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Clinical development of chimeric antigen receptor-T cell therapy for hematological malignancies

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Abstract

Cellular therapies have revolutionized the treatment of hematological malignancies since their conception and rapid development. Chimeric antigen receptor (CAR)-T cell therapy is the most widely applied cellular therapy. Since the Food and Drug Administration approved two CD19-CAR-T products for clinical treatment of relapsed/refractory acute lymphoblastic leukemia and diffuse large B cell lymphoma in 2017, five more CAR-T cell products were subsequently approved for treating multiple myeloma or B cell malignancies. Moreover, clinical trials of CAR-T cell therapy for treating other hematological malignancies are ongoing. Both China and the United States have contributed significantly to the development of clinical trials. However, CAR-T cell therapy has many limitations such as a high relapse rate, adverse side effects, and restricted availability. Various methods are being implemented in clinical trials to address these issues, some of which have demonstrated promising breakthroughs. This review summarizes developments in CAR-T cell trials and advances in CAR-T cell therapy.

Keywords: Cellular immunotherapy; Chimeric antigen receptor T cell; Hematological malignancy

Introduction

The rapid development of various cellular therapies, such as chimeric antigen receptor (CAR)-T cells, natural killer (NK) cells,[1,2] and engineered T cell receptor-T cells,[3-6] for hematological malignancies is beginning to diversify. CAR-T cell therapy is the most widely applied cellular therapy. CAR-T cells are modified T cells expressing an engineered receptor to recognize and eradicate malignancies.[7] The Food and Drug Administration (FDA) approved two CD19 CAR-T cells products for treating relapsed or refractory (r/r) acute lymphoblastic leukemia (ALL) and diffuse large B cell lymphoma (DLBCL) in 2017, which became a milestone for CAR-T cell therapy. Additionally, two more CD19-CAR-T products were approved in 2020 and 2021.[8] There have also been developments in treatments for other hematological malignancies, such as CAR-T cells targeting B cell maturation antigen (BCMA) for relapsed/refractory multiple myeloma (MM), for which two CAR-T cell products were approved for treatment in 2021 and 2022. Furthermore, many clinical and preclinical studies have focused on CAR-T cells to treat relapsed/refractory Hodgkin's lymphoma (HL),[9-11] T cell malignancies,[12] acute myeloid leukemia (AML),[13-15] and some rare diseases such as polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes syndrome.[16] For the rapid development of CAR-T cell clinical trials, China and the United States have played an important leading role. Although China started its CAR-T cell clinical program later than the United States, it has shown rapid growth in recent years. China has conducted many more clinical trials than the United States; however, several shortcomings remain, including small sample sizes and limited distributions.[17,18]

Although CAR-T cell therapy has improved the remission rate of hematological malignancies, many issues limit the adoption of CAR-T cell therapy.[19] First, a high relapse rate has been reported after treatment with CAR-T cells.[20,21] Moreover, many treatment-related adverse events include "on-target off-tumor" effects, neurotoxicity, and cytokine release syndrome (CRS).[19,22] Additionally, the high cost of treatment, the failure of CAR-T cell generation, and the fact that many patients are forced to stop treatment owing to disease progression or death are factors that limit the adoption of CAR-T cell therapy.[8,22] In clinical and preclinical trials, several approaches are being used to address these limitations, some of which have reported promising progress. This review

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summarizes the status of developments in CAR-T cell trials and the advances in CAR-T cell therapy.

Contrastive Analysis of CAR-T Cell Clinical Trials in the United States and China

In 2019, Gou *et al*[17] analyzed the distinction and similarity between clinical trials conducted in the United States and China by collecting detailed information on clinical trials in clinicaltrials.gov from 2007 to 2017. In 2020, Wei *et al*[18] conducted a comparative analysis between the United States and China regarding the development of CAR-T cell therapy in the past 5 years. Consequently, we selected the keywords "chimeric antigen receptor" and "CAR" on clinicaltrials.gov to search for newly registered trials from January 1, 2018 to March 31, 2022. After manually verifying the detailed information, 434 trials were retrieved, of which 277 trials were conducted in China, 126 trials were implemented in the United States, and the remaining 30 trials in other countries, including Japan, Israel, the United Kingdom, Canada, France, Germany, Italy, Spain, and Belgium [Figure 1A]. In the following section, we analyze these results to reveal the current status and progress of CAR-T cell therapy for hematological malignancies in recent years.

Increased number and distribution of CAR-T cell clinical studies in China and the United States

China has experienced rapid growth in the number of new trials since 2018, whereas clinical trials in the United States have had relatively stagnant growth. The number of new domestic CAR-T cells clinical trials was nearly twice as many as that in the United States for each year. In 2019 and 2020, 76 and 73 new CAR-T cell clinical trials were registered in China, respectively, while only 29 and 38 new trials were added in the United States during the same period [Figure 1B].

Although the number of CAR-T cell clinical trials in China far surpassed that in the United States, research centers were distributed geographically more widely in the United States. A total of 20 provinces and 26 cities in China conducted CAR-T cell trials from January 1, 2018 to March 31, 2022 with Hangzhou (47 trials), Beijing (44 trials), Shanghai (29 trials), Langfang (28 trials), and Shenzhen (23 trials) dominating. In the United States, 30 states had implemented CAR-T trials, mainly in California (64 trials), Texas (53 trials), New York (43 trials), Massachusetts (37trials) and Pennsylvania (33 trials).

Involved study center and patients of CAR-T cell clinical studies

Despite the rapid progress of CAR-T cell clinical trials in China in recent years, there are some discrepancies in the subject size of trials between the two countries. Specifically, 78% (215/277) of clinical trials in China involved ≤40 subjects, compared with only 56% (71/126) of those in the United States. China had only 10 projects that recruited >100 subjects, while the United States had 23 projects, twice as many as China [Figure 1C]. Additionally,

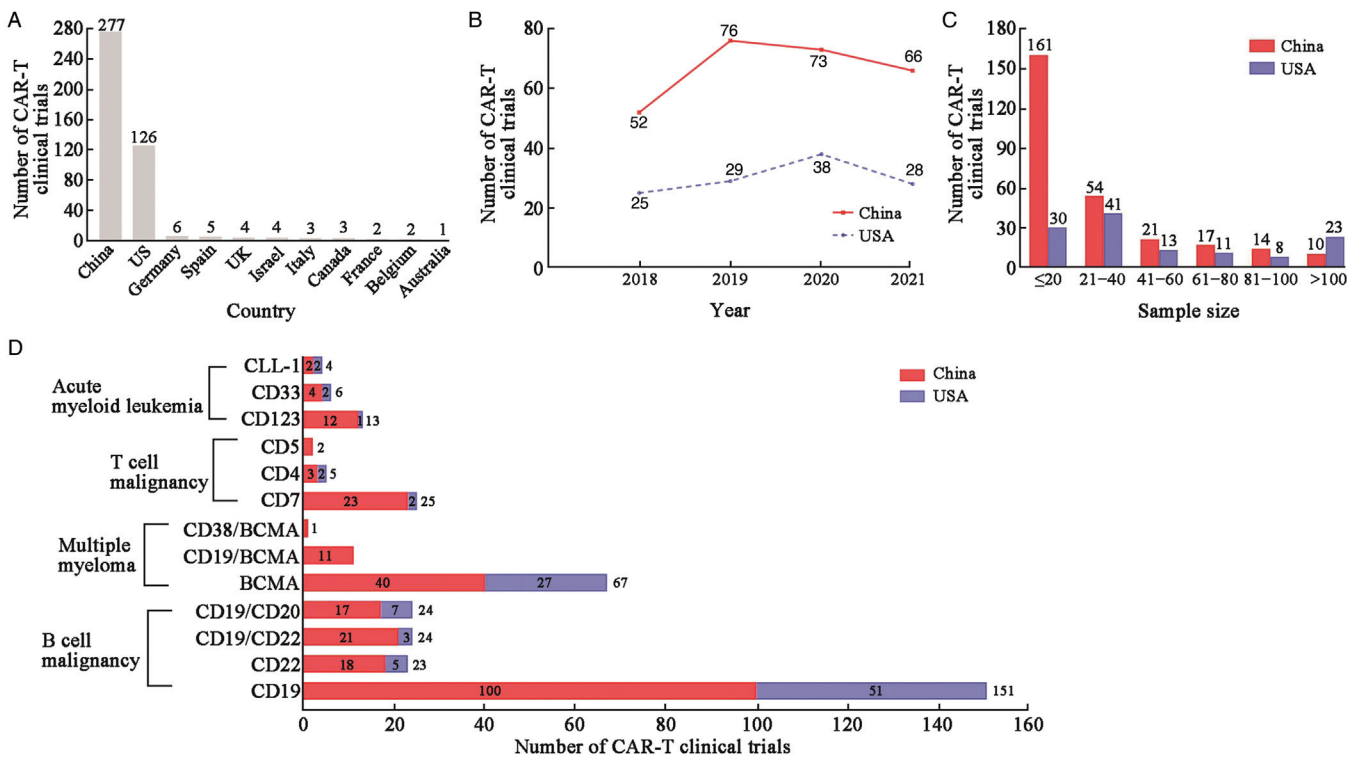

Figure 1: (A) Countries conducting CAR-T cell clinical trials posted on the clinicaltrials.gov website from January 1, 2018 to March 31, 2022. (B) Trend changes of annual number of newly registered clinical trials in China and the United States. (C) Subject sample size of CAR-T cell clinical trials in China and the United States. (D) Distribution of target antigens in China and the United States. BCMA: B cell maturation antigen; CAR-T: Chimeric antigen receptor-T; CLL-1: C-type lectin-like molecule 1; USA: The United States of America.

for involved study centers, domestic clinical trials are dominated by single-center clinical trials (84.4%). Conversely, multicenter clinical trials in the United States are currently dominant, accounting for 61% (77/126) of newly registered trials, including 23 multinational trials. While there are 28 projects involving >10 centers in the United States, China only has two such trials. Although China registered more clinical trials, a large cohort to study the long-term efficacy of CAR-T cells is lacking. Conversely, some cohort studies were conducted in the United States, such as ZUMA, KarMMa, and CARTITUDE.

