

# A giant step forward: chimeric antigen receptor T-cell therapy for lymphoma

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**Abstract** The combination of the immunotherapy (i.e., the use of monoclonal antibodies) and the conventional chemotherapy increases the long-term survival of patients with lymphoma. However, for patients with relapsed or treatment-resistant lymphoma, a novel treatment approach is urgently needed. Chimeric antigen receptor T (CAR-T) cells were introduced as a treatment for these patients. Based on recent clinical data, approximately 50% of patients with relapsed or refractory B-cell lymphoma achieved complete remission after receiving the CD19 CAR-T cell therapy. Moreover, clinical data revealed that some patients remained in remission for more than two years after the CAR-T cell therapy. Other than the CD19-targeted CAR-T, the novel target antigens, such as CD20, CD22, CD30, and CD37, which were greatly expressed on lymphoma cells, were studied under preclinical and clinical evaluations for use in the treatment of lymphoma. Nonetheless, the CAR-T therapy was usually associated with potentially lethal adverse effects, such as the cytokine release syndrome and the neurotoxicity. Therefore, optimizing the structure of CAR, creating new drugs, and combining CAR-T cell therapy with stem cell transplantation are potential solutions to increase the effectiveness of treatment and reduce the toxicity in patients with lymphoma after the CAR-T cell therapy.

**Keywords** chimeric antigen receptor T (CAR-T) cell; lymphoma; cytokine release syndrome (CRS); immune effector cell-associated neurotoxicity syndrome (ICANS)

## Introduction

The lymphoma comprises a heterogeneous group of lymphoid neoplasms, which originate from lymphocytes, and arises in the context of immune dysregulation. Generally, the lymphoma is divided into two subtypes, namely, Hodgkin lymphomas (HL) and non-Hodgkin lymphomas (NHL) in accordance with the morphology of tumor cells. The HL is characterized using the Reed–Sternberg cells, which are derived from B cells [1]. The NHL is derived from diverse cell types, including B, T, or natural killer (NK) cells. In 2019, newly diagnosed HL and NHL account for 0.46% and 4.21% of cases, respectively, in the United States [2]. In China, the incidence rate of

malignant lymphoma is 6.68/100 000 [3]. Furthermore, with the aid of a conventional first-line chemotherapy regimen (ABVD), the 5-year overall survival (OS) rate reaches 73% in patients with HL after 6–8 courses of treatment [4]. Moreover, 90.1% of young patients (aged 18 to 60 years) with good prognostic factors and 43.5% of elderly patients (aged 60 to 80 years) with newly diagnosed diffuse large B cell lymphoma (DLBCL) can obtain a long-term survival of 6 and 10 years after 6–8 courses of first-line chemotherapy, respectively [5,6]. However, 25% of patients with HL and 50% of patients with DLBCL fail to respond or relapse after the administration of the first-line chemotherapy. The majority of patients with relapsed or refractory (R/R) lymphoma eventually die due to the disease progression.

The conventional chemotherapy is proven useful in treating patients with lymphoma, but the addition of rituximab to the conventional chemotherapy regimen has increased the long-term survival by 20% and has the

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potential to cure about half of the patients with DLBCL [5,6]. About 85%–90% of NHL is derived from B cells [7], and the CD20 antigen presents on the surface of lymphoma cells. The rituximab is a milestone immunotherapy for cancer, representing the first monoclonal antibody able to target tumor cells. The rituximab binds with the CD20 on B lymphoma cells and activates antibody-dependent cell-mediated cytotoxicity to kill tumor cells. In short, rather than eliminating rapidly dividing cells by using cytotoxic drugs, the aim of immunotherapy is to utilize our own immune system for the elimination of cancerous cells from the body.

Recently, the chimeric antigen receptor T (CAR-T) cell, a genetically engineered immune cell product, has made remarkable progress in the treatment of hematological malignancies. The complete remission (CR) rate of R/R acute lymphocyte leukemia (ALL) and DLBCL can reach 90% [8,9] and 50% [10–12], respectively, by using the CD19 CAR-T cell therapy. Given their effectiveness in combating hematological malignancies, two commercial CD19-targeted CAR-T cell products are approved by the Food and Drug Administration (FDA) in 2017. As of November 2019, 887 CAR-T therapy-associated clinical trials are registered at ClinicalTrial.gov, and 268 trials are for lymphoma. Since then, the CAR-T therapy has become a popular topic in scientific research and clinical applications. In this study, we review the most recent data on CAR-T cell therapy and document the progress of research and clinical trials using this therapy.

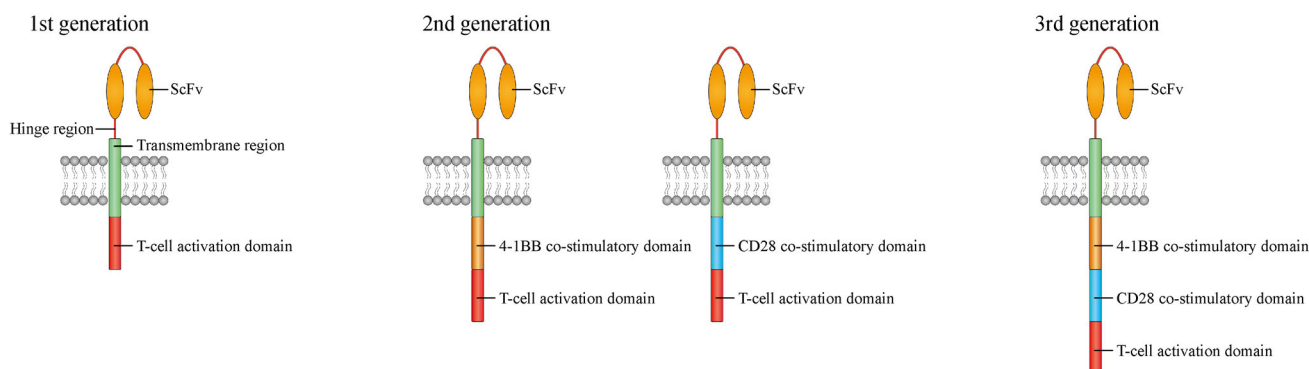
## Chimeric antigen receptors (CARs)

