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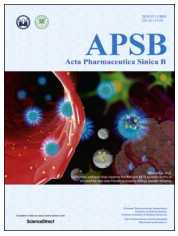

REVIEW

The application of CAR-T cell therapy in hematological malignancies: Advantages and challenges

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Abstract Chimeric antigen receptor T cell (CAR-T cell) therapy is a novel adoptive immunotherapy where T lymphocytes are engineered with synthetic receptors known as chimeric antigen receptors (CAR). The CAR-T cell is an effector T cell that recognizes and eliminates specific cancer cells, independent of major histocompatibility complex molecules. The whole procedure of CAR-T cell production is not well understood. The CAR-T cell has been used predominantly in the treatment of hematological malignancies, including acute lymphoblastic leukemia, chronic lymphocytic leukemia, lymphoma, and multiple myeloma. Solid tumors including melanoma, breast cancer and sarcoma offer great promise in CAR-T cell research and development. CD19 CAR-T cell is most commonly used, and other targets, including CD20, CD30, CD38 and CD138 are being studied. Although this novel therapy is promising, there are several disadvantages. In this review we discuss the applications of CAR-T cells in different hematological malignancies, and pave a way for future improvement on the effectiveness and persistence of these adoptive cell therapies.

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1. Introduction

T lymphocyte cells (T cells) play a key role in cell-mediated immune response. These cells are involved in monitoring and killing tumor cells or potentially malignant cells. During past years many therapies have been developed to culture, redirect, and/or enhance T cells against tumors[1]. Among them is the T cell-based adoptive immunotherapy, which is developing new means to deal with malignancies, especially hematologic cancers. This emerging therapy includes three models: tumor infiltrating lymphocytes, T cell receptor (TCR)-modified T cells and chimeric antigen receptor T cells (CAR-T cell)[2]. The first two techniques, compared with CAR-T therapy do not make a huge modification of the T cell *per se*, and therefore the efficacy is not substantial. Also, the process of production, the poor success rate, and the dependence upon vaccination limit their development[3,4]. As a promising therapeutic regimen, CAR-T cell therapy has stood the test of time for over 25 years[5]. Its TCR part is replaced by CAR which includes two domains: an extracellular and an intracellular domain. The extracellular domain is typically an antibody single-chain fragment (scFv) specifically against a cell surface antigen, while the intracellular domain includes fused signaling domains from a natural TCR complex and costimulatory molecules[6,7]. Different intracellular sections represent various CAR-T cell generations. The structure ranges from CD3z signaling domain alone in first generation CARs (lack of costimulatory signal) to those that possess the signaling endo-domains of costimulatory molecules like CD28, CD134 (OX40) or CD137 (4-1BB), which are fused with CD3z, in second and third generation CARs (Fig. 1). This structure imitates the costimulation signal when TCR combines with antigen-presenting cells to complete the process of activation[6,8,9]. All of these generate CAR-T cell specificity for a certain type of cancer cell and leads to their elimination[10]. Because a monoclonal antibody against a tumor antigen offers novel T cell specificity for certain types of cancer cells and bypasses the established antigen-presenting process, an important strength of this method is that the recognition is independent of the major histocompatibility complex[6,11]. Nevertheless, a novel generation of CARs has proven attractive to scientists. In addition to costimulatory signal(s) like CD28 and (or) CD137, this so-called "fourth generation of CARs" is also equipped with a "nuclear factor of activated T cell-responsive expression" element for an inducible transgenic product like IL-12 or other cytokine (Fig. 1). The specific recognition of a carcinogenic target by CAR-CD3 signaling stimulates the nuclear factor of activated T cell minimal promoter so IL-12 production and release result[12]. To avoid interaction between the promoter of CAR and inducible box, the two *trans*-genes are separated into different genomic sites[12]. The newest version is being tested in solid tumors, but current records of clinical trials are insufficient.

The whole procedure of CAR-T cell production is complicated[13] (Fig. 2). Firstly, T cells from peripheral blood are collected by phlebotomy or leukapheresis, followed by apheresis without addition of granulocyte colony stimulating factor[14]. The reason why granulocyte colony stimulating factor is excluded is that it may disrupt T-cell proliferation and responsiveness[15,16]. The separated T cells then are transfected with a CAR viral (retroviral or lentiviral) or nonviral vector, where a section of genome DNA is inserted artificially[14,16]. T-cell *ex vivo* expansion and purification is the subsequent and key step, determining the efficacy of this novel adoptive immunotherapy[14]. The ideal dose is 1 to 5 × 10^8 cells which, however, is not equal to the CAR-T cell count in human bodies[17,18]. Finally, tests of cell quality and sterility are necessary, which take 2–4 weeks to complete[16]. Before the transduced T cells are administered a conditioning treatment, including lymphodepleting, should be done 2 days ahead for a greater T cell expansion[14,16].

This kind of immunotherapy is commonly used in hematological malignancies such as acute lymphoblastic leukemia (ALL),