Involvement of targeting an antigen and disease in CAR-T cell clinical trials

Both in China and the United States, the majority of clinical trials involved patients with ALL and lymphoma with 187 trials (67.5%) in China and 85 trials (67.5%) in the United States, followed by MM and AML, although in the United States the primary indication of clinical trials is lymphoid malignancy with lymphoma predominating (72/85). Conversely, in China, ALL predominates among the indications (144/187). Furthermore, in the past 5 years, more researchers in China have focused on the effectiveness of CAR-T cells for AML than abroad. For example, 29 trials (10.5%) were conducted in patients with AML in China, while only four trials (3.2%) were implemented in the United States.

CD19 is the most commonly targeted antigen, followed by BCMA [Figure 1D]. In addition to CD19 and BCMA, trials on CAR-T cell therapy target CD20, CD22, CD123, CD33, C-type lectin-like molecule 1, CD5, CD7, CD4, CD30, CD38, orphan G protein-coupled receptor, class C group 5 and member D (GPRC5D), and dual target CD19/CD20, CD19/CD22, and BCMA/transmembrane activator and calcium-modulating cyclophilin ligand interactor (TACI) were conducted in both China and the United States. Additionally, there are some different target antigens between the two countries. For example, NK group 2D ligand, CD117, FMS-like tyrosine kinase 3,[23] and immunoglobulin-like transcript 3 were investigated in Chinese CAR-T trials, all of which treated AML, while signaling lymphocytic activation molecule family 7 (SLAMF7), CD138, and CD79a were used in the United States.

Genetic modification of CAR-T cells

Although CAR-T cell therapy achieves a high response rate, the high relapse rate and some adverse events, such as CRS and neurotoxicity, hinder the widespread use of this therapy. However, with the rapid development of gene-editing techniques, many potent properties circumvent these obstacles. In the past 5 years, nearly 30 new clinical trials in China and the United States were conducted using a gene-editing technique. In general, these trials are divided into three categories by purpose: (1) Introducing safety switches to prevent severe adverse events. Two molecules were extensively used: truncated epidermal growth factor receptor and doxycycline-inducible Casp9. (2) Alleviating immunological rejection. Knocking out the T cell receptor was attempted in both China and the United States to avoid graft-*vs*. -host disease. Moreover, human leukocyte antigen-I knock-out was attempted in China. (3) Enhancing efficacy. Inhibiting immune checkpoints is a well-known approach to enhance immune responses. In China, two trials edited CAR-T cells to express the programmed death 1 (PD1)/CD28 switch receptor, and the other five clinical trials knocked out PD1 or edited CAR-T cells to express an immune checkpoint inhibitor. However, there were no clinical trials designed to inhibit immune checkpoints in the United States. Additionally, other endogenous immunosuppressive mechanisms can be targeted for immunotherapy in addition to the immune checkpoint pathways, such as Casitas B-lineage lymphoma protein B (CBL-B), an E3 ubiquitin ligase, which was used in the United States. Another molecule tested in China was hematopoietic progenitor kinase1 (HPK1), a kinase associated with T cell exhaustion.

Efficacy of clinical trials

To review the efficacy of CAR-T cell therapy, we searched PUBMED using the keywords "chimeric antigen receptor or CAR" and then manually checked the articles. Finally, we found 74 reports published from 2018 to 2022, including 42 articles from China and 32 from the United States. The efficacy of CAR-T cell trials was reported, and the majority of these articles have shown that the complete remission or complete remission with incomplete hematological recovery (CR/CRi) rate surpassed 60% (30/42 of trials in China and 22/32 of trials in the United States).

Although the clinical efficacy appears comparable between the two countries, the available data indicate some problems. First, there is a gap in the subject sample size. While most published studies (20/42) involved <20 patients in China, only four published clinical trials involved <20 patients receiving CAR-T cell therapy in the US. Second, a long-term follow-up study remains lacking. Hence, more large-scale clinical trials and long-term follow-ups are needed to evaluate the efficacy of CAR-T cells manufactured in China.

Recent Progress of CAR-T Cell Therapy for Hematological Malignancies

CAR-T cell therapy for B cell malignancies

CAR-T cell therapy for non-Hodgkin's lymphoma (NHL) and ALL

In NHL and acute B cell lymphoblastic leukemia, CD19 is widely expressed on the surface of blasts and is a therapeutic target antigen for cellular therapy. In 2017, the FDA approved axicabtagene ciloleucel (Yescarta) for treating r/r large B cell lymphoma (LBCL) and tisagenlecleucel (Kymriah) for treating pediatric r/r ALL. In 2021, the National Medical Products Administration approved Yescarta for treating r/r LBCL in China. As the first product to be applied clinically for leukemia treatment, Kymriah has shown new indications in recent years, such as r/r DLBCL[24] and r/r follicular lymphoma[25] which relapsed after two or more lines of systemic treatment. Moreover, a significant breakthrough of Yescarta was

found. In ZUMA7,[26] 359 patients with r/r LBCL were included, all of whom had either failed to enter CR after first-line chemotherapy regimens or relapsed within 12 months of achieving CR. A total of 180 patients were eventually transfused with Yescarta, and 179 patients received the standard treatment regimen. In a median follow-up period of 24.9 months, Yescarta exhibited statistically significant clinical benefits. Patients who received Yescarta had a 6.3-month longer median event-free survival compared with those who received standard therapy (8.3 months *vs.* 2.0 months). Furthermore, the Yescarta group had a significantly higher CR rate than the standard therapy group (65% *vs.* 32%). Therefore, on April 1, 2022, the FDA approved Yescarta for treating LBCL patients who did not obtain CR at the end of the first-line chemotherapy regimen or who relapsed within 12 months after achieving CR, which represents the success of Yescarta as a second-line treatment option for LBCL.

The efficacy of Yescarta for treatment of relapsed/refractory indolent NHL (r/r iNHL) has also been investigated in the ZUMA-5 trial.[27] In the primary endpoint analysis, 74% (77/104) of patients achieved CR, including 66 patients with r/r follicular lymphoma and 11 patients with r/r marginal zone lymphoma. During the subsequent median follow-up of 23.3 months, 64 patients achieved persistent remission, while the overall survival (OS) rate at 18 months was up to 87.4%. Moreover, CAR-T cell treatment showed a good safety profile. Therefore, Yescarta is an effective treatment option for r/r iNHL patients.

In 2020 and 2021, the FDA approved brexucabtagene autoleucel (KTE-X19) and lisocabtagene maraleucel (liso-cel) for use in r/r mantle cell lymphoma (MCL) and r/r DLBCL therapy, respectively. However, most CAR-T cell therapies have been suggested to treat r/r lymphoma or pediatric r/r ALL, and none of these products have been suggested to treat adult r/r ALL. A phase I multi-center trial reported promising results in treating adult r/r ALL[28] with 17 of 20 patients achieving minimal residual disease (MRD)-negative CR at 1 month, indicating that CD19 CAR-T cells may be a salvage therapy for adult ALL patients. In the ZUMA-3 cohort study,[29,30] this conclusion was confirmed. The ZUMA-3 phase II trial included 71 patients with r/r acute B cell lymphoblastic leukemia,[30] 55 of whom had received KTE-X19. Finally, 39 patients (71%) reached CR/CRi, and 97% (38/39) of responders were MRD negative. In addition to the high treatment response rate, KTE-X19 demonstrated promising durable remission in adults with ALL. During a median follow-up period of 16.4 months, the median OS of patients who had CR/CRi was not reached. The median duration of response (mDOR) was up to 12.8 months for all patients, while the mDOR for patients who obtained CR/CRi was not reached.[30] Thus, the FDA approved KTE-X19 as a therapy alternative for r/r precursor B-cell lymphoblastic leukemia on October 1, 2021.

In addition to treating ALL, DLBCL, and MCL, CD19-CAR-T cells are being explored for new indications. TRANSCEND CLL 004[31] is a multicenter phase I/II trial. CD19 CAR-T cells showed high efficacy and a controlled safety profile in this study. Eventually, 23 patients with r/r chronic lymphocytic leukemia or small lymphocytic lymphoma (CLL/SLL) received liso-cel in this trial, of whom 45% (10/22) obtained CR/CRi. While 20 patients underwent MRD monitoring of blood and bone marrow samples, 75% (15/20) obtained MRD negativity. During a median follow-up period of 24 months, the median progression-free survival (PFS) was 18 months, while the mDOR was not reached. Thus, the high effectiveness of CD19-CAR-T cell therapy resulted in an improved prognosis for r/r CLL/SLL patients.

China has also made a great contribution to manufacturing novel CD19 CAR-T cells. Relmacabtagene autoleucel (JWCAR029) is a kind of CD19 CAR-T cell with a 4-1BB costimulatory domain manufactured in China. A phase II multicenter clinical trial[32] demonstrated high clinical efficacy of JWCAR029 for r/r DLBCL. The CR rate reached 51.7% by the cut-off date. Furthermore, the rate of adverse events was relatively low with both severe CRS and neurotoxicity at 5.1% [Table 1]. Thus, in 2021, the National Medical Products Administration approved JWCAR029 for commercial use in treating r/r LBCL in China. Additionally, there are many other novel CD19 CAR-T cells manufactured in China, such as CNCT19 with a novel single-chain variable fragments (scFv)-HI19α[33,34] (NCT04586478 and NCT04230473) and XYF-19 CAR-T cells eliminating endogenous HPK1 by clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (NCT0403756).[35]

Table 1: Published CD19 CAR-T cell clinical trials.

