cell subsets and proliferation, it led to a stronger antigen-specific tumor cell killing and cytokine release *in vitro* (Beane et al., 2015).

Menger et al. (2016) used TALEN to knock out *PD-1* on tumor antigen-specific T cells isolated from the melanoma and fibrosarcoma mice. They found that *PD-1* knockout T cells were able to persist in the tumor site longer, and could control the tumor progression better than non-engineered T cells. The CRISPR-Cas9 system was also applied to knock out the *PD-1* gene in human primary T cells and the enhanced function of gene modified T cells was also found (Schumann et al., 2015; Su et al., 2016, 2017). Recently, we, as well as another group, used CRISPR-Cas9 to knock out *PD-1* in CAR-T cells. The *PD-1* expression on CAR-T cell surface was significantly reduced while the proliferation and immune phenotype were not significantly affected. The gene edited CAR-T cells showed a stronger anti-tumor effect both *in vivo* and *in vitro*. Moreover, the

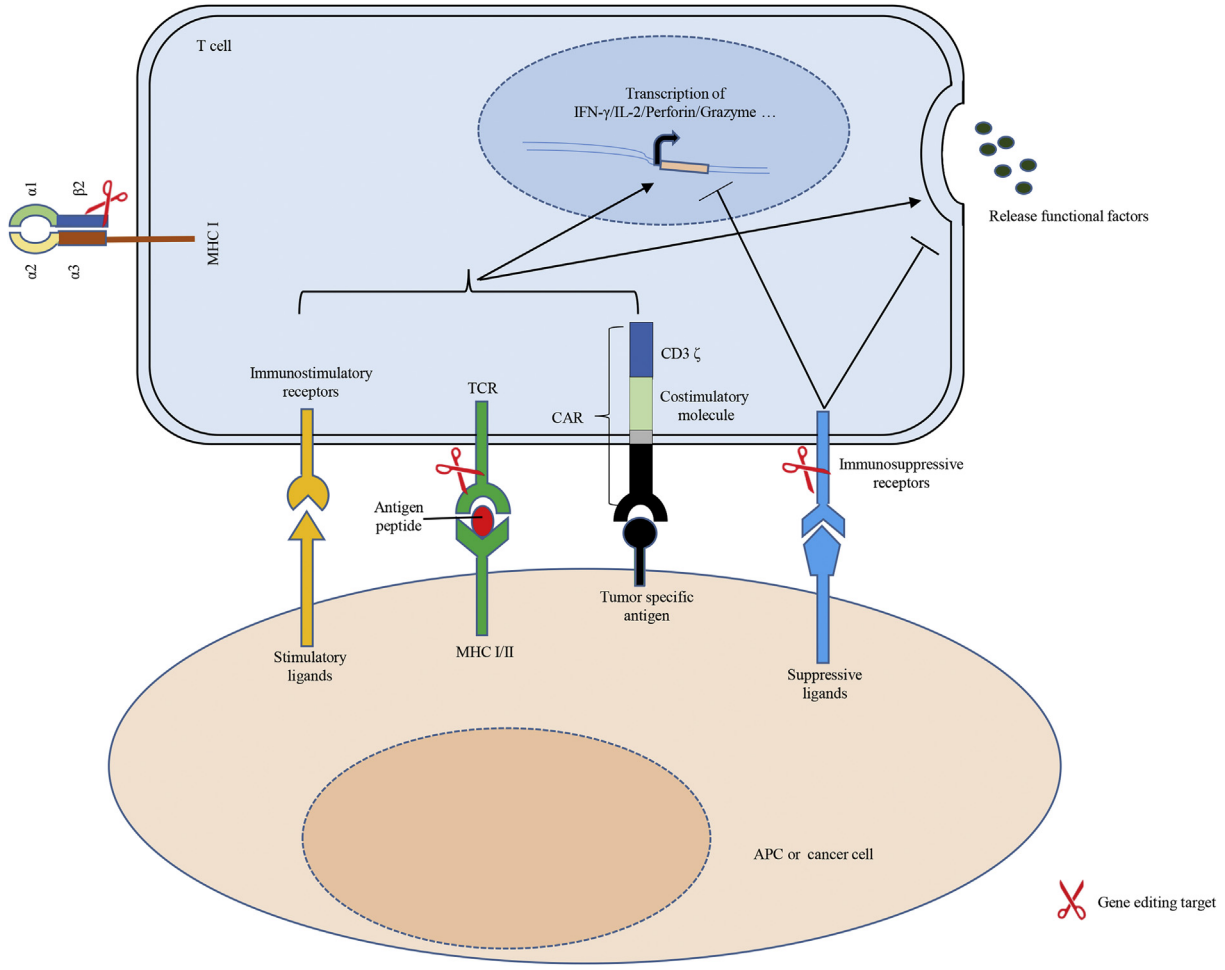

Fig. 2. Gene editing in T cells for cancer treatment. Antigens expressed on cancer cell surface or presented by MHC molecule can be recognized by CAR or TCR. Upon activation, T cells release functional cytokines (IFN-γ, IL-2) and factors like perforin and grazyme to eliminate cancer cells. However, in tumor microenvironment (TME), immune inhibitory factors suppress the function of T cells. By eliminating the immunosuppressive receptors (such as PD1, CTLA4, TIM3, LAG3) by gene editing, T cells are rendered more resistant to the immunosuppressive effect of TME. To generate universal cells, TCR on allogeneic CAR-T cells is removed by gene editing to avoid GVDH. Knocking out β2 microglobulin subunit eliminates MHC class I molecules on T cell surface.

advantage of using CRISPR-Cas9 platform is that multiple genes can be knocked out simultaneously (Liu et al., 2017; Ren et al., 2017a, 2017b).

In addition to PD-1, many preclinical studies have indicated that TIM-3 and LAG-3 play a synergistical role with PD-1 in regulating immune response and mediating tumor escape, as blocking PD-1 and LAG-3 or TIM-3 has synergistically enhanced antitumor effects (Woo et al., 2012; Lloyd et al., 2013). More recently, we used CRISPR-Cas9 to knock out *LAG-3* gene in T and CAR-T cells with high efficiency without dramatically affecting its viability and immune phenotype during *in vitro* culture. However, we did not find a positive effect on CAR-T effector function following *LAG-3* disruption in *in vitro* cytotoxicity assay and