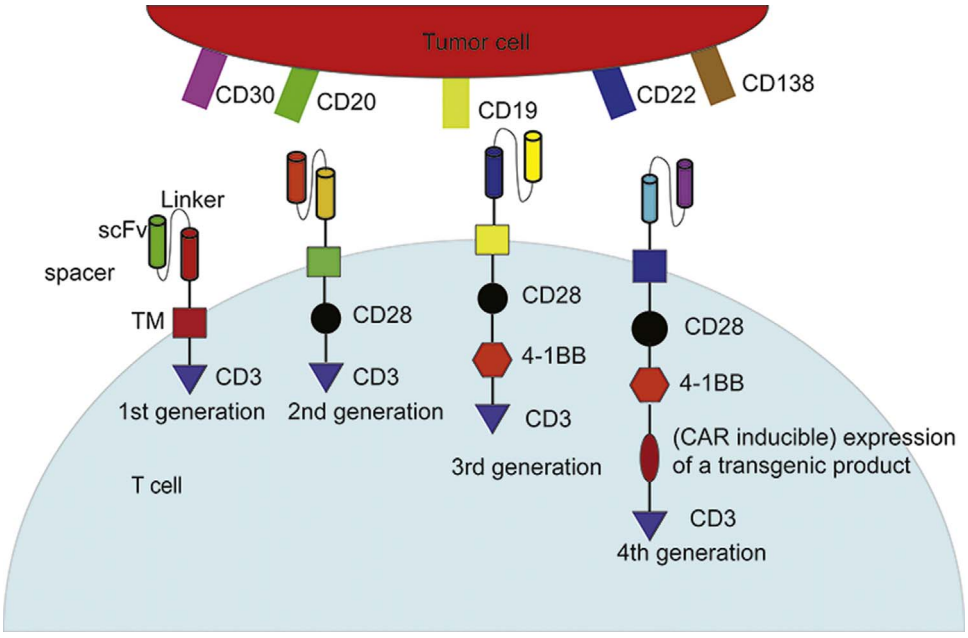

Figure 1 Illustration of basic structure of 4 generations of chimeric antigen receptor T cells (CAR-T cell) and common targets on tumor cells. The whole structure of CARs consisted of an antibody single-chain fragment (scFv, extracellular segment) specifically against a cell surface antigen as well as one or several fused signaling domain(s) from natural TCR complex and costimulatory molecules (intracellular segment). Different intracellular segments represent various CAR-T cell generations. scFv, single-chain fragment. TM, transmembrane region.

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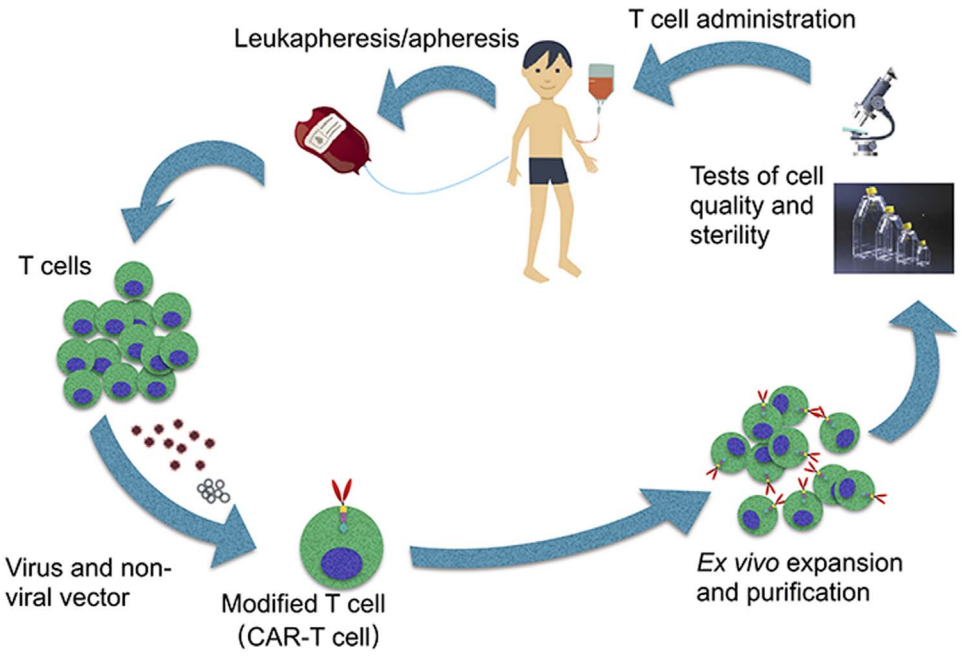

Figure 2 Flow chart of the whole procedure of chimeric antigen receptor T cell (CAR-T cell) production. Firstly, T cells from peripheral blood are collected *via* leukapheresis, followed by apheresis. Then the T cells are transduced by viral (retroviral or lentiviral) or nonviral vector loading genes of CAR inserted artificially. Next step, the cultured T cells are expanded and purified. Ultimately, cell quality and sterility will be examined before the cell products are infused into patients.

chronic lymphocytic leukemia (CLL), lymphoma, and multiple myeloma (MM)[19]. The most common target is CD19 and the total response is optimistic for ALL[20,21]. Other targets such as CD20, CD30, CD138 are showing some success as well[22–24]. Solid tumors are becoming another battleground for CAR-T cell regimen, including melanoma, sarcoma and breast cancer[25–27]. Contrary to hematologic tumors, the majority of treatment in solid tumors is unsuccessful due to insufficient and untypical molecular targets for CAR-T cells to attack and control the microenvironment of tumor[28–31]. Despite many issues about safety and efficacy, this technique is indisputably a promising tool for the future adoptive cancer immunotherapy. Here, we provide a framework mainly for understanding the applications of CAR-T cells in different hematological cancers, and also discuss future directions that will undoubtedly inform the improvement of the effectiveness of these adoptive cell therapies.

2. Applications of CAR-T cells in various hematological malignancies

2.1. CAR-T cell in acute lymphoblastic leukemia and chronic lymphocytic leukemia

2.1.1. CAR-T cell therapy in acute lymphoblastic leukemia

So far treatment of ALL, especially fatal relapsed/refractory (r/r) B-ALL is the most suitable for CAR-T therapy[32]. During the treatment of ALL, the most effective CAR is anti-CD19, an essential biomarker of B cell lineage showing higher expression in B-ALL, while anti-CD20 and immunoglobulin light chains are also potential targets[6,33–36] (Fig. 1). The first generation of CAR incorporated only a CD3ζ chain and failed to generate potent antitumor effects[37] with relatively short persistence[38]. This prompted scientists to upgrade, triggering creation of the second generation of CAR. Despite a better efficacy of the second generation CAR-T cell with either CD28 or 4-1BB, combining them might be a superior choice, which may give rise to a third generation of CAR-T cell.