Study	Number of patients	Disease	CAR-T products	CR rate (%)	OS rate (median OS) (% [months])	CRS rate		NT rate	
						All grade CRS rate (% [*n*/*M*])	≥Grade 3 CRS rate (% [*n*/*M*])	All grade NT rate (% [*n*/*M*])	≥Grade 3 NT rate (% [*n*/*M*])
Locke *et al*[26]	359	r/r DLBCL	Axicabtagene ciloleucel	65	61 (24)	92 (157/170)	6 (11/170)	60 (102/170)	21 (36/170)
Jacobson *et al*[27]	148	r/r iNHL	Axicabtagene ciloleucel	76	87 (18)	82 (121/148)	7 (10/148)	59 (87/148)	19 (28/148)
Ying *et al*[32]	58	r/r DLBCL	Relmacabtagene autoleucel	51.7	NA	47.5 (28/59)	5.1 (3/59)	20.3 (12/59)	5.1 (3/59)
Shah *et al*[30]	55	r/r Adult ALL	Brexucabtagene autoleucel	71	71 (12)	89 (49/55)	24 (13/55)	60 (33/55)	25 (14/55)
Roddie *et al*[28]	20	r/r Adult ALL	AUTO1	85	64 (12)	55 (11/20)	0	20 (4/20)	15 (3/20)
Siddiqi *et al*[31]	23	r/r CLL/SLL	Lisocabtagene maraleucel	45	NA	74 (17/23)	9 (2/23)	39 (9/23)	22 (5/23)

ALL: Acute lymphoblastic leukemia; CAR-T: Chimeric antigen receptor-T; CLL: Chronic lymphocytic leukemia; CR: Complete remission; CRS: Cytokine release syndrome; DLBCL: Diffuse large B cell lymphoma; NA: Not available; NT: Neurotoxicity; r/r: Relapsed/refractory; iNHL: Indolent non-Hodgkin's lymphoma; OS: Overall survival; SLL: Small lymphocytic lymphoma.

CAR-T cell therapy for HL

Although most HL patients achieve durable complete remission after first-line treatment, there is a 15% relapse rate. This group of patients has a poor prognosis after intense chemotherapy and bone marrow transplantation, and requires new treatment options.[11] CD30 is expressed in Reed-Sternberg cells of HL and is rarely detected on the surface of normal cells, making CD30 an essential target for HL immunotherapy. A recent phase 1 clinical trial reported[10] that the overall response rate of CD30-CAR-T cell therapy for r/r HL patients was 72% (23/32) with 19 patients achieving CR and four patients achieving partial remission (PR). Of all patients, one patient who remained in CR at the start of treatment maintained CR for >3 years after receiving CD30 CAR-T cells, which demonstrated that the high effectiveness of CD30 CAR-T can have clinical benefits for patients. It has also been suggested that augmenting the cytotoxicity of CD30 CAR-T cells against HL requires facilitating the migration of CAR-T cells into the tumor or assisting CAR-T cells to counteract the immunosuppressive environment in the tumor.[11] Currently, a trial is investigating the clinical efficacy of C-C chemokine receptor type 4-expressing CD30 CAR-T cells (NCT03602157), a regimen that has been shown to promote CAR-T cells' migration into tumors and enhance elimination in *in vivo* and *in vitro* trials.[36]

CAR-T cell therapy for MM

BCMA is a member of the tumor necrosis factor receptor superfamily, which is expressed on the surface of malignant plasma cells and some normal mature B cells.[37-39] Further, overexpression and activation of BCMA are related to the pathogenesis of MM. Therefore, BCMA is an essential target for MM patients.[39] In 2019, a phase I clinical trial reported that ABECM (idecabtagene vicleucel) provided a clinical benefit in patients with manageable adverse events.[38] A recent phase II clinical trial[40] confirmed this conclusion. In total, 140 patients were involved, 128 of whom received ABECMA with 73% (94/128) of patients achieving a treatment response, of whom 33% (42/128) achieved CR. Over a median follow-up period of 13.3 months, the median OS and median PFS were 19.4 months and 8.8 months, respectively, for all patients, with a 12-month extension of the median PFS for patients who achieved CR. Consequently, in 2021, the FDA approved ABECM for treating r/r MM.

Some clinical trials[41-43] have demonstrated that a novel CAR-T cell therapy, ciltacabtagene autoleuce l (LCAR-B38M), which was designed to target two distinct BCMA epitopes, resulted in a considerable early response rate and duration of remission, and manageable safety profile [Table 2]. Because of these clinical trials, the FDA approved ciltacabtagene autoleucel for r/r MM treatment in 2022. A meta-analysis reported[44] that different structures of BCMA CAR-T cells affect the objective response rate, CR, and PFS. Therefore, the means to construct a more suitable CAR for more effective treatment of patients may be a concern for future research.

Despite the growing maturity of BCMA CAR-T cell development and the lasting clinical benefits that they provide, there are many clinical cases of BCMA-negative relapse. Consequently, it is also necessary to find more targets for treating r/r MM.[45]*SLAMF7*, a member of the SLAM family, is widely expressed on the surface of malignant plasma cells in MM, making it a targeted antigen.[45] A preclinical trial demonstrated that SLAMF7 CAR-T cells eliminate tumor cells rapidly, resulting in a durable response. However, SLAMF7 is also expressed on the surface of normal NK cells and a proportion of lymphocytes. Therefore, SLAMF7 CAR-T cells also exert an "on-target off-tumor" effect to eradicate SLAMF7$^+$ normal cells,[45] which may limit the application of SLAMF7 CAR-T cells. However, introducing a suicide gene has ensured their safety without compromising effectiveness.[46]

GPRC5D is mainly expressed on the surface of malignant MM cells and hair follicle cells and is therefore a safe therapeutic target.[47]*In vivo* and *in vitro*, GPRC5D CAR-T and BCMA-CART cells have comparable efficacy, whereas GPRC5D CAR-T cells have also shown adequate clearance of MM in a mouse model of BCMA-negative relapse. Additionally, GPRC5D CAR-T cells did not exert off-target effects. Therefore, GPRC5D has the potential to be an essential target for MM treatment and not only a salvage treatment after BCMA-negative relapse.[47] Some

Table 2: Published reports of BCMA CAR-T cells.

Study	Number of patients	CAR-T products	CR rate (%)	OS rate (median OS) (% [months])	CRS rate		NT rate	
					All grade CRS rate (% [*n*/*M*])	≥Grade3 CRS rate (% [*n*/*M*])	All grade NT rate (% [*n*/*M*])	≥Grade 3 NT rate (% [*n*/*M*])
Raje *et al*[38]	33	Idecabtagene vicleucel	45	NA	76 (25/33)	6 (2/33)	42 (14/33)	3 (1/33)
Munshi*et al*[40]	128	Idecabtagene vicleucel	33	NA	84 (107/128)	5 (7/128)	18 (23/128)	3 (4/128)
Zhao *et al*[41]	57	Ciltacabtagene autoleucel	68	NA	89 (51/57)	7 (4/57)	2 (1/57)	0
Xu *et al*[42]	17	Ciltacabtagene autoleucel	76	82 (12)	100 (17/17)	41 (7/17)	NA	NA
Berdeja *et al*[43]	97	Ciltacabtagene autoleucel	65	89 (12)	95 (92/97)	5 (5/97)	21 (20/97)	9 (9/97)

BCMA: B cell maturation antigen; CAR-T: Chimeric antigen receptor-T; CR: Complete remission; CRS: Cytokine release syndrome; NA: Not available; NT: Neurotoxicity; OS: Overall survival.

clinical trials are also in progress (NCT04555551 and NCT05016778).

CAR-T cell therapy for T cell malignancies

Antigens of malignant T cells are often expressed on the surface of normal T cells and CAR-T cells, and therefore the "on-target off-tumor" effect will not only affect T cells themselves, but also eliminate CAR-T cells, drastically limiting the use of CAR-T cell therapy for T cell malignancies.[48] CD7 is expressed in most lymphoblastic T cell leukemias.[49] An *in vivo* and *in vitro* study[48] demonstrated that CD7 CAR-T cells effectivity eliminate $CD7^+$ T-ALL. Hence, CD7 may be a target in CAR-T cell therapy for T lymphocytic malignancies, but there are many problems such as accurately isolating normal T cells from peripheral blood for CAR-T cell generation. Therefore, it has been proposed that r/r T-ALL can be treated using allogeneic CAR-T cells.[50] In a domestic phase I clinical trial,[49] 20 patients received donor-derived CAR-T cells, 90% (18/20) of whom achieved CR, including 17 patients who achieved MRD negativity (flow cytometry-MRD <0.01%). Additionally, controllable adverse effects such as graft-*vs*.-host disease and T cell reduction were observed in the trial. Therefore, donor-derived CAR-T cells may have clinical benefits for patients, and a follow-up phase II clinical trial (NCT04689659) is in progress.

CAR-T cell therapy for AML

Although CAR-T cell therapy has shown an inspiring response rate in lymphoid malignancies, the development of CAR-T cells in the treatment of r/r AML has faced some obstacles, one of which is the selection of targeted antigen. Because the targeted antigens of AML cells are expressed on the surfaces of both AML blasts and normal hematopoietic stem cells, they can easily cause "on-target off-tumor" effects.[3,4] CD33 and CD123 have emerged as promising targets for AML in many newly registered clinical trials.