CARs are genetically engineered transmembrane protein that can recognize specific tumor antigens and activate

immune effector cells. CAR structures are made up of antigen-recognizing, transmembrane, costimulatory, and T cell-activating domains. The antigen-recognizing domain is frequently derived from a single-chain variable fragment (scFv) region, which can bind to a specific tumor antigen (Fig. 1B). The antigen-recognizing domain is fused to intracellular domains (including the costimulatory and T cell activation domains) via a transmembrane domain mainly derived from CD8 $\alpha$  or CD28. Researchers have designed different antigen-recognizing domains in accordance with specific tumor antigens to target different types of tumor cells. Until now, the FMC63 has been the most widely used CD19-specific monoclonal antibody in CAR-T cells and targets CD19-positive B cell malignancies. Notably, the only two FDA-approved CAR-T cell products are based on FMC63 [11,12]. Additionally, costimulatory domains, such as CD28 and 4-1BB, have replaced antigen-presenting cells (APC) in providing second signal in the T cell activation signaling pathway, with the T cell activation signaling pathway, with the T cell activation domain CD3 $\zeta$  providing the first signal in the T cell activation signaling pathway. When CARs bind to specific tumor antigens, the intracellular domain provides dual signals to activate CAR-T cells, leading to a tumor-killing effect and the proliferation of CAR-T cells [13].

## Generation of CAR-T cells

The first attempts to generate a chimeric T cell receptor (TCR) composed of the TCR constant region and the V-region domain derived from the immunoglobulin are performed in the late 1980s [14,15]. In 1993, Eshhar *et al.* have developed the first-generation CAR, consisting of the scFv and the CD3 $\zeta$  to overcome the low efficiency of single cell transduction with two separate retroviral vectors

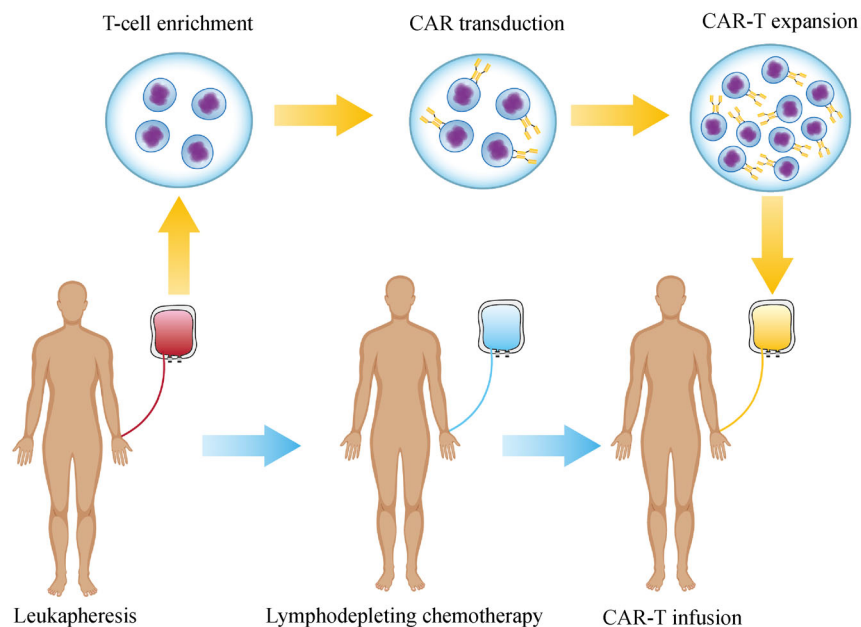


**Fig. 1** Generation of CAR structures. (A) First-generation CARs contain antigen-recognizing (scFv), transmembrane, and T cell activation (CD3 $\zeta$ ) domains. (B) Second-generation CARs, to which the costimulatory domains (CD28 or 4-1BB) are added with respect to the first-generation CAR, show high levels of antitumor activity. (C) Third-generation CARs include two costimulatory domains (CD28 and 4-1BB).