Studies have reported data from clinical trials with CD19-targeted CAR-T cells for adults and children inflicted by r/r B-ALL[17,20,39–41]. All showed promising complete remission (CR) and partial remission (PR) rates. In one clinical study, following conditioning therapy (cyclophosphamide), CD19 CAR-T cells were infused, and 15 out of 16 patients required a qualified amount of T cells; the CR rate was 88%[39]. Delightfully, the CR was of high quality as few detectable disease indicators were detected by high-sensitive molecular assays such as deep-sequencing or real-time polymerase chain reaction[32]. Studies involving children and young adult patients (aged 1–30 years old) have found that the CR rate for the 20 B-ALL patients was 70% and the molecular CR rate was 60%. The limited persistence of CAR-T cells (approximately 2 months) is counterbalanced by the rapid remission of patients and post-treatment allogeneic stem-cell transplant[17,32]. In another clinical trial[20,41], patients received conditioning treatment, including both fludarabine and cyclophosphamide completed 1 week ahead of adoptive transfer of CAR-T cells. The CR rate was 90% and the molecular CR rate was 73%. Other research teams have also carried out clinical studies of ALL[42,43].

In addition, some studies have suggested that the defined composition of $CD4^+$ and $CD8^+$ CAR-T cell in one intravenous infusion can reveal factors that facilitate the evaluation of efficacy, adverse effects, cell expansion and the persistence of mixed products[21,44,45]. This is important for additional therapies such as lymphodepletion and anti-tumor drug use[21,44,46,47], which may clarify the relationship between CAR-T cell dose, cell expansion *in vivo* and the toxicity risk in order to adjust infusing dose to reduce the possibility of cytokines release syndrome (CRS) and neurotoxicity[48]. Although CD19 is an ideal target for CAR-T cell

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Table 1 Selected clinical trials of CAR-T cell therapy in ALL.

Institute	CAR (target & generation)	Sample		Effective No. of participants	Outcome[a]	Publishing Year and Ref.
		Number (M/F)	Age[*]			
Memorial Sloan-Kettering Cancer Center	CD19 2nd CD28	16 (12/4)	Adult	15[b]	CR rate: 88%	2014[39]
Fred Hutchinson Cancer Research Center	CD19 2nd 4-1BB	29[c]	Not available	26	CR rate: 93%	2015[48]
National Cancer Institute	CD19 2nd CD28	21[d] (14/7)	14.71 ± 6.64	21	CR rate :66.7%	2014[17,32]
University of Pennsylvania	CD19 2nd 4-1BB	30 (18/12)	Children & Adult	30	CR rate: 90%	2014[20,41]
University of Pennsylvania	CD19 2nd 4-1BB	27[c]	Adult	27	3 CR in cohort 1 and 2; 3 CR in cohort 3; 75%CR and 8.3%PR in cohort 4	2016[43]
Hebei Yanda Lu Daopei Hospital	CD19 2nd 4-1BB	42 (28/14)[e]	Children & adult	40[f]	CR rate: 90%	2017[42]
		9 (4/5)[e]	Children & adult	9	All patients achieved MRD$^-$	2017[42]
Peking University People's Hospital	CD19 4th (CD28/4-1BB/CD27/ inducible apoptotic caspase9	6 (1/5)	26.50 ± 13.62	5[g]	5 achieved minimal residual disease (MRD)-negative remission[h]	2017[66]

Abbreviations: aGVHD, acute Graft-versus-host disease; ALL, acute lymphoblastic leukemia; BM, bone marrow; CAR T cell, chimeric antigen receptor T cell; CR, complete remission; M/F, male and female; MRD, minimal residual disease; PR, partial remission; Ref, reference; SEM, standard error of mean.

[a]The denominator in the calculation is the total sample number.

[b]One patient had only gross extramedullary disease (no detectable disease in the BM).

[c]No gender indicated.

[d]Twenty ALL patients.

[e]This clinical trial has two groups: one includes 42 primary refractory/hematological relapsed and 9 refractory minimal residual disease (MRD) by flow cytometry B-ALL patients.

[f]Two patients died from treatment-related mortality early in the trial (on days 21 and 24).

[g]One patient was discharged automatically without evaluation after developing severe thrombotic microangiopathies.

[h]Four of five responsive patients relapsed after 2–7 months, and one died of sepsis following MRD-negative remission after a second infusion. None of the other second infusion recipients achieved a second complete remission. Two and one patient developed grade 2 and 3 aGVHD, respectively.

[*]Ages of patients are expressed as mean ± SEM if the data are available.

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Table 2 Selected clinical trials of CAR-T cell therapy in CLL.

Institute	CAR (target & generation)	Sample		Effective No. of participants	Outcome[b,d]	Publishing year and Ref.
		Number (M/F)	Age[*]			
University of Pensylvania	CD19 2nd 4-1BB	14 (12/2)	66.90 ± 8.10	14	CR rate: 28%[a,c]; PR rate: 28%[b,d]	2015[48,55]
University of Pensylvania	CD19 2nd 4-1BB	30[h]	Adult	23	CR rate: 22%; PR rate: 17%	2014[18]
National Cancer Institute	CD19 2nd CD28	15[e] (8/7)	51.67 ± 11.22	15[e]	CR rate: 53%	2014[53]
Memorial Sloan Kettering Cancer Center	CD19 2nd CD28	10[f] (8/2)	63.90 ± 8.49	9[a,f]	No CR	2011[54]
National Cancer Institute	CD19 2nd CD28	20 (11/9)	50.93 ± 12.86	20[g]	CR rate: 30%; PR rate: 10%, no aGVHD by CAR-T cell infusion	2016[67]
Fred Hutchinson Cancer Research Center	CD19 3rd CD28/4-1BB	24[h]	59.54 ± 7.87	24	CR rate: 16.7%; PR rate: 54.2%	2017[62]

Abbreviations: a GVHD, acute Graft-versus-host disease; ALL, acute lymphoblastic leukemia; CAR-T cell, chimeric antigen receptor T cell; CLL, chronic lymphocytic leukemia; CR, complete remission; M/F, male and female; PR, partial remission; Ref, reference; SEM, standard error of mean.