CD33, whose expression is detected in 70% of patients, is also expressed in 90% of leukemic cells. However, normal human hematopoietic stem cells also express CD33, while $CD34^+$ $CD33^-$ hematopoietic stem cells are simultaneously present in the human body. Therefore, CD33 has become a target for immunotherapy in AML patients.[3,51] Moreover, the FDA has approved the drug-conjugated anti-CD33-antibody gemtuzumab ozogamicin for treating AML, which also facilitates the emergence of CD33 CAR-T cell therapy.[3] As reported by Liu *et al*,[51] CD33 CAR-T cells show promising cytotoxicity for $CD33^+$ leukemia stem cells *in vitro*, and they concluded that CD33 CAR-T cells can be used in clinical application. However, in a previous trial,[52] all patients who received CD33 CAR-T cells showed no response, which may be related to the small dose (0.3×10^6/kg) received by the patients. Moreover, some projects are investigating the clinical effects of CD33 CAR-T cells (NCT04835519 and NCT03971799). Additionally, in a previous trial,[51] CAR-T cells exhibited cytotoxicity when cocultured with $CD33^+$ hematopoietic stem cells. Therefore, clinical trials in the future will need to focus on reducing bone marrow toxicity.

CD123, the transmembrane interleukin (IL)-3 receptor alpha chain, is highly expressed on AML cells and is a good target. In a recent study, CD123 CAR-T cells showed a powerful tumor cell-killing ability, reaching a 90% lysis rate. Furthermore, they also inhibited the proliferation of AML progenitor cells to some extent, which corresponded to previous findings. Additionally, conflicting with other studies, the CD123 CAR-T cells they constructed did not show any inhibitory effect on bone marrow hematopoiesis.[53] They hypothesized that this might be associated with the CAR structure. More trials are needed to validate this assumption.

In general, CAR-T cell therapy has achieved great breakthroughs in recent years, such as becoming a second-line treatment option of r/r B-ALL and finding some new target antigens. Some clinical trials have also demonstrated great outcomes when treating HL and CLL. However, reports on long-term efficacy are lacking. Additionally, although CAR-T cell therapy has achieved great progress in treating AML and T cell malignancies in xenograft models, many challenges remain in extending CAR-T cell therapy to AML and T cell malignancies, such as apheresis[49,52] and potential myelosuppression.

Limitations of CAR-T Therapy and Solutions

Despite current progress, many challenges remain, such as a high relapse rate, unsatisfactory duration of efficacy, and some treatment-related toxicity. In the subsequent sections, we summarize the breakthroughs in addressing these problems in recent years.

Treatment-related toxicity

During treatment, neurotoxicity and CRS are the most frequent adverse events. The incidence of CRS can reach 90%, and neurotoxicity can reach approximately 60%. However, the incidence of severe side effects is approximately 10%. Although the use of IL-6 antagonists, glucocorticois,[22] suicide genes, and safety switches, such as truncated epidermal growth factor receptor and doxycycline-inducible Casp9,[46,54-56] can be effective in treating these side effects, there is no effective prophylaxis for these side effects.

IL-1 induces the release of IL-6.[57,58] Moreover, IL-6 is a hallmark cytokine of CAR-T-related CRS and neurotoxicity.[22,58,59] Accordingly, IL-1 secretion may initiate CRS.[58,59] Norelli *et al*[58] reported that the IL-1 receptor antagonist anakinra may be an effective prophylaxis for CRS and neurotoxicity, resulting in extended OS *in vivo*. Consequently, some clinical trials (NCT04359784 and NCT04150913) were conducted to evaluate whether anakinra can prevent CRS and neurotoxicity.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) also contributes to the development of neurotoxicity.[59,60] Furthermore, GM-CSF neutralization or GM-CSF knockout alleviates CRS and neuroinflammation

significantly *in vivo*[60] with enhanced anti-tumor activity of CAR-T cells.[60,61] A phase I, open-label, multicenter clinical trial (ZUMA-19) was implemented to assess the safety of an anti-GM-CSF antibody combination with CD19 CAR-T cells. Another promising progression of preventing CRS and neurotoxicity is reducing the affinity of CAR-T cells. Roddie *et al*[28,62] designed novel CD19 CAR-T cells with a rapid binding off-rate, decreasing the affinity of CAR-T cells. They demonstrated that low-affinity CD19 CAR-T cells had low toxicity with an increased anti-tumor activity and durable response compared with traditional CD19 CAR-T cells.[28,62] Although the mechanism of this phenomenon is unclear, they hypothesized that it might be due to shorter cell–cell contact, resulting in lower cytokine release and T cell exhaustion. A large phase Ib/II clinical trial was conducted to assess the safety and efficacy of these novel CD19 CAR-T cells in adult ALL (NCT04404660).

Another severe side effect is the "on-target, off-tumor" effect. Many targeting antigens are highly expressed on both tumor and normal cells, such as CD19 in the B-lineage, CD5 and CD7 in the T-lineage,[48,49] and some myeloid antigens.[3] Therefore, when CAR-T cells recognize tumor cells, they may also impair normal cells in the body, which may cause immune suppression[49] and bone marrow suppression in severe cases.[51,53,63] However, there is a lack of effective methods to deal with this side effect, and it has been proposed that it can be circumvented by screening for more specific antigens.[19] Achieving precise targeting of tumor cells is possible by constructing multi-specific CAR-T cells with "AND" logic gates or "NOT" logic gates.[64] These novel CAR-T cells show more accurate tumor cell identification.[64] However, there is a lack of efficacy comparisons with conventional CAR-T cells. Therefore, it is unclear whether these novel CAR-T cells provide lasting clinical benefits to patients and which populations are more suitable for such CAR-T cells. Thus, more clinical trials are needed to study them.

Relapse after treatment

Although CAR-T cell therapy improves the remission rate of patients with hematological malignancies, the high post-treatment relapse rate also prevents its further promotion.[21] There are two significant relapse mechanisms: antigen-negative and antigen-positive relapse. There are several mechanisms of antigen-negative escape, including antigen escape, lineage switching, and downregulation of the antigen.[19-21,65] Regarding antigen-positive relapse, it is mainly related to the short persistence of CAR-T cells *in vivo* and rapid onset of immune exhaustion.[7,13]

Overcoming antigen-negative relapse

To overcome antigen escape, multi-specific CAR-T cells or mixed CAR-T cell sequential therapy has been considered potentially effective in preclinical studies.[12,20,21,66-69] In a phase I clinical trial in the United States,[70] all r/r ALL patients (n = 17) had a therapeutic response to CD19/CD22-bispecific CAR-T cells, with 82% (14/17) of them achieving CR. In a phase I/II clinical trial conducted in China,[69] 71% (20/28) of patients infused with CD19/CD20-bispecific CAR-T cells achieved CR with four of the treated patients showing CD19 negativity in FCM and immunohistochemical staining, two of whom achieved CR and one of whom achieved partial remission. In subsequent clinical trials, CD19/CD20 CAR-T cells resulted in a lower negative relapse rate than CD19-CAR-T cells.[71] Lineage switching means that patients may relapse with genetically related, but distinct phenotypes of malignancy, with the most common being AML. This phenomenon may be associated with mixed-lineage leukemia gene rearrangement.[20] $CD123^+$ blasts are detected in some B-ALL patients, which may be associated with antigen-negative relapse. Some previous studies have demonstrated that combing CD19 with CD123 may enhance anti-tumor efficacy and prevent CD19-negative recurrence in a xenograft model.[72]

In terms of r/r MM, the combination of CD38 or CD19 with BCMA has shown encouraging clinical efficacy. In a single-arm, single center phase II clinical trial conducted by Yan *et al*,[73] they evaluated the effectiveness of sequential treatment with CD19 and BCMA. They enrolled 21 patients, of whom 95% (20/21) achieved a treatment response, including 12 patients who achieved CR and stringent CR (sCR). In another domestic clinical study, they reported comparable results. After sequential treatment with CD19 CAR-T and BCMA CAR-T cells, 90% (9/10) of patients achieved a therapeutic response.[74] Mei *et al*[75] reported the results of a phase I clinical trial using CD38/BCMA for r/r MM. In preclinical studies, CD38/BCMA CAR-T cells showed more potent cytotoxicity than BCMA CAR-T cells. CD38/BCMA CAR-T cells showed cytotoxicity against CD38-negative or BCMA-negative cells. Subsequently, 23 patients received CD38/BCMA CAR-T cells, of which 87% (20/23) responded to treatment, including 12 sCR patients, four very good PR patients, and four PR patients. Additionally, all patients who reached PR or a better treatment response were negative for MRD, including 15 CD38-negative patients and one BCMA-negative patient, suggesting that CD38/BCMA CAR-T cells effectively eradicate CD38- or BCMA-expressing cells *in vivo*.

In addition to the promising efficacy of CD38 or CD19 in combination with BCMA, CAR-T cells based on a proliferation-inducing ligand (APRIL), the ligand of TACI/BCMA, have been reported to have favorable therapeutic effects. Lee *et al*[76] found that APRIL CAR-T cells demonstrated effective cytotoxicity when co-cultured with myeloma cells. In a mouse model of MM, APRIL CAR-T cells showed potent anti-tumor effects. Additionally, in an antigen-escape model, APRIL CAR-T cells showed cytolysis of tumors expressing only BCMA or TACI, whereas BCMA CAR-T cells had only an anti-$BCMA^+$ tumor activity. Two other clinical trials of APRIL CAR-T are in progress (NCT05020444 and NCT04657861), but the results remain unknown.