[16] (Fig. 1A). However, the first-generation CAR-T cells have failed to eliminate NALM-6 tumor cells (human pre-B ALL cell line) *in vivo* [17,18]. Subsequently, Sadelain *et al.* and Campana *et al.* have introduced the costimulatory domains CD28 or 4-1BB into the first generation of CAR-T cells (Fig. 1B) [18,19]. With the addition of the costimulatory domain into the CARs, this second generation of CAR-T cells show high treatment efficacy in B cell hematological malignancies. In 2010, Rosenberg *et al.* have reported the treatment of a patient with advanced follicular lymphoma (FL) through the CAR-T cellular therapy. Astonishingly, this patient has obtained partial remission (PR) status for 32 weeks [20] and is the first patient with lymphoma who has been treated successfully with the CAR-T therapy. Costimulatory domains (e.g., CD28, 4-1BB, and OX40) are introduced to the third generation of CAR-T cells, and the preclinical data actually show that third-generation CARs have higher cytolytic efficacy than second-generation CARs (Fig. 1C) [21–28]. For instance, Till *et al.* have reported that a patient with FL and two patients with mantle cell lymphoma (MCL) have received anti-CD20 CAR-T cells containing CD28 and 4-1BB costimulatory domains and achieved objected response (OR) [29]. At present, many researchers are focused on designing effective and safe CAR-T cells via the addition of genes encoding cytokines [30], costimulatory ligands [31], a “safety switch” [32,33], or universal CAR [34–38].

## Process of the CAR-T cell therapy in clinics

First, lymphocytes are collected from patients or donors via the leukapheresis. T cells are harvested and activated by antibody-coated beads. Usually, the *CAR* gene is transferred into T cells via the transgenic technology or the electroporation [39–41] by lentivirus [8,11] or retrovirus [12]. CAR-T cells are sufficiently expanded *ex vivo* for clinical use. Generally, patients receive the lymphodepleting chemotherapy (fludarabine and cyclophosphamide) before the CAR-T cell infusion (Fig. 2). Compared with administering cyclophosphamide alone, the combined lymphodepleting regimen of fludarabine and cyclophosphamide can effectively increase the expansion of CAR-T cells [42]. The lymphocyte depletion is a crucial step in establishing a favorable cytokine profile, eliminating immunosuppressor cells, and destroying the tumor microenvironment (TME) for improved CAR-T expansion *in vivo* [43–45]. Until now, only two CD19-targeted CAR-T cell products, namely, tisagenlecleucel and axicabtagene ciloleucel, have been approved worldwide by respective regulatory state health departments. However, no available commercial CAR-T cell product exists for HL and NHL derived from NK and T cells. The discovery of a new tumor-specific antigen is indispensable to broaden CAR-T therapy indications in different types of lymphoma and other solid tumors, such as anti-CD30 CAR-T cells for HL. The CAR-T therapy has shown an incredibly high CR



**Fig. 2** Process of CAR-T cell therapy in clinics. Lymphocytes were collected from patients or donors via the leukapheresis. After T cell enrichment and activation, the *CAR* gene was transduced into T cells. The CAR-T cells were expanded adequately *ex vivo* for clinical use. Patients normally received the lymphodepleting chemotherapy prior to the CAR-T cell infusion.

rate (about 90%) in R/R ALL, but a CR rate of only 50% in the R/R large B cell lymphoma. This phenomenon is likely to be because of the presence of the TME that limits the infiltration and the expansion of CAR-T cells in lymphoma tissues. However, many new solutions, such as the modification of the CAR structure, the combination of novel medications, and the optimization of treatment strategies (bridging CAR-T to allogeneic hematopoietic stem cell transplantation (HSCT)) have been proposed to improve the remission rate and prolong the survival period in preclinical and clinical studies. Thus, in this article, we will review the clinical progression of the CAR-T cellular therapy associated with lymphoma.

## Data of commercialized CAR-T cell products from clinical trials

### Tisagenlecleucel: JULIET clinical trial

The tisagenlecleucel utilizes the FMC63, 4-1BB, and CD3 $\zeta$  as antigen-recognizing, costimulatory, and T cell-activating domains, respectively. The tisagenlecleucel is generated via lentivirus transgenic methods and approved by the FDA for the treatment of R/R ALL and B-NHL. In the JULIET clinical trial (NCT02445248), a single-group, open-label, multicenter, international phase 2 study for tisagenlecleucel in R/R DLBCL, 93 adult patients have received the tisagenlecleucel infusion at a median dose of  $3 \times 10^8$  CAR-T cells after the lymphodepleting conditioning regimen. The best objective response rate is 52% (40% CR rate and 12% PR rate) without any treatment-related mortality (TRM). In the median follow-up period of 14 months, the median OS and 12-month OS rate among patients who received an infusion is 12 months and 49%, respectively. The 12-month progression-free survival (PFS) rate is estimated at 90% and the median PFS has not been reached among the patients who show a complete response (CR) at three months. For patients with a durable response, the *CAR* gene can be detected for up to two years [11].

### Axicabtagene ciloleucel: ZUMA-1 clinical trial

The axicabtagene ciloleucel, the first commercialized CAR-T cell product for R/R B-NHL, is composed of FMC63, CD28, and CD3 $\zeta$  via retrovirus transgenic methods. In the ZUMA-1 clinical trial, a multicentered phase 2 clinical study is conducted for the axicabtagene ciloleucel for the treatment of R/R DLBCL, primary mediastinal B cell lymphoma, and transformed FL. Among 108 patients who have received the axicabtagene ciloleucel treatment, 82% have achieved OR, and 54% have achieved CR. Two cases have TRM. Subsequently, 101 patients are assessed for survival analysis and followed up for a median

of 27.1 months. For patients who have achieved CR, PR, and stable disease (SD) at 3 months, the estimated PFS rates at 24 months are 72%, 75%, and 22%, respectively. The median OS is not reached, whereas the OS rate at 24 months is estimated at 50.5%. A long PFS is associated with a high peak concentration of CAR-T cells and a great area under curve of CAR-T cell concentration within 28 days after infusion. By 24 months, the *CAR* gene-marked cells are detected in 21 out of 32 (66%) ongoing response patients [12].