murine xenograft model (Zhang et al., 2017). Since the tumor model we used does not recapitulate the immunosuppressive environment of primary tumor, these results are far from conclusive. Future efforts are required to explore CAR-T cells with immune checkpoint knockout in better tumor microenvironment models, such as patient-derived xenograft (PDX) model generated using primary solid tumor samples (Fig. 2) (Aparicio et al., 2015; Hoffman, 2015).

3.2. Generating universal CAR-T cells

Currently, CAR-T cells are mainly produced using patient's own T cells (autologous transfer). This procedure is expensive and time-consuming, which involves isolating, modifying and expanding T cells for each patient. Furthermore, for newborn, elder or cachexia patients, it is often difficult to obtain enough T cells with good quality to generate patient-specific CAR-T cells. Overall, the lack of standard CAR-T generation procedure may hinder the wide application of CAR-T therapy.

One potential solution is to generate universal CAR-T cells using T cells derived from healthy donors (allogeneic adoptive transfer). To achieve this, the αβTCR on allogeneic CAR-T cells needs to be eliminated to avoid graft-versus-host-disease (GVHD), and human leukocyte antigens class I (HLA-Is) on CAR-T cells need to be removed to minimize their immunogenicity. To this end, efficient multiplex gene editing in T cells is required to ablate the expression of TCRs, MHC, or other molecules.

The TCRα chain is encoded by a single gene *TRAC*, and the TCRβ chain is encoded by two *TCRB* genes. The two chains form a dimer to maintain the expression and function of TCR on the surface of T cells. Therefore, knocking out the *TRAC* gene is the simplest strategy to eliminate TCR on T cell surface. At present, the main gene editing techniques ZFN (Torikai et al., 2012), TALEN (Berdien et al., 2014; Poirot et al., 2015; Valton et al., 2015) and CRISPR-Cas9 systems (Liu et al., 2017; Ren et al., 2017a, 2017b) have all been successfully applied to knock out TCR on CAR-T cells. TCR knockout CAR-T cells maintain antigen-specific tumor cell killing function both *in vitro* and *in vivo*. The immune response to allogeneic antigen was significantly reduced and did not cause GVHD in mice (Poirot et al., 2015). MHC class I molecules could be eliminated on T cell surface to avoid or delay rejection of transferred cells by knocking out β2 microglobulin (B2M) which is essential for cell-surface expression of HLA-I heterodimers (Torikai et al., 2013; Liu et al., 2017; Ren et al., 2017a, 2017b).