To overcome antigen downregulation, there are several methods, such as enhancing the affinity of CAR-T cells and increasing antigen destiny. In a preclinical study, γ-secretase (GS) inhibitors prevented GS from cleaving surface BCMA and enhanced BCMA CAR-T cell

recognition of myeloma cells and the anti-tumor effect. However, when applied to CD19 CAR-T cells, high concentrations of GS inhibitors resulted in reversible inhibition of the CAR-T cells' function. Additionally, the increase of surface BCMA was also reversible, indicating that it is necessary to maintain an effective concentration below the inhibitory level when used with CAR-T cells.[37]

Overcoming antigen-positive relapse

The most important means to overcome positive relapse is to extend the persistence of CAR-T cell and overcome T cell exhaustion and the immunosuppressive microenvironment.[21] Combining with immune checkpoint inhibitors, constructing human-derived CARs, and receiving consolidative allogeneic hematopoietic stem cell transplantation (allo-HSCT) improve CAR-T cell persistence and efficacy.

In the tumor microenvironment, tumor cells suppress T cell functions by binding to immune checkpoint molecules on T cells, in which the PD-1/PD-L1 pathway plays a significant role.[77] Similarly, the PD-1/PD-L1 pathway restrains CAR-T cell functions, which is a mechanism leading to positive relapse.[78-80] Studies have shown that targeting the PD-1/PD-L1 pathway improves the effectiveness of CAR-T cells. Blocking PD-1 binding to PD-L1 by various methods, such as the combination of antagonists, has been proven to enhance CAR-T cell efficacy.[81,82] CAR-T cells treated with autocrine PD-1/PD-L1 inhibitors show enhanced expansion and cytolysis in both *in vivo* and *in vitro* assay.[78] Some related clinical trials are also in progress (NCT03790891 and NCT03910842). Additionally, other approaches, such as knocking down PD-1 (NCT04213469) and/or competing for PD-L1 by expressing mutant PD-1 (NCT04163302 and NCT04162119), are being explored in several clinical trials.

Another approach to target PD-1/PD-L1 is to block transmission of inhibitory signals. Under normal conditions, PD-1 inhibits T cell activation by combining with PD-L1 and delivering inhibitory signals to T cells. By binding the PD-1 extracellular domain to the CD28 costimulatory domain, the inhibitory signal can be converted to an activating signal.[81] In both *in vivo* and *in vitro* experiments, CAR-T cells expressing PD-1/CD28 showed more robust cytolysis activity than CAR-T cells alone or CAR-T cells combined with an anti-PD-1 antibody.[81] Furthermore, in a domestic clinical trial,[81] 17 patients were transfused with these CAR-T cells, and 41.2% (7/17) of them achieved complete remission, and the median OS was not reached during the follow-up. There are some trial programs investigating the efficacy of CAR-T cells with the PD-1/CD28 switch receptor (NCT04850560 and NCT03932955), but larger clinical trials to assess their efficacy are lacking. Additionally, expressing PD-1 dominant negative receptor (PD1-DNR) effectively overcomes the immunosuppressive effects of the PD1/PD-L1 pathway, and CAR-T cells expressing PD1-DNR have exhibited enhanced proliferation and cytotoxicity in *in vivo* and *in vitro* experiments.[79] There is a relative lack of trials using CAR-T cells expressing PD1-DNR to treat hematological tumors.

To overcome exhaustion, other endogenous immunosuppressive mechanisms can be targeted for immunotherapy in addition to the immune checkpoint pathway. CBL-B, an E3 ubiquitin ligase, is associated with T cell exhaustion.[83] A preclinical study has demonstrated that deletion of *CBLB* in CAR-T cells confers them with resistance to exhaustion.[83] Moreover, a clinical trial in the United States transfected messenger RNA encoding the *CBLB*-targeting megaTAL enzyme to edit the *CBLB* gene, whereas another pathway was assessed in China. A previous study has reported that high expression of HPK1 mediates T cell exhaustion.[35] Knocking out *HPK1* is also an effective method to alleviate CAR-T cell exhaustion and enhance CAR-T cell proliferation and anti-tumor effects *in vivo*. *HPK1* was also knocked out in a clinical trial.

Cytokines are essential for T cell amplification, persistence, and activation. Thus, some CAR-T cells are edited to secrete cytokines. IL-18, IL-7, C-C motif chemokine ligand 19 (CCL19), and IL-15 have been studied extensively. IL-18 enhances CAR-T cell amplification and anti-tumor activity by increasing *T-bet* expression and silencing *forkhead box O1* in solid tumor models.[84] IL-7 enhances the survival and proliferation of T cells by activating signal transducer and activator of transcription 5.[85,86] Moreover, *in vivo*, activating the IL-7 signaling pathway dramatically improves the anti-tumor effect of CAR-T cells in solid tumor models.[87] CCL19 is a leukocyte chemoattractant that may help C-C chemokine receptor type 7-positive T cells, the receptor of CCL19, migrate to the tumor microenvironment to enhance cytotoxicity.[86,88,89] Furthermore, IL-7 and CCL19 are necessary for the formation and persistence of the T-zone in lymphoid organs. In terms of IL-15, a previous study has demonstrated that CAR-T cells expressing membrane-bound IL-15 prevent $CD19^+$ leukemia engraftment and achieve sustained survival *in vivo* with a memory stem cell phenotype.[90]

Researchers have gradually discovered that host immunity to CAR-T cells influences the persistence and efficacy of CAR-T cells and that murine-derived scFv, such as FMC63 for CD19 CAR-T, correlate with the immunogenicity of CAR. Therefore, researchers began using humanized scFv to construct CARs.[91] In an early phase I clinical trial in China,[92] 24 patients received human-derived CD19 CAR-T cells, including four patients who relapsed after receiving murine-derived CAR-T cells. As a result, 83.3% (20/24) of patients achieved CR/CRi at one month after treatment, and 90% (18/20) of them achieved negative FCM-MRD, while three of four patients who did not achieve CR/CRi had a previous murine-derived CAR-T cell infusion. During a median follow-up period of 471 days, the OS rate at 1 and 3 years was 63% and 42%, respectively. In another domestic phase I trial,[93] 18 r/r MM patients were treated with fully human-derived BCMA CAR-T cells (CT103A), four of whom had received murine-derived CAR-T cell therapy. Eventually, 72.2% (13/18) of patients achieved CR/sCR, including three murine CAR-exposed patients. Moreover, anti-CAR-T antibodies were found in only one patient. Thus, human-derived CAR-T cells also provide clinical benefits

to patients and reduce the immune response to CAR-T in patients. However, there is a lack of comparisons between the efficacies of human- and murine-derived CAR-T cells. Therefore, it is unclear whether this reduction in the host immune response further enhances efficacy. However, the efficacy of these two products in patients who previously received murine-derived CAR-T cells is very different, which may be related to the different diseases of the patients. Consequently, it is necessary to conduct a clinical trial to compare the long-term efficacy of murine-derived CAR-T cells and humanized CAR-T cells.

Another approach to extend the persistence of CAR-T cells is receiving allo-HSCT as consolidated therapy. A clinical trial in China involved 58 patients.[94] After receiving CD19 CAR-T cells, 47 patients achieved MRD-negative CR, 21 of whom received allo-HSCT. Seventeen patients after allo-HSCT remained disease-free during the follow-up, and only two patients had CD19-negative relapse. Additionally, there was a significant difference in event free survival (EFS) and relapse free survival (RFS) between patients who received allo-HSCT and patients who did not. Hence, consolidative allo-HSCT following CAR-T cell therapy may be an effective method to maintain EFS and RFS in MRD-negative patients.

Availability of CAR-T cell therapy

The six CAR-T cell products approved by the FDA are all autologous CAR-T cells. However, the process of CAR-T cell treatment is complicated and lengthy, and includes apheresis, CAR-T cell generation, lymphodepletion, and infusion. Many patients withdraw before infusion because of infection, disease progression, production failure, or unqualified production. The high cost of treatment also prevents the promotion of CAR-T cell treatment.[8] Thus, using universal CAR-T (UCAR-T) cells from healthy donor sources has become a new direction for CAR-T cell therapy. Similar to the target of autologous cells, CD19 remains a popular target sites for UCAR-T cells. Many projects still investigate the efficacy of CD19 UCAR-T cells (NCT04035434, NCT04629729, NCT04154709, NCT04026100, NCT04264039, and NCT05105867). In addition to CD19, the clinical efficacy of UCAR-T cells against CD7 (NCT04538599), BCMA (NCT04244656, NCT03752541), CS1 (NCT04142619), CD22 (NCT04150497), and CD123 (NCT04796441) is being evaluated. Although most UCAR-T cells are mono-targeted cells, dual-targeted UCAR-T cells have also shown good efficacy. Hu *et al*[95] reported the therapeutic efficacy of CD19/CD22 UCAR-T cells in a phase I clinical trial. Despite the trial's small size, it also suggests that CD19/CD22 UCART cells improve the prognosis of ALL patients. Alternatively, there is a larger single center clinical study investigating the clinical effects of CD19/CD20 and CD19/CD22 (NCT03398967).

Accelerating the production of CAR-T cells is another effective method. Jackson *et al*[96] reported that using an automated system reduces cell manufacturing time to 12 days, decreases the cost, and expedites treatment. They found that supplementation with IL-7/IL-15 promoted a stem cell-like memory T cell-like phenotype that is associated with enhanced self-renewal and effector differentiation. Another study confirmed this conclusion.[97] However, the clinical efficacy of this novel technique remains unclear.