[a]One patient (ALL) was yet to be treated with modified T cells.

[b]The denominator in the calculation is the total sample number.

[c]One died after 21 months post-treatment period.

[d]Only one is alive with disease.

[e]Including 4 CLL patients.

[f]There were 8 CLL patients.

[g]There are 5 CLL patients.

[h]No gender indicated.

[*]Ages of patients are expressed as mean ± SEM if the data are available.

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treatment in ALL, studies have found that "antigen escape" is a potential obstacle in the development of immunotherapy, thereby making it imperative to optimize, including recognition and identification of additional targets[49,50]. Fortunately, CD22 is another potential target for CAR-T cell, and recently, two different anti-CD22 agents have been tested against B-ALL in clinical trials to make up the deficiency of anti-CD19 therapy[37].

2.1.2. *CAR-T cells therapy in chronic lymphocytic leukemia*

CLL is a chronic malignancy with a varying clinical course and prognosis for chemotherapy[6]. However, the only current approach to curing CLL is allogeneic stem-cell transplantation[51]. Recently, CD19 CAR-T cells were used to treat patients with relapsed and risky CLL and some response to the CAR-T cell in CLL patients with equal CR and PR rate have been reported[52,53]. In addition to CD19, several other targets such as the tyrosine-protein kinase *trans*-membrane receptor have been explored[36]. In the past several years several studies have explored the effect of CAR-T cells in CLL patients[18,48,53–56].

Because CLL pathogenesis leads to early immune deficiency, the efficacy of CAR-T cell therapy will be limited by difficulties in expansion of T cells *ex vivo* from CLL patients and their proliferative response *in vivo*. Finding agents that enhance the ability to prevent the above phenomenon is of necessity[55,57]. Ibrutinib, an irreversible inhibitor of bruton tyrosine kinase, may not only avoid negative effects on the T cell but could also improve its antitumor capability[57]. To test the effect of ibrutinib on the T cell in CLL patients, Fraietta et al.[57] tested the phenotype and function of T cells in a cohort study of CLL patients during their treatment course with ibrutinib. The result showed that five cycles of ibrutinib therapy enhanced the expansion of CD19-directed CAR-T cells (CTL019, a second-generation of CD19 CAR-T cell), and decreased the expression of programmed cell death protein 1 (an immunosuppressive molecule) on T cells; and increased the expression of CD200 in B-cell CLL[57]. CD200/CD200 receptor is important to regulate antitumor immunity[58–60]. CD200 overexpression in CLL leads to the functional impairment of $CD8^+$ T-cell responses[61]. Importantly, when CAR-T cell is exposed to ibrutinib, its function is not altered *in vitro* but survival, CAR-T cell engraftment, and tumor clearance are indeed improved in human xenograft models of resistant ALL and CLL when both the cells and agent are administered concurrently[57]. Nevertheless, a case in which ibrutinib was ineffective and CAR-T cell was less effective in treating CLL patients has been reported[62].

Additionally, studies have found that CAR-T cells can be used to treat patients with a relapse of B-cell malignancies after allogeneic hematopoietic stem cell transplantation (Allo-HSCT). Traditionally, donor lymphocyte infusions of natural allogeneic lymphocytes derived from transplant donors are used to treat B-cell malignancies after allo-HSCT[63,64]. However, the main side effect is graft-*versus*-host disease (GVHD) and the acute type occurs in about 1/3 of the patients receiving donor lymphocyte infusions. GVHD is a predominant cause of the 6–11% mortality rate from donor lymphocyte infusion[64,65]. Thus, studies have tested the effect of CAR-T cell infusion in patients suffering from relapsed B-ALL and CLL after allo-HSCT[66,67]. Both of the trials suggested that donor-derived CAR-T cell infusion seems to be effective and safe for relapsed B cell malignancies after allo-HSCT, although larger clinical studies are needed. All the studies reviewed above and others involved in ALL and CLL are summarized in Table 1[17,20,32,39,41–43,48,66] and Table 2[18,48,53–55,62,67].

2.2. *CAR-T cell therapy in lymphoma*

Patients with disease deterioration after primary and secondary therapies for lymphoma have been found to have a poor prognosis even though they have shown improvements in cytotoxic chemotherapy regimens and monoclonal antibody therapies[68]. However, there is a novel and effective treatment alternative for patients who have shown no resolution after multiple lines of chemotherapy[16]. CAR-T cells are among the latest advanced immunotherapies for relapsed or chemotherapy-refractory B-cell non-Hodgkin lymphoma (NHL)[69].