Commercialized CAR-T cell products display high efficacy in eradicating lymphoma and achieving long-term persistence *in vivo*. Except the transgenic vector, the main difference between the tisagenlecleucel and the axicabtagene ciloleucel is the costimulatory domain, which is 4-1BB and CD28, respectively. The CD28 is a member of the immunoglobulin family of costimulatory receptors and regulates the IL-2 production and enhances the survival and the differentiation of T cells [46]. The 4-1BB (CD137), a member of the tumor necrosis factor (TNF) receptor superfamily, upregulates antiapoptotic molecules and cytokines, enhances the effector cell's function, and helps in the proliferation of memory T cells [47]. In a xenograft model study conducted by Carpenito *et al.*, CD28- and 4-1BB-based CAR-T cells show the same antitumor activity, but the 4-1BB-based CAR has performed better in enhancing the persistence of T cells *in vivo* compared with the CD28-based CAR [24]. In addition, CAR-T cells with CD28 and 4-1BB costimulatory domains regulate different metabolism pathways. The CD28-based CAR-T cells yield a higher proportion of effector memory T cells and rely more on anaerobic glycolysis than 4-1BB based CAR-T cells do to fulfill the metabolic demand of the effector cell proliferation in the rapid metabolic pathway. By contrast, the 4-1BB-based CAR-T cells promote the outgrowth of central memory T cells, depend more on fatty acid oxidation than CD28 based CAR-T cells do, and enhance mitochondrial biogenesis with long-term persistence [48].

## Various types of CAR-T targeting antigens for lymphoma

### Anti-CD20 CAR-T

Undeniably, the CD20 is a classical immunotherapy target antigen for lymphoma because the CD20 is greatly expressed on B cells. For instance, rituximab, the monoclonal antibody against CD20, is a milestone immunotherapy and the first-line therapy for most of the B-NHL. Preclinical studies have found that anti-CD20 CAR-T cells show high levels of antitumor activity *in vitro* [49] and *in vivo* [50]. The CD20 antibody is widely used for B-NHL patients but may be a great challenge for

patients with R/R lymphoma who have previously undergone the CD20-targeting immunotherapy.

Han *et al.* have conducted a single-center phase I/IIa clinical trial to assess the efficacy and the safety of anti-CD20 CAR-T for patients with R/R B-NHL. A total of 14 patients with R/R CD20<sup>+</sup> DLBCL and 3 patients with CD20<sup>+</sup> indolent lymphoma are recruited into this study. Each patient has received the anti-CD20 CAR-T cell infusion at a median dose of  $5 \times 10^6$ – $15 \times 10^6$  per kg. Prior to the anti-CD20 CAR-T cell infusion, 17 patients have received the rituximab treatment but experience disease progression. In this study, 8 and 6 out of 17 patients have achieved CR and PR, respectively. The best overall OR and CR rates are 82% and 47%, respectively. At a median follow-up period of 20 months, the median PFS and the estimated 2-year PFS rate in 12 eligible patients for survival analysis with response are 10 months and 41.7%, respectively [51].

### Anti-CD22 CAR-T

Similar to the CD19, the CD22 is restrictedly expressed on the surface of B cells and has shown promising antitumor activity in preclinical trials [52]. However, clinical data on the use of anti-CD22 CAR-T for the treatment of lymphoma have not been published up until now. In a clinical trial conducted by Shah *et al.* (NCT02315612), patients with R/R CD22<sup>+</sup> ALL and R/R CD22<sup>+</sup> lymphoma are enrolled. According to their published data, a 14-year old patient with refractory DLBCL who has received the anti-CD22 CAR-T cell therapy prior to the anti-CD19 CAR-T treatment has achieved SD [53]. Moreover, 21 patients with R/R ALL, including 15 patients who have previously received the anti-CD19 CAR-T cellular therapy, have received a median dose of  $1 \times 10^6$  per kg anti-CD22 CAR-T cell infusion. Twelve out of 21 (57%) patients have achieved CR. Among the 12 patients who have achieved CR, three patients have maintained ongoing remission at 21, 9, and 6 months after the anti-CD22 CAR-T infusion, but eight patients have relapsed at a median of 6 months after receiving the anti-CD22 CAR-T infusion. Furthermore, a patient has died of sepsis immediately after recovering from the cytokine release syndrome (CRS) and bone marrow suppression [54]. Until now, 11 anti-CD22 CAR-T cell therapy clinical trials for lymphoma are registered at [clinicaltrials.gov](http://clinicaltrials.gov).