Conclusion

CAR-T cell clinical trials are growing rapidly in China and the United States. Despite recent innovation in CAR-T cell therapy in China, there are still certain drawbacks.[18] For example, most clinical trials have small sample sizes and are single-center. Additionally, a large cohort to study long-term efficacy is lacking in China. Seven CAR-T cell products have been applied to clinical treatment of MM, NHL, and B-ALL with Yescarta successfully becoming a second-line treatment option for r/r LBCL. Additionally, some CAR-T cell products have been proven to benefit patients with CLL and HL. However, in terms of T-ALL and AML, the efficiency of CAR-T cells remains poor. Hence, in the future, more clinical trials are needed to promote CAR-T cell therapy for other hematological malignancies. CAR-T cell therapy also has many problems such as a high relapse rate, poor accessibility, and severe side effects. Clarifying the mechanism and promoting some approaches for clinical treatment will further optimize the therapy in future studies.

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Conflicts of interest

None.

References

1. Gong Y, Klein Wolterink RGJ, Wang J, Bos GMJ, Germeraad WTV. Chimeric antigen receptor natural killer (CAR-NK) cell design and engineering for cancer therapy. J Hematol Oncol 2021;14:73. doi: 10.1186/s13045-021-01083-5.
2. Yilmaz A, Cui H, Caligiuri MA, Yu J. Chimeric antigen receptor-engineered natural killer cells for cancer immunotherapy. J Hematol Oncol 2020;13:168. doi: 10.1186/s13045-020-00998-9.
3. Hofmann S, Schubert ML, Wang L, He B, Neuber B, Dreger P, *et al*. Chimeric antigen receptor (CAR) T cell therapy in acute myeloid leukemia (AML). J Clin Med 2019;8:200. doi: 10.3390/jcm8020200.
4. Xu Z, Huang X. Cellular immunotherapy for hematological malignancy: recent progress and future perspectives. Cancer Biol Med 2021;18:966–980. doi: 10.20892/j.issn.2095-3941.2020.0801.
5. He J, Xiong X, Yang H, Li D, Liu X, Li S, *et al*. Defined tumor antigen-specific T cells potentiate personalized TCR-T cell therapy and prediction of immunotherapy response. Cell Res 2022;32:530–542. doi: 10.1038/s41422-022-00627-9.
6. Shafer P, Kelly LM, Hoyos V. Cancer therapy with TCR-engineered T cells: current strategies, challenges, and prospects. Front Immunol 2022;13:835762. doi: 10.3389/fimmu.2022.835762.

7. Larson RC, Maus MV. Recent advances and discoveries in the mechanisms and functions of CAR T cells. Nat Rev Cancer 2021;21:145–161. doi: 10.1038/s41568-020-00323-z.
8. Lin H, Cheng J, Mu W, Zhou J, Zhu L. Advances in universal CAR-T cell therapy. Front Immunol 2021;12:744823. doi: 10.3389/fimmu.2021.744823.
9. Ramos CA, Ballard B, Zhang H, Dakhova O, Gee AP, Mei Z, *et al*. Clinical and immunological responses after CD30-specific chimeric antigen receptor-redirected lymphocytes. J Clin Invest 2017;127:3462–3471. doi: 10.1172/jci94306.
10. Ramos CA, Grover NS, Beaven AW, Lulla PD, Wu MF, Ivanova A, *et al*. Anti-CD30 CAR-T cell therapy in relapsed and refractory Hodgkin lymphoma. J Clin Oncol 2020;38:3794–3804. doi: 10.1200/jco.20.01342.
11. Grover NS, Savoldo B. Challenges of driving CD30-directed CAR-T cells to the clinic. BMC Cancer 2019;19:203. doi: 10.1186/s12885-019-5415-9.
12. Dai Z, Mu W, Zhao Y, Cheng J, Lin H, Ouyang K, *et al*. T cells expressing CD5/CD7 bispecific chimeric antigen receptors with fully human heavy-chain-only domains mitigate tumor antigen escape. Signal Transduct Target Ther 2022;7:85. doi: 10.1038/s41392-022-00898-z.
13. You L, Han Q, Zhu L, Zhu Y, Bao C, Yang C, *et al*. Decitabine-mediated epigenetic reprograming enhances anti-leukemia efficacy of CD123-targeted chimeric antigen receptor T-cells. Front Immunol 2020;11:1787. doi: 10.3389/fimmu.2020.01787.
14. Zhang H, Wang P, Li Z, He Y, Gan W, Jiang H. Anti-CLL1 chimeric antigen receptor T-cell therapy in children with relapsed/refractory acute myeloid leukemia. Clin Cancer Res 2021;27:3549–3555. doi: 10.1158/1078-0432.Ccr-20-4543.
15. Petrov JC, Wada M, Pinz KG, Yan LE, Chen KH, Shuai X, *et al*. Compound CAR T-cells as a double-pronged approach for treating acute myeloid leukemia. Leukemia 2018;32:1317–1326. doi: 10.1038/s41375-018-0075-3.
16. Xu J, Wang Q, Xu H, Gu C, Jiang L, Wang J, *et al*. Anti-BCMA CAR-T cells for treatment of plasma cell dyscrasia: case report on POEMS syndrome and multiple myeloma. J Hematol Oncol 2018;11:128. doi: 10.1186/s13045-018-0672-7.
17. Gou L, Gao J, Yang H, Gao C. The landscape of CAR T-cell therapy in the United States and China: a comparative analysis. Int J Cancer 2019;144:2043–2050. doi: 10.1002/ijc.31924.
18. Wei J, Guo Y, Wang Y, Wu Z, Bo J, Zhang B, *et al*. Clinical development of CAR T cell therapy in China: 2020 update. Cell Mol Immunol 2021;18:792–804. doi: 10.1038/s41423-020-00555-x.
19. Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. Blood Cancer J 2021;11:69. doi: 10.1038/s41408-021-00459-7.
20. Majzner RG, Mackall CL. Tumor antigen escape from CAR T-cell therapy. Cancer Discov 2018;8:1219–1226. doi: 10.1158/2159-8290.Cd-18-0442.
21. Shah NN, Fry TJ. Mechanisms of resistance to CAR T cell therapy. Nat Rev Clin Oncol 2019;16:372–385. doi: 10.1038/s41571-019-0184-6.
22. Xu X, Huang S, Xiao X, Sun Q, Liang X, Chen S, *et al*. Challenges and clinical strategies of CAR T-cell therapy for acute lymphoblastic leukemia: overview and developments. Front Immunol 2020;11:569117. doi: 10.3389/fimmu.2020.569117.
23. Wang Y, Xu Y, Li S, Liu J, Xing Y, Xing H, *et al*. Targeting FLT3 in acute myeloid leukemia using ligand-based chimeric antigen receptor-engineered T cells. J Hematol Oncol 2018;11:60. doi: 10.1186/s13045-018-0603-7.
24. Schuster SJ, Tam CS, Borchmann P, Worel N, McGuirk JP, Holte H, *et al*. Long-term clinical outcomes of tisagenlecleucel in patients with relapsed or refractory aggressive B-cell lymphomas (JULIET): a multicentre, open-label, single-arm, phase 2 study. Lancet Oncol 2021;22:1403–1415. doi: 10.1016/s1470-2045(21)00375-2.
25. Fowler NH, Dickinson M, Dreyling M, Martinez-Lopez J, Kolstad A, Butler J, *et al*. Tisagenlecleucel in adult relapsed or refractory follicular lymphoma: the phase 2 ELARA trial. Nat Med 2022;28:325–332. doi: 10.1038/s41591-021-01622-0.
26. Locke FL, Miklos DB, Jacobson CA, Perales MA, Kersten MJ, Oluwole OO, *et al*. Axicabtagene ciloleucel as second-line therapy for large B-cell lymphoma. N Engl J Med 2022;386:640–654. doi: 10.1056/NEJMoa2116133.
27. Jacobson CA, Chavez JC, Sehgal AR, William BM, Munoz J, Salles G, *et al*. Axicabtagene ciloleucel in relapsed or refractory indolent non-Hodgkin lymphoma (ZUMA-5): a single-arm, multicentre, phase 2 trial. Lancet Oncol 2022;23:91–103. doi: 10.1016/s1470-2045(21)00591-x.
28. Roddie C, Dias J, O'Reilly MA, Abbasian M, Cadinanos-Garai A, Vispute K, *et al*. Durable responses and low toxicity after fast off-rate CD19 chimeric antigen receptor-T therapy in adults with relapsed or refractory B-cell acute lymphoblastic leukemia. J Clin Oncol 2021;39:3352–3363. doi: 10.1200/jco.21.00917.
29. Shah BD, Bishop MR, Oluwole OO, Logan AC, Baer MR, Donnellan WB, *et al*. KTE-X19 anti-CD19 CAR T-cell therapy in adult relapsed/refractory acute lymphoblastic leukemia: ZUMA-3 phase 1 results. Blood 2021;138:11–22. doi: 10.1182/blood.2020009098.
30. Shah BD, Ghobadi A, Oluwole OO, Logan AC, Boissel N, Cassaday RD, *et al*. KTE-X19 for relapsed or refractory adult B-cell acute lymphoblastic leukaemia: phase 2 results of the single-arm, open-label, multicentre ZUMA-3 study. Lancet 2021;398:491–502. doi: 10.1016/s0140-6736(21)01222-8.
31. Siddiqi T, Soumerai JD, Dorritie KA, Stephens DM, Riedell PA, Arnason J, *et al*. Phase 1 TRANSCEND CLL 004 study of lisocabtagene maraleucel in patients with relapsed/refractory CLL or SLL. Blood 2022;139:1794–1806. doi: 10.1182/blood. 2021011895.
32. Ying Z, Yang H, Guo Y, Li W, Zou D, Zhou D, *et al*. Relmacabtagene autoleucel (relma-cel) CD19 CAR-T therapy for adults with heavily pretreated relapsed/refractory large B-cell lymphoma in China. Cancer Med 2021;10:999–1011. doi: 10.1002/cam4.3686.
33. An N, Tao Z, Li S, Xing H, Tang K, Tian Z, *et al*. Construction of a new anti-CD19 chimeric antigen receptor and the anti-leukemia function study of the transduced T cells. Oncotarget 2016;7:10638–10649. doi: 10.18632/oncotarget.7079.
34. Gu R, Liu F, Zou D, Xu Y, Lu Y, Liu B, *et al*. Efficacy and safety of CD19 CAR T constructed with a new anti-CD19 chimeric antigen receptor in relapsed or refractory acute lymphoblastic leukemia. J Hematol Oncol 2020;13:122. doi: 10.1186/s13045-020-00953-8.
35. Si J, Shi X, Sun S, Zou B, Li Y, An D, *et al*. Hematopoietic progenitor kinase1 (HPK1) mediates T cell dysfunction and is a druggable target for T cell-based immunotherapies. Cancer Cell 2020;38:551–566.e11. doi: 10.1016/j.ccell.2020.08.001.
36. Di Stasi A, De Angelis B, Rooney CM, Zhang L, Mahendravada A, Foster AE, *et al*. T lymphocytes coexpressing CCR4 and a chimeric antigen receptor targeting CD30 have improved homing and antitumor activity in a Hodgkin tumor model. Blood 2009;113:6392–6402. doi: 10.1182/blood-2009-03-209650.
37. Pont MJ, Hill T, Cole GO, Abbott JJ, Kelliher J, Salter AI, *et al*. γ-Secretase inhibition increases efficacy of BCMA-specific chimeric antigen receptor T cells in multiple myeloma. Blood 2019;134:1585–1597. doi: 10.1182/blood.2019000050.
38. Raje N, Berdeja J, Lin Y, Siegel D, Jagannath S, Madduri D, *et al*. Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. N Engl J Med 2019;380:1726–1737. doi: 10.1056/NEJMoa1817226.
39. Shah N, Chari A, Scott E, Mezzi K, Usmani SZ. B-cell maturation antigen (BCMA) in multiple myeloma: rationale for targeting and current therapeutic approaches. Leukemia 2020;34:985–1005. doi: 10.1038/s41375-020-0734-z.
40. Munshi NC, Anderson LD Jr, Shah N, Madduri D, Berdeja J, Lonial S, *et al*. Idecabtagene vicleucel in relapsed and refractory multiple myeloma. N Engl J Med 2021;384:705–716. doi: 10.1056/NEJMoa2024850.
41. Zhao WH, Liu J, Wang BY, Chen YX, Cao XM, Yang Y, *et al*. A phase 1, open-label study of LCAR-B38M, a chimeric antigen receptor T cell therapy directed against B cell maturation antigen, in patients with relapsed or refractory multiple myeloma. J Hematol Oncol 2018;11:141. doi: 10.1186/s13045-018-0681-6.
42. Xu J, Chen LJ, Yang SS, Sun Y, Wu W, Liu YF, *et al*. Exploratory trial of a biepitopic CAR T-targeting B cell maturation antigen in relapsed/refractory multiple myeloma. Proc Natl Acad Sci U S A 2019;116:9543–9551. doi: 10.1073/pnas.1819745116.
43. Berdeja JG, Madduri D, Usmani SZ, Jakubowiak A, Agha M, Cohen AD, *et al*. Ciltacabtagene autoleucel, a B-cell maturation antigen-directed chimeric antigen receptor T-cell therapy in patients with relapsed or refractory multiple myeloma (CARTITUDE-1): a phase 1b/2 open-label study. Lancet 2021;398:314–324. doi: 10.1016/s0140-6736(21)00933-8.
44. Roex G, Timmers M, Wouters K, Campillo-Davo D, Flumens D, Schroyens W, *et al*. Safety and clinical efficacy of BCMA CAR-T-cell therapy in multiple myeloma. J Hematol Oncol 2020;13:164. doi: 10.1186/s13045-020-01001-1.