There are several types of CARs modified on the surface of T cells either autonomously or allogeneically, and the anti-CD19 CAR-T cell is the earliest and the most traditional. For lymphoma, the first-generation of CAR-T cells were not effective in preventing proliferation, persistence and homing compared to the second and third generation[68,70,71]. Two different studies have reported preclinical results showing superior proliferative and antitumor activities of the second and third generation T cells, with the CD28 or 4-1BB cytoplasmic signaling domains, both *in vitro* and *in vivo*[72–74]. Despite a positive effect of CD28 as an early signal in improving cell expansion and persistence, some trials have suggested that using CAR containing 4-1BB as a late costimulatory signal yields more remarkable expansion and anti-tumor activity in indolent B cell malignancies[56]. For example, CTL019 therapy has shown great effect in some patients with advanced r/r follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL). Among 8 eligible patients, 4 people had responses at different levels: 3 CRs (CR rate is 13%) and 1 PR (PR rate is 4%), with a 50% 3-month overall response rate. Four patients with DLBCL had progressive disease before or at initial response assessment, and there was no treatment-related mortality[14,75]. The longest ongoing clinical response in this study was more than 350 days for FL, and 400 days for DLBCL. CD28-containing CD19 CAR-T cells have continued to show promise for patients. For instance, studies have shown that 75% of patients who suffered from splenic marginal zone lymphoma achieved PR[76,77], and this was also shown in other corroborative studies[53,78,79]. These patients were all maintained for several months (ranging from 7 to over 18 months)[77].

Besides CD19, other surface biomarkers are also essential. CD20, a tetra-trans-membrane protein presents in more than 90% of B-cell lymphomas and is a well-established target for NHL treatment[80]. Currently, first–generation anti-CD20 CAR-T cell therapy has been used in several studies[70]. In one clinical trial study 7 participants suffering from lymphomas were treated. Two patients in this study achieved CR, 1 subject got a PR while the disease in another 4 patients was stable [81]. To test whether the second-generation of CAR-T treatment is effective in DLBCL patients, a study in 2014 using anti-CD20 CAR-T cell with 4-1BB reported a promising effect of this novel treatment[80]. In this study, 7 patients with refractory advanced $CD20^+$ DLBCL were recruited. Among them, 5 patients were burdened with bulky tumors and the other 2 were not. Except for 1 subject, the other 6 patients received preconditioning chemotherapy for disease control or the tumor was debulked before anti-CD20 CAR-T cell infusion. One of the two patients without a bulky tumor burden achieved a 14-month long lasting and ongoing CR without

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Table 3 Selected clinical trials of CAR-T cell therapy in lymphoma.

Institute	CAR (target & generation)	Sample		Effective No. of participants	Outcome[a]	Publishing year & Ref.
		Number (M/F)	Age[*]			
University of Pennsylvania	CD19 2nd 4-1BB	23 (14/9)	Adult	20[b]	CR rate: 13%; PR rate: 4%;	2014[14,75]
National Cancer Institute	CD19 2nd CD28	8[c,d]	55.88±5.77	8[c]	5 PRs[e]; 1 CR[e]; 1 SD[e]	2012[76,77]
National Cancer Institute	CD19 2nd CD28	15[f] (8/7)	51.67±11.22	15[f]	CR rate: 53%; PR rate: 26%; SD rate: 7%	2014[53]
National Cancer Institute	CD19 2nd CD28	9 (8/1)	Adult	9	1 CR[e]; 5 PRs[e]	2014[78]
Fred Hutchinson Cancer Research Center & National Cancer Institute	CD20 1st CD3z	9 (8/1)	Adult	7[g]	2 CRs[e]; 1 PR[e]; 4SDs[e]	2008[81]
Fred Hutchinson Cancer Research Center	CD19 (the generation is unknown)	28[h,d]	Adult	24[i]	In 12 patients received lymphodepletion with Cy-based regimens without fludarabine, the CR rate is 8.3% and PR rate is 41.7%; In 16 patients received lymphodepletion with addition of fludarabine, the CR rate is 42% and PR rate is 25%	2015[79]
		6[h,d]	Adult	6	3 CR ;1 PR	2015[79]
Chinese PLA General Hospital	CD20 2nd 4-1BB	7 (6/1)	65	6[j]	1 CR[g]; 3PRs[g]; 2 PDs[g]	2014[80]
Chinese PLA General Hospital	CD30 2nd 4-1BB	18 (13/5)	31	18	PR rate: 39%; SD rate: 33%	2016[23]
Baylor College of Medicine	κ-lightchain	13[d]	Not available	10[c]	CR rate: 15%; PR rate: 30%	2013[88]

Abbreviations: CLL, chronic lymphocytic leukemia; CR, complete remission; M/F, male and female; PD, progress disease; PR, partial remission; Ref, reference; SD, stable disease; SEM, standard error of mean.

[a]The denominator in the calculation is the total sample number.

[b]Three patients were removed from the trial before therapy due to progressive disease.

[c]There are 5 lymphoma patients.

[d]No gender indicated.

[e]In this study, we use the absolute value instead of rate in that the response rate is meaningless (sample size is less than 10).

[f]There are 11 lymphoma patients.

[g]The reason why 2 of nine patients was untreated is unknown.

[h]In this clinical trial, there are two main groups, one is non-Hodgkin lymphoma and the other one is chronic lymphacytic leukemia.

[i]Four patients were not available, among which 2 died early.

[j]One participant died of severe hemorrhage of the alimentary tract.

[*]Ages of patients are expressed as mean ± SEM if the data are available.

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Table 4 Selected clinical trials of CAR-T cell therapy in multiple myeloma.

Institute	CAR (target & generation)	Sample size		Effective No. of participants	Outcome	Publishing year & Ref.
		Number (M/F)	Age*			
Chinese PLA General Hospital	CD138 2nd 4-1BB	5(1/4)	Adult	5	4SD[a]	2015[24]
National Cancer Institute	BCMA 2nd CD28	12[d]	Not available	12	1PR and 2 SD in group of "0.3 × 10^6/kg CAR-T cell"; 3 SD in group of "1 × 10^6/kg CAR-T cell"; 1PR and 2 SD in group of "3 × 10^6/kg CAR-T cell"; 1 CR, 1 PR and 1 SD in group of "9 × 10^6/kg CAR-T cell"[b]	2016[105]
Bluebird Bio	BCMA 2nd 4-1BB (bb2121)	9[d]	Not available	9	2CRs in a cohort of 15*10^7 CAR-T cells; 1 PR in a cohort of 5.0*10^7 CAR-T cell, 1 PR in the cohort of 15*10^7 CAR-T cells and 2 PR in a cohort of 45*10^7 CAR-T cell; 1 SD in the cohort of 5.0*10^7 CAR-T cell and another one is in the cohort of 45*10^7 CAR-T cell	2017[109]
University of Pennsylvania	BCMA 2nd 4-1BB	11[d]	Not available	6[c]	1CR ;1PR ;1 SD	2016[110]

Abbreviations: BCMA, B cell maturation antigen; CAR-T cell, chimeric antigen receptor T cell; CR, complete remission; M/F, male and female; PR, partial remission; Ref, reference; SD, stable disease.