### Anti-CD30 CAR-T

The CD30, a transmembrane receptor and a member of the TNF receptor superfamily, is universally expressed on the surface of HL, anaplastic large cell lymphoma (ALCL), and lymphomatoid papulosis cells. The CD30 is also expressed on the surface of other lymphomas derived from B or T cells, such as DLBCL, primary mediastinal B cell

lymphoma, mycoses fungoides, peripheral T cell lymphoma, and adult T cell leukemia/lymphoma [55–58]. The CD30 is an ideal target antigen because it is restrictedly expressed on tumor cells and on a small subset of lymphocytes [59], which may lead to a controllable risk of on-target off-tumor toxicity. In a preclinical study, anti-CD30 CAR-T cells exhibit a powerful tumor-killing effect in *in vitro* and *in vivo* settings [60]. A phase 1 dose escalation clinical trial is conducted on 7 patients with R/R HL and 2 patients with R/R ALCL. Patients have received a median dose of  $0.2 \times 10^8$ – $2 \times 10^8$  per m<sup>2</sup> CAR-T cells without a lymphodepleting conditioning regimen. As a result, 3 of 5 patients, including one R/R ALCL patient who has received a dose of  $2 \times 10^8$  per m<sup>2</sup> CAR-T cells, have successfully achieved CR. The CR status of the patients with ALCL is maintained for nine months after four courses of the anti-CD30 CAR-T cell infusion. Moreover, two other patients with HL have remained CR for over 24 and 36 months. Patients treated with low doses have not achieved OR [61]. In another open-label phase 1 clinical trial, 17 patients with R/R HL and 1 patient with R/R ALCL are enrolled. All patients have received a dose of  $1 \times 10^7$ – $3 \times 10^7$  per kg anti-CD30 CAR-T cells. In this trial, seven patients have achieved PR, and six patients have achieved SD with an OR rate of 39%. With a follow-up of 3–14 months, the median PFS obtained is 6 months [62]. In these two early-phase clinical trials, anti-CD30 CAR-T cells display promising levels of antitumor activity for the CD30<sup>+</sup> lymphoma.

### Anti-CD37 CAR-T

The CD37, a cell surface glycoprotein belonging to the transmembrane 4 superfamily, is expressed in most B cell lymphomas and some types of T cell lymphoma, including DLBCL, FL, MCL, Burkitt's lymphoma (BL), chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), cutaneous T cell lymphoma, and peripheral T cell lymphoma [63,64]. Recently, Maus *et al.* and Wälchli *et al.* have constructed anti-CD37 CAR-T cells successfully and reported their efficiency in eradicating the B cell lymphoma *in vitro* and *in vivo*, including patient-derived xenograft model [65,66]. Furthermore, Wälchli *et al.* have found that the anti-CD37 CAR-expressing B cell does not prevent the killing capacity of the anti-CD37 CAR-T, which may show superior safety by avoiding the risk of CD19 relapse due to accidental CAR transduction on patient B cells [66,67]. A phase 1 clinical trial of anti-CD37 CAR T cells is expected to start in February 2020 (NCT04136275).

### Anti-Igk CAR-T

As a component of membrane immunoglobulin, the  $\kappa$  or the  $\lambda$  light chain is expressed on the surface of mature B

lymphocyte and neoplastic counterparts, including BL, DLBCL, FL, MCL, hairy cell leukemia, marginal zone B cell lymphoma, prolymphocytic leukemia, and lymphoplasmacytic lymphoma (LPL). The CLL/SLL is also derived from mature B lymphocytes, but the expression of surface light chains on CLL/SLL cells remains limited, which is most likely due to the deletion of the B cell receptor complex that contains immunoglobulin, CD79a, and CD79b. In addition to immature lymphocytes, also known as lymphoblasts, the thymic B and the plasma cells lack the surface expression of the light chain and their neoplastic counterparts, including B lymphoblastic leukemia/lymphoma, primary mediastinal B cell lymphoma, and plasma cell disorders [68,69]. Considering that each lymphocyte expresses either the  $\kappa$  or the  $\lambda$  light chain but not both, anti- $\kappa$  or anti- $\lambda$  CAR-T cells are able to eradicate lymphoid malignancies and preserve part of the mature B lymphocyte to prevent the incidence of hypogammaglobulinemia. Dotti *et al.* have first constructed anti- $\kappa$  CAR-T cells with CD28 costimulatory domain and proven their efficiency and safety in a preclinical study [70]. Dotti *et al.* have conducted a phase 1 clinical trial to evaluate the efficiency and the safety of anti- $\kappa$  CAR-T and recruited 9 patients with NHL (including 2, 2, 2, 2, and 1 patients with LPL, transformed FL, DLBCL, CLL/SLL, and MCL, respectively) and 7 patients with MM. Patients without lymphopenia have received the cyclophosphamide conditioning followed by anti- $\kappa$  CAR-T infusion. Of 16 patients, 2 patients with transformed FL and 1 patient with LPL have achieved CR and PR, respectively, and 1 patient with CLL/SLL and 4 patients with MM have achieved SD. In this study, because most patients of NHL have the B cell aplasia and the polyclonal hypogammaglobulinemia at the baseline, the potential advantage of targeting the  $\kappa$  light chain and avoiding hypogammaglobulinemia requires further investigation [71].