45. Gogishvili T, Danhof S, Prommersberger S, Rydzek J, Schreder M, Brede C, *et al*. SLAMF7-CAR T cells eliminate myeloma and confer selective fratricide of SLAMF7+ normal lymphocytes. Blood 2017;130:2838–2847. doi: 10.1182/blood-2017-04-778423.
46. Amatya C, Pegues MA, Lam N, Vanasse D, Geldres C, Choi S, *et al*. Development of CAR T cells expressing a suicide gene plus a chimeric antigen receptor targeting signaling lymphocytic-activation molecule F7. Mol Ther 2021;29:702–717. doi: 10.1016/j.ymthe.2020.10.008.
47. Smith EL, Harrington K, Staehr M, Masakayan R, Jones J, Long TJ, *et al*. GPRC5D is a target for the immunotherapy of multiple myeloma with rationally designed CAR T cells. Sci Transl Med 2019;11:eaau7746. doi: 10.1126/scitranslmed.aau7746.
48. Gomes-Silva D, Srinivasan M, Sharma S, Lee CM, Wagner DL, Davis TH, *et al*. CD7-edited T cells expressing a CD7-specific CAR for the therapy of T-cell malignancies. Blood 2017;130:285–296. doi: 10.1182/blood-2017-01-761320.
49. Pan J, Tan Y, Wang G, Deng B, Ling Z, Song W, *et al*. Donor-derived CD7 chimeric antigen receptor T cells for T-cell acute lymphoblastic leukemia: first-in-human, phase I trial. J Clin Oncol 2021;39:3340–3351. doi: 10.1200/jco.21.00389.
50. Cooper ML, Choi J, Staser K, Ritchey JK, Devenport JM, Eckardt K, *et al*. An "off-the-shelf" fratricide-resistant CAR-T for the treatment of T cell hematologic malignancies. Leukemia 2018;32:1970–1983. doi: 10.1038/s41375-018-0065-5.
51. Liu Y, Wang S, Schubert ML, Lauk A, Yao H, Blank MF, *et al*. CD33-directed immunotherapy with third-generation chimeric antigen receptor T cells and gemtuzumab ozogamicin in intact and CD33-edited acute myeloid leukemia and hematopoietic stem and progenitor cells. Int J Cancer 2022;150:1141–1155. doi: 10.1002/ijc.33865.
52. Tambaro FP, Singh H, Jones E, Rytting M, Mahadeo KM, Thompson P, *et al*. Autologous CD33-CAR-T cells for treatment of relapsed/refractory acute myelogenous leukemia. Leukemia 2021;35:3282–3286. doi: 10.1038/s41375-021-01232-2.
53. El Khawanky N, Hughes A, Yu W, Myburgh R, Matschulla T, Taromi S, *et al*. Demethylating therapy increases anti-CD123 CAR T cell cytotoxicity against acute myeloid leukemia. Nat Commun 2021;12:6436. doi: 10.1038/s41467-021-26683-0.
54. Wang Q, He F, He W, Huang Y, Zeng J, Zi F, *et al*. A transgene-encoded truncated human epidermal growth factor receptor for depletion of anti- B-cell maturation antigen CAR-T cells. Cell Immunol 2021;363:104342. doi: 10.1016/j.cellimm.2021.104342.
55. Yu S, Yi M, Qin S, Wu K. Next generation chimeric antigen receptor T cells: Safety strategies to overcome toxicity. Mol Cancer 2019;18:125. doi: 10.1186/s12943-019-1057-4.
56. Zhou X, Tu S, Wang C, Huang R, Deng L, Song C, *et al*. Phase I trial of fourth-generation anti-CD19 chimeric antigen receptor T cells against relapsed or refractory B cell non-Hodgkin lymphomas. Front Immunol 2020;11:564099. doi: 10.3389/fimmu.2020.564099.
57. Tahtinen S, Tong AJ, Himmels P, Oh J, Paler-Martinez A, Kim L, *et al*. IL-1 and IL-1ra are key regulators of the inflammatory response to RNA vaccines. Nat Immunol 2022;23:532–542. doi: 10.1038/s41590-022-01160-y.
58. Norelli M, Camisa B, Barbiera G, Falcone L, Purevdorj A, Genua M, *et al*. Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells. Nat Med 2018;24:739–748. doi: 10.1038/s41591-018-0036-4.
59. Gust J, Ponce R, Liles WC, Garden GA, Turtle CJ. Cytokines in CAR T cell-associated neurotoxicity. Front Immunol 2020;11:577027. doi: 10.3389/fimmu.2020.577027.
60. Sterner RM, Sakemura R, Cox MJ, Yang N, Khadka RH, Forsman CL, *et al*. GM-CSF inhibition reduces cytokine release syndrome and neuroinflammation but enhances CAR-T cell function in xenografts. Blood 2019;133:697–709. doi: 10.1182/blood-2018-10-881722.
61. Cox MJ, Manriquez Roman C, Tapper EE, Siegler EL, Chappell D, Durrant C, *et al*. GM-CSF disruption in CART cells modulates T cell activation and enhances CART cell anti-tumor activity. Leukemia 2022;36:1635–1645. doi: 10.1038/s41375-022-01572-7.
62. Ghorashian S, Kramer AM, Onuoha S, Wright G, Bartram J, Richardson R, *et al*. Enhanced CAR T cell expansion and prolonged persistence in pediatric patients with ALL treated with a low-affinity CD19 CAR. Nat Med 2019;25:1408–1414. doi: 10.1038/s41591-019-0549-5.
63. Mardiana S, Gill S. CAR T cells for acute myeloid leukemia: state of the art and future directions. Front Oncol 2020;10:697. doi: 10.3389/fonc.2020.00697.
64. Han X, Wang Y, Wei J, Han W. Multi-antigen-targeted chimeric antigen receptor T cells for cancer therapy. J Hematol Oncol 2019;12:128. doi: 10.1186/s13045-019-0813-7.
65. Guo Z, Tu S, Yu S, Wu L, Pan W, Chang N, *et al*. Preclinical and clinical advances in dual-target chimeric antigen receptor therapy for hematological malignancies. Cancer Sci 2021;112:1357–1368. doi: 10.1111/cas.14799.
66. Schneider D, Xiong Y, Wu D, Nölle V, Schmitz S, Haso W, *et al*. A tandem CD19/CD20 CAR lentiviral vector drives on-target and off-target antigen modulation in leukemia cell lines. J Immunother Cancer 2017;5:42. doi: 10.1186/s40425-017-0246-1.
67. Hegde M, Mukherjee M, Grada Z, Pignata A, Landi D, Navai SA, *et al*. Tandem CAR T cells targeting HER2 and IL13Rα2 mitigate tumor antigen escape. J Clin Invest 2016;126:3036–3052. doi: 10.1172/jci83416.
68. Yang M, Tang X, Zhang Z, Gu L, Wei H, Zhao S, *et al*. Tandem CAR-T cells targeting CD70 and B7-H3 exhibit potent preclinical activity against multiple solid tumors. Theranostics 2020;10:7622–7634. doi: 10.7150/thno.43991.
69. Tong C, Zhang Y, Liu Y, Ji X, Zhang W, Guo Y, *et al*. Optimized tandem CD19/CD20 CAR-engineered T cells in refractory/relapsed B-cell lymphoma. Blood 2020;136:1632–1644. doi: 10.1182/blood.2020005278.
70. Spiegel JY, Patel S, Muffly L, Hossain NM, Oak J, Baird JH, *et al*. CAR T cells with dual targeting of CD19 and CD22 in adult patients with recurrent or refractory B cell malignancies: a phase 1 trial. Nat Med 2021;27:1419–1431. doi: 10.1038/s41591-021-01436-0.
71. Zhang Y, Wang Y, Liu Y, Tong C, Wang C, Guo Y, *et al*. Long-term activity of tandem CD19/CD20 CAR therapy in refractory/relapsed B-cell lymphoma: a single-arm, phase 1-2 trial. Leukemia 2022;36:189–196. doi: 10.1038/s41375-021-01345-8.
72. Ruella M, Barrett DM, Kenderian SS, Shestova O, Hofmann TJ, Perazzelli J, *et al*. Dual CD19 and CD123 targeting prevents antigen-loss relapses after CD19-directed immunotherapies. J Clin Invest 2016;126:3814–3826. doi: 10.1172/jci87366.
73. Yan Z, Cao J, Cheng H, Qiao J, Zhang H, Wang Y, *et al*. A combination of humanised anti-CD19 and anti-BCMA CAR T cells in patients with relapsed or refractory multiple myeloma: a single-arm, phase 2 trial. Lancet Haematol 2019;6:e521–e529. doi: 10.1016/s2352-3026(19)30115-2.
74. Yan L, Qu S, Shang J, Shi X, Kang L, Xu N, *et al*. Sequential CD19 and BCMA-specific CAR T-cell treatment elicits sustained remission of relapsed and/or refractory myeloma. Cancer Med 2021;10:563–574. doi: 10.1002/cam4.3624.
75. Mei H, Li C, Jiang H, Zhao X, Huang Z, Jin D, *et al*. A bispecific CAR-T cell therapy targeting BCMA and CD38 in relapsed or refractory multiple myeloma. J Hematol Oncol 2021;14:161. doi: 10.1186/s13045-021-01170-7.
76. Lee L, Draper B, Chaplin N, Philip B, Chin M, Galas-Filipowicz D, *et al*. An APRIL-based chimeric antigen receptor for dual targeting of BCMA and TACI in multiple myeloma. Blood 2018;131:746–758. doi: 10.1182/blood-2017-05-781351.
77. Liu X, Ranganathan R, Jiang S, Fang C, Sun J, Kim S, *et al*. A chimeric switch-receptor targeting PD1 augments the efficacy of second-generation CAR T cells in advanced solid tumors. Cancer Res 2016;76:1578–1590. doi: 10.1158/0008-5472.Can-15-2524.
78. Rafiq S, Yeku OO, Jackson HJ, Purdon TJ, van Leeuwen DG, Drakes DJ, *et al*. Targeted delivery of a PD-1-blocking scFv by CAR-T cells enhances anti-tumor efficacy in vivo. Nat Biotechnol 2018;36:847–856. doi: 10.1038/nbt.4195.
79. Cherkassky L, Morello A, Villena-Vargas J, Feng Y, Dimitrov DS, Jones DR, *et al*. Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. J Clin Invest 2016;126:3130–3144. doi: 10.1172/jci83092.
80. Wang Z, Li N, Feng K, Chen M, Zhang Y, Liu Y, *et al*. Phase I study of CAR-T cells with PD-1 and TCR disruption in mesothelin-positive solid tumors. Cell Mol Immunol 2021;18:2188–2198. doi: 10.1038/s41423-021-00749-x.
81. Liu H, Lei W, Zhang C, Yang C, Wei J, Guo Q, *et al*. CD19-specific CAR T cells that express a PD-1/CD28 chimeric switch-receptor are effective in patients with PD-L1-positive B-cell lymphoma. Clin Cancer Res 2021;27:473–484. doi: 10.1158/1078-0432.Ccr-20-1457.