[a]In this study, we use the absolute value instead of rate in that the response rate is meaningless (sample size is less than 10).

[b]One patient only accepted the dose of 3 × 10^6/kg CAR-T cell.

[c]Five patients not receiving treatment because of screen fail ($n = 2$), rapid multiple myeloma progression/renal failure ($n = 2$), and self choice ($n = 1$).

[d]No gender indicated.

*Ages of patients are expressed as mean ± SEM if the data are available.

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preconditioning regimen, and another gained a 6-month tumor regression. 3 of 5 with bulky tumors got 3- to 6-month tumor regression[80].

CD30, another potential target, is a member of the TNFR superfamily. It is an antigen to the Ki-1 antibody which binds to Reed-Sternberg cells in Hodgkin lymphoma (HL)[82,83]. CD30-positive lymphocytes are mainly found around the follicular areas of lymphoid tissues, but is less common in germinal centers[84]. For lymphoma, CD30 is expressed in classical HL, anaplastic large cell lymphoma, DLBCL, primary mediastinal B-cell lymphoma, and peripheral T-cell lymphoma[83,85,86]. Recently, Wang et al.[23] reported that 18 patients with r/r HL, who received a conditioning chemotherapy prior to the CD30 CAR-T cell infusion showed progression in regard to their conditions before CAR-T cell infusion. Almost 7 patients achieved PR and 6 maintained stable disease (SD). In all these patients their lymph node lesions responded better than extra-nodal lesions, and the response in lung lesions seemed to be relatively poor. Analysis of biopsy tissues by real-time quantitative polymerase chain reaction and immunohistochemistry revealed CAR-T cells migrating into the targeted sites, and reduction in the expression of CD30 in tumors[23]. Moreover, introduction of EBV-specific CAR-T cells that recognize and kill Epstein-Barr virus (EBV)-infected cells may secure against the relapse of EBV-related B-cell NHLs, including BL and DLBCL[87].

In regard to κ-light chain, it is a feasible choice for B-cell lymphoma in that mature malignant B cells express either a κ or λ light immunoglobulin chain. Anti-κ/λ CAR-T cells, hence, can target one kind of malignant B cell and avoid damaging normal B cells[70]. In one dose-escalation study, 10 r/r κ^+ NHL, or CLL patients were infused with autologous CAR-κ T cells and any other treatments were discontinued at least 4 weeks ahead of T-cell infusion. This study reported that among the 5 NHL patients, 2 entered CR (after 2 and 3 infusions at dose level 1 and 3, respectively), 1 patient achieved PR, and 2 other subjects progressed[88]. The clinical trials aforementioned are summarized in Table 3[14,23,53,75–79,81,88].

2.3. CAR-T cell in multiple myeloma

Multiple myeloma (MM) is a bone-marrow-derived refractory malignancy leading to anemia, immunosuppression with repeated infections, hypercalcemia, bone lesions and renal failure[89]. Despite chemotherapy, autologous hematopoietic stem cell transplantation or the use of other immune-modulatory agents, this disease remains incurable because of the heterogeneous cytogenetic and molecular abnormalities of myeloma[90,91]. However, the fact that myeloma can subside as a result of the graft-*versus*-myeloma effect in allogeneic stem cell transplantation provides insight into the role of T-cell-based immunotherapy[92,93]. Studies have found a lower, but a more frequent expression of CD19 on myeloma cells[94,95]. Because myeloma cells hardly express CD19 on their surface, anti-CD19 CAR-T cells cannot perform well to kill the malignant cells; instead, they harm some healthy tissues other than tumors[96]. Two studies targeted this population *via* the use of CTL019 cells, reporting a remission in a 43-year-old patient with 9 prior lines of treatment with nearly no sign of CRS[94,97]. It is therefore evident that more specific targets presented on tumor cells *versus* those on non-vital, healthy tissues should be explored to achieve a broader application in MM[91,96].

Another molecule involved in this event is CD138, which is a membrane protein and belongs to the Syndecan family of heparan sulfate proteoglycans[98]. In hematopoietic tissues, malignant and differentiated plasma cells express this biomarker[99]. It is also present in neoplastic, mature epithelial cells and other normal tissues[100]. By virtue of its expression in nearly all MM patients, CD138 is used as a primary diagnostic marker[101]. The result of a clinical trial using second-generation of anti-CD138 CAR-T cell treatment of 5 refractory MM subjects showed that, after 7-month follow-up treatment, the conditions of at least 4 patients were stable and one patient with advanced plasma cell leukemia had a reduction of myeloma cells in peripheral blood[24]. This shows that the CAR-T cells had entered the bone marrow[98]. Thus, CD138 CAR-T cell therapy for MM is well-tolerated and potentially has anti-tumor immunity[24].