## Toxicity of the CAR-T cell therapy

### CRS

The CRS is the most prominent adverse effect after the CAR-T infusion. Clinically, the CRS is markedly characterized by fever, hypoxemia, hypotension, tachycardia, coagulation dysfunction, and vital organ dysfunction. Furthermore, in laboratory findings, the CRS is marked by elevated cytokine levels, including IL-1, IL-2, IL-6, and IFN- $\gamma$ . When CAR-T cells recognize tumor-specific antigens, they release IL-2, soluble IL-2R $\alpha$ , IFN- $\gamma$ , IL-6, soluble IL-6R, and GM-CSF and activate the mononuclear phagocytic system. APCs, which are macrophages, then secrete abundant levels of IL-1RA, IL-6, IL-8, IL-10, soluble IL-6R, IFN- $\alpha$ , CXCL9, CXCL10, CCL3, and

CCL4. A high baseline disease burden and elevated clinical biomarkers, such as CRP and ferritin, are associated with severe CRS [72–77]. However, specific biomarkers to measure the severity of CRS are still not available. Therefore, until now, the management according to the CRS grading is based on the initial criteria, consisting of a combination of several clinical symptoms proposed by Lee *et al.* [78]. Besides, other clinical centers, such as the MD Anderson Cancer Center, Memorial Sloan Kettering Cancer Center, and University of Pennsylvania, have suggested their own CRS grading system [77,79,80]. The American Society for Transplantation and Cellular Therapy (ASTCT) has assigned experts from different clinical centers to agree on a set of consensual criteria for the CRS grading for the evaluation of different CAR-T cell toxicity in various clinical centers [81].

The CRS requires careful surveillance and experienced supportive care, including nonsteroidal anti-inflammatory drugs for fever, intravenous hydration, vasopressor for hypotension, and supplemental oxygen for hypoxia. Furthermore, the IL-6 receptor antagonist, tocilizumab, is prescribed for the treatment of moderate or severe CRS. However, no concrete evidence for the prophylactic or preemptive use of tocilizumab has been found because its effect on the T cell expansion and persistence remains unknown. For the treatment of the CRS refractory to symptomatic management and tocilizumab, steroids are strongly suggested, even though their lymphotoxicity may have a negative effect on CAR-T cells. Other cytokine-modulating agents, such as IL-6 antibody (siltuximab) [82], IL-1 receptor antagonist (anakinra) [73], and GM-CSF antibody (lenzilumab) [83] are also under preclinical and clinical evaluations. Plasmapheresis is another potential modality for the CRS management that allows the direct elimination of excessive cytokines [84].

Until today, infection and CRS are difficult to differentiate on the basis of subjective symptoms and objective evidence. Therefore, after receiving the CAR-T treatment, every febrile patient should undergo the broad-spectrum antibiotic therapy immediately. Moreover, CAR-T patients commonly experience pancytopenia [85]. However, the mechanism of the pancytopenia after the CAR-T infusion remains unclear and may be related to the myelosuppressive effect of the lymphodepleting chemotherapy and the interaction of CAR-T cells with immature B cell precursors in the bone marrow. Patients with a prognosis complicated with CRS and pancytopenia require G-CSF and transfusion support [86].

### Immune effector cell-associated neurotoxicity syndrome (ICANS)

The neurotoxicity related to the CAR-T therapy is once termed the CAR-related encephalopathy syndrome (CRES). With an increasing number of CRES-like

neurotoxicity cases reported in clinical trials that use blinatumomab (a bispecific T cell engager (BiTE) antibody) [87], the term CRES is no longer suitable for various immunotherapies, including BiTE, other engineered T cells, and checkpoint inhibitors. Experts from ASTCT have unified the neurotoxicity grading system related to immunotherapy and proposed the term “immune effector cell-associated neurotoxicity syndrome” to pertain to the adverse events occurring after immunotherapy.

The ICANS is an adverse event characterized by a pathologic process involving the central nervous system (CNS) after immunotherapy, including the CAR-T cell therapy. The ICANS can be manifested as decreased attention, changes in mental state, confusion, disorientation, hallucination, aphasia, ataxia, delirium, coma, encephalopathy, and cerebral edema [88,89]. The ICANS is a life-threatening side effect and limits the deployment of the CAR-T treatment. The JCAR015, a commercialized CAR-T therapy that is once pending approval from FDA, is withdrawn from phase 2 clinical trials soon after five cases of fatal ICANS are reported. In this retrospective analysis, the early rapid expansion and activation of JCAR015 with a CD19-recognizing, a CD28 costimulatory, and a CD3 $\zeta$ -activating domains with high levels of cytokines may be responsible for the lethal ICANS [90]. The ICANS may be related to cerebral microangiopathy and the CNS infiltration of targeted cells. A lymphodepleting condition regimen and a large quantity of inflammatory cytokines can lead to the damage of microvessels in CNS, which can eventually lead to the disruption of the blood–brain barrier (BBB).

Several clinical trials have demonstrated that CAR-T cells are able to penetrate the BBB and eliminate targeted cells in the CNS. The presence of severe ICANS is also correlated to severe CRS [91–94]. When CAR-T cells recognize CNS-infiltrating targeted cells, such as lymphoma and leukemic cells, through the disrupted BBB, CAR-T cells and bystander immune cells release cytokines and chemokines to recruit a large number of CAR-T cells and other immune cells to the site, thus inducing a cascade reaction. With high levels of cytokines, chemokines, and immune cells, a focalized cytokine storm in the CNS develops.

As mentioned previously, an intensive lymphodepleting condition regimen, prior intrathecal chemotherapy, and CNS infiltration are potential high-risk factors for the occurrence of ICANS. Nonetheless, as a type of monoclonal antibody, the tocilizumab is unlikely to penetrate the BBB to inhibit cytokine storms in the CNS. Therefore, despite the high risk of lymphotoxicity, the dexamethasone is commonly used for severe ICANS due to its ability to penetrate the CNS and BBB. Locke *et al.* have found that the prophylactic use of tocilizumab in 31 patients with R/R NHL receiving axicabtagene ciloleucel results in a low incidence of severe CRS but not ICANS. These data

provide preliminary evidence that the prophylactic or the preemptive use of tocilizumab may not benefit the incidence of severe ICANS [95].