Another group and we used CRISPR-Cas9 technology to simultaneously knock out TCR and HLA molecules in CAR-T cells. In addition, we simultaneously knocked out immune checkpoint PD-1 to develop more powerful universal CAR-T cells (Fig. 2) (Liu et al., 2017; Ren et al., 2017a, 2017b).

It is noteworthy that the absence of HLA class I molecules may elicit an NK response against allogeneic T cells. One possible solution is to engineer T cells with overexpression of other HLA class I molecules, such as HLA-G or HLA-E to avoid NK cell activation. Instead of knocking out HLA class I molecules, other groups knocked out *CD52* (Poirot et al., 2015) or deoxycytidine kinase (*dCK*) gene (Valton et al., 2015) to make CAR-T cells resistant to alemtuzumabor purine nucleotide analogues. These drugs are commonly used for lymphodepleting chemotherapy, which can ensure the removal of patients' lymphocytes while maintaining infused allogeneic CAR-T cells.

Recently, in a first-in-human clinical application, TALEN-edited CD19 CAR-T cells lacking TCR and CD52 were successfully used to treat relapsed refractory $CD19^{+}$ B cell acute lymphoblastic leukemia. Within 28 days, molecular remissions were achieved in both patients, followed by allogeneic stem cell transplantation, demonstrating the clinical efficacy of universal CAR-T cells. It's noteworthy that despite less than 1% of infused CAR-T cells with TCR expression, both patients developed symptoms of skin GVHD, highlighting the need for further improvement (Qasim et al., 2017).

More recently, Ren et al. (2017a,b) developed a one-shot CRISPR system by constructing multiple sgRNAs in the CAR lentiviral vector, which can efficiently knock out four genes simultaneously. They used the CRISPR-Cas9 system to generate Fas receptor knockout and *PD-1* and *CTLA-4* knockout universal CAR-T cells. The Fas receptor (also known as CD95) induces T cell apoptosis by binding to its ligand FasL (Gorak-Stolinska et al., 2001; Pignatti et al., 2001; Maher et al., 2002) and can impair CAR-T functions (Kunkele et al., 2015). Ren et al. (2017b) found that *Fas* knockout CAR-T cells could better remove tumor from tumor-bearing mice and prolong the survival of mice (Ren et al., 2017b).

Another important application of gene editing is to develop CAR-T cells with site-specific integration of transgene. The *CAR* gene is mostly introduced into the T cell genome by retroviral or lentiviral transduction, which has a potential risk of tumorigenesis due to the random insertion in the genome and the highly variable CAR expression levels (von Kalle et al., 2014). Researchers have integrated the exogenous gene into human lymphocytes and hematopoietic stem cell in *CCR5* locus and adeno-associated virus site 1 (AAVS1) which is a safe harbor in human genome that allows for stable expression of transgene without affecting surrounding genes (Lombardo et al., 2011; Sather et al., 2015). Notably, two recent studies introduced *CAR* transgenes into the *TRAC* locus using CRISPR-Cas9 or engineered homing endonuclease (Eyquem et al., 2017; MacLeod et al., 2017). Interestingly, it not only resulted in more uniform CAR expression in T cells, but also enhanced T cell potency with reduced tonic signaling, terminal differentiation, and exhaustion.

4. Concluding remarks

Gene editing technologies hold great promise for enhancing T cell therapy, bringing hope for functional cure of HIV infection and tumors. However, the clinical applications of gene editing T cell therapies still face some questions. The safety of genetically modified T cells is the primary concern. Although a large number of studies have focused on improving the specificity of gene editing and minimizing off-target effects (Gabriel et al., 2011; Frock et al., 2015; Kim et al., 2015; Tsai et al., 2015; Wang et al., 2015; Kleinstiver et al., 2016; Slaymaker et al., 2016), it remains to be determined what level of accuracy is required for each particular clinical application. On the other hand, how the immune system will respond to the genetically modified cells is not clear. However, based on a large number of preclinical and clinical studies, and the recent result of first-in-human clinical application of universal CAR-T therapy, gene editing technologies combined with T cell therapy have shown great promises. With the rapid development of gene editing techniques, such as the development of novel CRISPR systems (Shmakov et al., 2015; Zetsche et al., 2015; Mei et al., 2016)

and the discovery of DNA-mediated nuclease (Swarts et al., 2014), gene editing in T cells will be further perfected and play an increasingly important role in T cell therapy.

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