B-cell maturation antigen (BCMA, CD269) is another molecule that has been identified and is a promising immunotherapeutic target in MM[102,103]. Expressed only in lymphoid tissue of healthy individuals and on mature B cells or plasma cells, BCMA enhances the survival of long-lived plasma cells in MM patients[102,104]. Patients that were recently enrolled in an anti-BCMA/CD269 CAR-T cell treatment in MM received CAR-BCMA T cells in a dose-escalation trial[105]. In addition, this study reported that all patients were pretreated with cyclophosphamide and fludarabine to enhance the activity of adoptive transferred T cells as previously reported[105–108]. In these 6 patients treated at the lowest 2 dose levels, low anti-myeloma activity or toxicity was noticed. Moreover, at the third dose level 1 patient obtained a very-good PR. Furthermore, two chemotherapy-resistant patients were treated on the fourth dose level with anti-BCMA CAR-T and achieved stringent CR lasting for 17 weeks before relapse. Ongoing promising PR[105] corroborative studies have also shown a similar therapeutic outcome[109,110]. The above mentioned studies and the 2 corroborative studies are further summarized Table 4[24,105,109,110]. Some bench studies have brought exciting stories of new approaches to treat MM[111,112], and progress have been achieved *in vitro* and hopefully further experimental and clinical trials will usher in a new era of MM immunotherapy.

3. Advantages and disadvantages in the application of CAR-T cell therapy in hematological malignancies

3.1. Advantages of CAR-T cell therapy in hematological malignancies

In contrast with common adaptive immune cells, CAR-T cells have unique specificity and can eliminate cancer cells containing the corresponding TAAs. To some extent this technique will avoid unnecessary killing of healthy tissues. Furthermore, CAR-T cells can recognize cell surface molecules without the help of HLA expression. Its advantage over the former one is that tumors often avoid T cell immune surveillance by hiding HLA or other molecules involved in antigen processing and presentation[70,113,114]. In addition, the flexibility of intracellular signaling domains within CARs permits the cell to counteract the down-regulation of co-stimulatory molecules directly or indirectly caused by cancer cells[115]. It is worthwhile to mention that CAR-T cell can recognize potential antigens in nearly all forms including carbohydrate, lipid, protein antigens, which can be combined specifically by antibodies[30].

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3.2. *Disadvantages of CAR-T cell therapy in hematological malignancies*

When treating hematological malignancies using CAR-T cells, it turns out that activating the antitumor immunity of engineered T cell results in broad and strong cytokine-driven effects, including CRS, macrophage activation syndrome, and hemophagocytic lymphohistiocytosis. Among them, CRS is a clinical response to raise cytokine levels and includes symptoms such as hypotension, fevers, neurological changes and hypoxia[52,56,77,116]. Risk-adapted CAR-T cell dosing strategies have been carried out in response to the greater incidence of severe CRS observed among patients who showed greater baseline disease burden, and were treated with higher doses of CAR-T cells[21,43,117]. Systemic corticosteroids are used as first-aid treatment for patients with life-threatening CRS to lessen or even eliminate the hyper-proliferative activated CAR-T cells. But the downside has been revealed as well: rapid ablation of engineered T cells will depress the antitumor efficacy of CD19 CAR-T cells and trigger subsequent disease progression or relapse[39,49,118]. The investigation of cytokines in several studies surprisingly identified IL-6 as a major cytokine induced by CAR therapy. Meanwhile, IL-6 also stems from apoptotic B cells or activated macrophages and moves to lysed tumor cells *via* chemotaxis[11]. Thus, the anti-IL-6 receptor antagonist antibody tocilizumab was successfully designated to weaken the cytokine-induced side effect, a treatment now being applied more systematically to counteract cytokine-release syndrome[33,39].

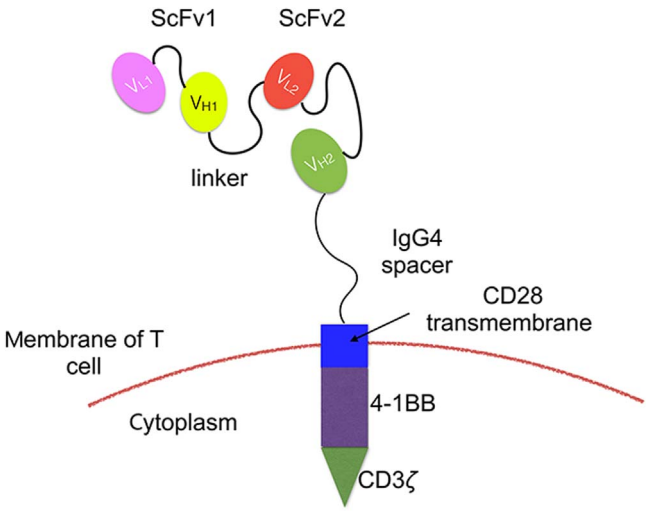

Figure 3 The structure of TanCAR with two different scFvs "hand-in-hand". The TanCAR is a type of bispecific T cell dealing with antigens escaping or multiple tumor antigens in relapsed B cell malignancies. The two antibodies on the CAR part are connected in tandem so that one CAR-T cell can combine two kinds of tumor antigen at the same time or binds to the tumor cell expressing either kinds of targeted antigen.

Neurotoxicity is another serious potential toxicity arising from CAR-T cell therapy and has been observed in several patients treated with CD19 CAR-T cells[119]. Symptoms of neurotoxicity include visual hallucinations, delirium, dysphasia, epilepsy or seizure[120]. Endothelial dysfunction, including vascular instability, capillary leak, blood-brain barrier disruption and disseminated intravascular coagulation are clinical evidence of neurotoxicity[121]. For medical imaging results, an abnormal brain MRI scan can suggest neurotoxicity by showing vasogenic edema, leptomeningeal enhancement, and/or multifocal microhemorrhages[121]. Similar to CRS, IL6, IFN-γ and TNF are prime culprits in inducing acute neurotoxicity so treatment can also apply tocilizumab to suppress IL6-mediated inflammatory pathways[121].