### **Localized CRS and tumor lysis syndrome**

Lymphoma involves lymph nodes, bone marrow, CNS, skin, gastrointestinal tract, cardiovascular system, respiratory tracts, liver, spleen, urinary system, and other vital organs. During the antitumor activity of CAR-T cell, these cells tend to recruit bystander immune cells and induce an inflammatory cascade reaction at the tumor site. As a result, the tumor tissues and the nearby tissues become swollen. In some cases, when the lymphoma is adjacent to the respiratory tracts, veins, gastrointestinal tracts, liver, or urinary system, these tissues may swell after the infusion of CAR-T cells and may compress the corresponding tracts or cavities, thereby causing dyspnea, venous reflux disorder, ileus, jaundice, back pain, and oliguria [96]. Furthermore, if the tumor cells invade a hollow organ thoroughly, perforation and bleeding may occur [97]. As mentioned previously, the pancytopenia commonly occurs after the CAR-T cell infusion. Furthermore, if the prognosis of a patient with perforation or bleeding is complicated by the pancytopenia, this patient is likely to die due to infection or severe bleeding. Physical evaluations using whole-body PET-CT scans, brain MRIs, or lumbar punctures are essential to identify the lymphoma involvement sites before the CAR-T treatment to prevent unnecessary adverse events.

### **B cell aplasia and hypogammaglobulinemia**

CAR-T cells eradicate neoplastic cells and normal cells with target expression on surface, known as the on-target off-tumor effect. The B cell aplasia is a specific manifestation of the on-target off-tumor effect for CAR-T cells targeting the pan-B antigen. As progenitors of plasma cells, once B cells are eliminated, the regeneration of plasma cells is interrupted followed by the dysfunction of immunoglobulin production, leading to hypogammaglobulinemia [98]. As a consequence of hypogammaglobulinemia, patients may be exposed to infection for a long time after the CAR-T therapy. The intravenous immunoglobulin can be used to correct hypogammaglobulinemia and prevent opportunistic infections. Limited evidence has shown that some CD19<sup>+</sup> long-lived plasma cells are able to evade the CAR-T cell attack and persist in secreting antibodies in a B cell-independent manner [99]. However, even if the pre-existing humoral immunity is preserved after the anti-CD19 CAR-T therapy, patients (especially children) who have not completed a vaccination program need to restart vaccination after an appropriate amount of time. Given the lack of evidence for such special population, the current best choice consists of vaccination

until the B cell recovery [100]. The B cell aplasia represents circumstantial evidence of the persistent efficiency and existence of CAR-T cells. The persistent B cell aplasia implies that CAR-T cells *in vivo* exert immune surveillance for target antigen-positive lymphoma cells and are correlated with a durable clinical response [101].

## Current challenges and prospects of the CAR-T therapy

### Broadening the therapeutic indications of the CAR-T therapy for lymphoma

The success of the CAR-T cell therapy in the treatment of B-NHL is remarkable. However, the clinical results of the CAR-T therapy for lymphoma have been derived from several pivotal clinical trials involving DLBCL due to the high morbidity of DLBCL in B-NHL. Other than CD19-, CD20-, or CD22-positive R/R B-NHL, diseases, such as precursor B cell lymphoblastic lymphoma, BL [102], MCL, FL, and CLL/SLL, should also be considered in CAR-T clinical trials. The ZUMA-2, a phase II multicenter global clinical trial, has assessed the efficacy and the safety of the use of axicabtagene ciloleucel for the treatment of patients with R/R MCL. A total of 28 patients with R/R CD19<sup>+</sup> MCL are recruited, and each patient has received the anti-CD19 CAR-T cell infusion at a median dose of  $2 \times 10^6$  per kg. The OR and the CR rates are 86% and 57%, respectively. With a median follow-up period of 13.2 months, the estimated 1-year duration of response, PFS rate, and OS rate are 86%, 71%, and 86%, respectively [103]. Universal CAR-T cells from third-party donors, allogenic CAR-T from haploidentical donors and HLA-matched donors, or universal CAR-NK cells from cord blood [104] represent a potential strategy for patients who are unable to receive the autologous CAR-T treatment due to rapid disease progression, failure of CAR-T generation, or pre-existing immunodeficiency conditions, such as acquired immune deficiency syndrome (AIDS).

The CAR-T cellular therapy is approved for the third-line salvage therapy. With a reduction in the cost of the CAR-T manufacturing and an improvement in the safety of its clinical use, the CAR-T cell therapy can be upgraded to a second- or even first-line therapy [105]. In this case, patients with lymphoma can be cured using the CAR-T treatment in the early stage and does not have to undergo conventional chemotherapy regimens. The CD30, a much-anticipated new target antigen, may broaden the indications of CAR-T therapy for R/R HL, ALCL, lymphomatoid papulosis, and other T cell lymphomas. However, further phase 2 clinical trials should be performed to evaluate its efficacy and safety. Furthermore, new target antigens for NK/T cell NHLs, including CD4 (NCT03829540) [106], CD7 (NCT03690011) [107],

CD30 (NCT04083495), and CD37 (NCT04136275) [65], are suggested.