Although CAR-T cell has relative specificity as noted above, every regimen may result in tissue damage at different levels because the molecular biomarkers can be expressed in some normal tissues or organs, especially the lymphatic tissues. This phenomenon is referred to as on-target/off–tumor toxicity, which includes B cell aplasia in anti-CD19/CD20 CAR-T cell treatment[20,77,122]. Also, antigen escape, typically CD19-negative

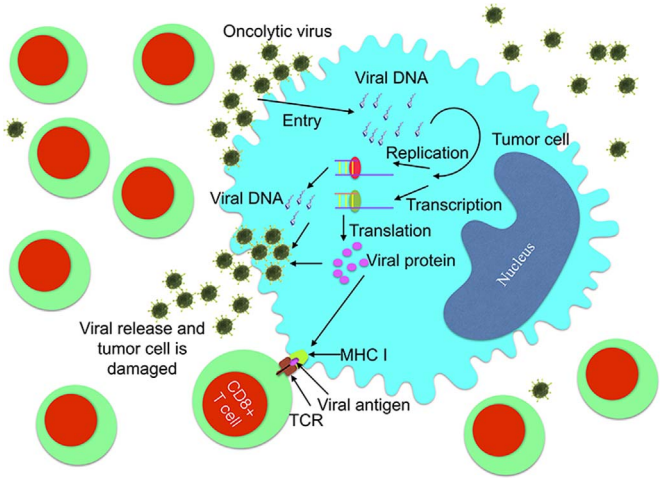

Figure 4 Oncolytic virus can help T cells to kill tumor cells in two ways. 1. Direct attack through viral entry, replication, transcription, and release, leading to tumor lysis while sparing normal cells; 2. Indirect damaging *via* integrating its genome to that of tumor cells for further expression on surface of tumor cells and the cell can be recognized by T cell through MHC (major histocompatibility complex) molecule. Moreover, these viruses can be equipped with chemokine genes to "attract" CAR-T cells, prolong their persistence and let them complete directly attack tumor cell.

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relapse of B cell malignancy, may challenge the success rate of CARs in blood cancer[17,20,21,41].

4. Outlook

CAR-T cell therapy has made great progress in recent decades, however, several challenges still need to be solved. The main issues are how to improve the effectiveness and persistence of adoptive cells and to reduce the adverse effects of treatment.

On one hand, to improve the treatment effect, CAR-T cells can be further equipped to improve their effectiveness and persistence by adding new co-stimulatory domains or CAR parts[123]. Since glucocorticoid-inducible tumor necrosis factor receptor-related protein (GITR, CD357) and 4-1BB are both nuclear factor-κB (NF-κB)-inducing members of the TNFR family and GITR is a costimulatory molecule, we suggest that further research is needed on the effect of adding this domain, and combining them with CD28 and 4-1BB to evaluate the effect of T-cells[124]. Plus, reconstructing the CAR part is a new approach to antigen escape or coexistence of multiple tumor antigens in lymphoma, MM and other type of malignancies. Bi-specific CAR-T cell is a promising treatment and it includes two formations: First, one CAR is composed of two different scFvs "hand-in-hand" (TanCAR) (Fig. 3) or two distinct CARs with different scFvs on one single T cell (dual-signaling CAR)[125,126]; second, T cell pools with different typical CARs infused sequentially or simultaneously[127]. Nevertheless, challenges still exist in recognizing the coexisting targets on a tumor cell and selecting the proper epitope in TanCAR therapy[128,129]. In addition, oncolytic viruses can infect the host, replicate and damage certain tumor cells without harming normal cells[130,131]. Evidence has shown that tumor cells can be killed *via* the new viral proteins integrated in its cytoplasm presented by the major histocompatibility complex class I molecules, and thereafter being recognized and destroyed by CD8 T cells [130]. In addition, tumor cells can also be killed by the release of progeny viral particles[130] (Fig. 4). Applications for this type of tumor cell killing using viruses such as Myxoma virus, adenovirus serotype 5 and *Coxsackievirus* A21 have been reported in MM[132–134]. B- and T-lymphoma or leukemia-derived cells/xenografts have also been used as preclinical models[135–137]. Studies have reported the use of oncolytic virus equipped with chemokine genes to recruit CAR-T cells, prolong their persistence and directly attack tumor cells[138,139]. This is a promising approach which can be tested during hematological malignancies.

On the other hand, avoiding uncontrolled T cell proliferation, cytokine storm, or "on-target/off-tumor" effects during CAR-T cell treatment is essential. Some researchers suggest that suicide-gene (also known as "elimination genes") systems can be activated to destroy infused CAR-T cells to regulate the expansion of CAR-T cells and restrict toxicity[123,140]. To date, some methods have been proved: suicide gene therapy with the *Herpes simplex* thymidine kinase/ganciclovir suicide system in the context of allo-HSCT has shown its safety and effectiveness[141], and other non-immunogenic suicide systems have been developed, especially through a modified caspase-9 member of the intrinsic apoptosis pathway. A new version of FK506-binding protein, demonstrating a propyl isomerase activity has been combined with caspase-9[142,143]. However, such suicide strategy will shorten the duration of treatment by thoroughly eliminating CAR-T cell[144]. Switchable CAR-T cell can make up this defect by inserting a small molecular complex between the scFv and T cell surface to "turn on" the cell function and discontinue adding it to switch off[144]. Another way to switch the cell between "on and off" would be to engineer a soluble intercellular molecule that contains a tumor antigen-specific antibody, a secondary moiety binding to CARs. This structure allows the researcher to switch the cell function by changing the concentration of the intermediary molecules. This novel format is being tested[145].

5. Conclusions

CAR-T cell therapy has made great progress to deal with hematological malignancies, especially ALL, CLL and lymphoma while the effectiveness, cell persistence and adverse effects become bottlenecks to the widespread use of this approach. Innovative bench research is required to strengthen the effectiveness of this therapy; that will give physicians and patients the information and therapeutics to eliminate these malignancies.

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