The main obstacles in the identification of novel target antigens for NK/T cell NHLs are fratricide between CAR-T cells due to the presence of pan-T cell antigens [108] and T cell aplasia, which is also known as the on-target off-tumor effect. The knockout of pan-T cell antigen genes by using gene editing techniques after the transduction of anti-pan-T cell antigen *CAR* gene may be an effective method to avoid the CAR-T fratricide. Moreover, the long-term T cell aplasia may lead to a similar condition to that observed in the AIDS. This condition may cause undesirable effects, including opportunistic infection and secondary tumors. Hence, the use of anti-NK/T cell NHL CAR-T therapy requires additional considerations because it may result in long-term T cell aplasia.

### Enhancing the efficacy and the persistence of CAR-T cells and overcoming the tumor-protecting effect for long-term survival

To date, about half of the patients with R/R B cell lymphoma who have received the CAR-T cell therapy in clinical trials have achieved CR. However, the other half of these patients have either relapsed or become refractory to the CAR-T cell therapy. An unsatisfactory CR rate related to the short PFS has become one of the major problems associated with the use of the CAR-T cell therapy for lymphoma. Compared with the high CR rate of R/R ALL and other precursor B cell-derived hematological malignancies, the heterogeneity of lymphoma cells and the TME may contribute to the low CR rate of patients with lymphoma after the CAR-T therapy [109]. The expression of the targeted antigens on lymphoma cells varies from one cell to another. Some lymphoma cells present low-density or no targeted antigens. This low affinity to CAR-T cells results in resistance to the tumor-killing effect of CAR-T cells [54,110–113]. Although high-density targeted antigen lymphoma cells are eliminated by CAR-T cells, low-density targeted antigen lymphoma cells tend to proliferate again and result in a relapse from short-term response or progressive diseases. Three strategies have been suggested under clinical trial evaluations to overcome the heterogeneity of antigen expression: (1) optimize the sequence of extracellular domains to increase the affinity of CARs and tumor-specific antigen [114]; (2) combine two or more monospecific CAR-T cells to cover broad-spectrum lymphoma cells [115,116]; (3) generate multispecific CAR-T cells that express multiple CARs on a single CAR-T cell (bicistronic CAR) [117] or a single CAR with two or more recognizing domains (tandem CAR) [34,118–121].

In addition, the TME in lymphoma is another important factor that leads to resistance to the tumor-killing effect of CAR-T cells. The gene expression in baseline tumor



samples, such as tumor-associated macrophages (i.e., CXCL2 and CXCL8), myeloid-derived suppressor cells (i.e., CXCL12, CCL3, CCL4, and CCL5), immunosuppressive cytokines (i.e., IL10 and TGF $\beta$ 1), tumor-associated dendritic cells (i.e., CD33 and CD14), and tumor-associated fibroblasts (i.e., FAP, TNC, CSPG4, PDGFRA, S100A4, ASPN, STC1, and ITGAM) in patients with PR R/R B-NHL is higher than that in patients with CR R/R B-NHL after the CAR-T treatment. Compared with those of patients with CR, the baseline tumor samples of patients with PR are characterized with low levels of the gene expression of chemokines (i.e., CCR6, CCR10, CXCR3, and CXCR4) and adhesion molecules (i.e., CD226, ITGAE, TNFRSF18). Increased tumor-associated macrophage infiltration and decreased tumor-infiltrating T cells in lymphoma are correlated with negative remission after the CAR-T therapy [122].

Besides, CD80, CD86, PDL1, PDL2, MHC class II, Galectin 9, and CEACAM1, which are largely expressed by antigen presenting cells or target cells, may cause the CAR-T cell exhaustion, which also results in poor T cell expansion or short-term T cell persistence [123]. In a clinical trial for R/R CLL, the preinfusion of CAR-T cells from the upregulated pathway of nonresponder patients is associated with apoptosis and exhaustion. The upregulation of coinhibitory molecules, such as PD-1, TIM-3, and LAG-3, on CAR-T cells indicates that the immune checkpoint pathway plays an important role in regulating the inhibitory effect on CAR-T cells [124]. The addition of an immune checkpoint blockade may reverse the exhaustion of CAR-T cells and maintain the tumor-killing effect and the persistence of CAR-T cells [125–127].

Other strategies for immune checkpoints, such as armored CAR-T cells secreting PD-L1 antibody [128], bispecific CAR-T targeting PD-L1, and tumor-specific antigen [129], represent potential solutions to counteract the CAR-T cell exhaustion. Moreover, the modification of condition regimens, such as intensive chemotherapy with autologous HSCT [130,131] and radiation [132,133], prior to the infusion of CAR-T cells may destroy the TME and improve the clinical outcomes of the CAR-T therapy.

### Reducing life-threatening adverse events in the CAR-T cell therapy

Another limitation of the CAR-T therapy is its adverse effects. As mentioned previously, the CRS is a major side effect of the CAR-T therapy. In addition to the application of cytokine monoclonal antibodies, the use of other small molecule agents, such as dasatinib [134,135], and the modification of the CAR-T cell structure, such as the development of a safety switch, should be explored in future studies [32,33]. Improving the understanding of the CAR-T among doctors and nurses is the key to decrease the mortality rate of the CAR-T therapy. With a safe and

effective CAR-T cell therapy, immunotherapy will play an indispensable role in the treatment of lymphoma in the future.

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## Compliance with ethics guidelines

Houli Zhao, Yiyun Wang, Elaine Tan Su Yin, Kui Zhao, Yongxian Hu, and He Huang declare that they have no conflict of interest. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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