82. Adusumilli PS, Zauderer MG, Rivière I, Solomon SB, Rusch VW, O'Cearbhaill RE, *et al*. A phase I trial of regional mesothelin-targeted CAR T-cell therapy in patients with malignant pleural disease, in combination with the anti-PD-1 agent pembrolizumab. Cancer Discov 2021;11:2748–2763. doi: 10.1158/2159-8290.Cd-21-0407.
83. Kumar J, Kumar R, Kumar Singh A, Tsakem EL, Kathania M, Riese MJ, *et al*. Deletion of Cbl-b inhibits CD8+ T-cell exhaustion and promotes CAR T-cell function. J Immunother Cancer 2021;9: e001688. doi: 10.1136/jitc-2020-001688.
84. Chmielewski M, Abken H. CAR T cells releasing IL-18 convert to T-Bet high FoxO1low effectors that exhibit augmented activity against advanced solid tumors. Cell Rep 2017;21:3205–3219. doi: 10.1016/j.celrep.2017.11.063.
85. Pang N, Shi J, Qin L, Chen A, Tang Y, Yang H, *et al*. IL-7 and CCL19-secreting CAR-T cell therapy for tumors with positive glypican-3 or mesothelin. J Hematol Oncol 2021;14:118. doi: 10.1186/s13045-021-01128-9.
86. Adachi K, Kano Y, Nagai T, Okuyama N, Sakoda Y, Tamada K. IL-7 and CCL19 expression in CAR-T cells improves immune cell infiltration and CAR-T cell survival in the tumor. Nat Biotechnol 2018;36:346–351. doi: 10.1038/nbt.4086.
87. Zhao Z, Li Y, Liu W, Li X. Engineered IL-7 receptor enhances the therapeutic effect of AXL-CAR-T cells on triple-negative breast cancer. Biomed Res Int 2020;2020:4795171. doi: 10.1155/2020/4795171.
88. Yan Y, Zhao W, Liu W, Li Y, Wang X, Xun J, *et al*. CCL19 enhances CD8+ T-cell responses and accelerates HBV clearance. J Gastroenterol 2021;56:769–785. doi: 10.1007/s00535-021-01799-8.
89. Duan D, Wang K, Wei C, Feng D, Liu Y, He Q, *et al*. The BCMA-targeted fourth-generation CAR-T cells secreting IL-7 and CCL19 for therapy of refractory/recurrent multiple myeloma. Front Immunol 2021;12:609421. doi: 10.3389/fimmu.2021.609421.
90. Hurton LV, Singh H, Najjar AM, Switzer KC, Mi T, Maiti S, *et al*. Tethered IL-15 augments antitumor activity and promotes a stem-cell memory subset in tumor-specific T cells. Proc Natl Acad Sci U S A 2016;113:E7788–E7797. doi: 10.1073/pnas.1610544113.
91. Wagner DL, Fritsche E, Pulsipher MA, Ahmed N, Hamieh M, Hegde M, *et al*. Immunogenicity of CAR T cells in cancer therapy. Nat Rev Clin Oncol 2021;18:379–393. doi: 10.1038/s41571-021-00476-2.
92. Wang S, Wang X, Ye C, Cheng H, Shi M, Chen W, *et al*. Humanized CD19-targeted chimeric antigen receptor T (CAR-T) cells for relapsed/refractory pediatric acute lymphoblastic leukemia. Am J Hematol 2021;96:E162–E165. doi: 10.1002/ajh.26123.
93. Wang D, Wang J, Hu G, Wang W, Xiao Y, Cai H, *et al*. A phase 1 study of a novel fully human BCMA-targeting CAR (CT103A) in patients with relapsed/refractory multiple myeloma. Blood 2021;137:2890–2901. doi: 10.1182/blood.2020008936.
94. Jiang H, Li C, Yin P, Guo T, Liu L, Xia L, *et al*. Anti-CD19 chimeric antigen receptor-modified T-cell therapy bridging to allogeneic hematopoietic stem cell transplantation for relapsed/refractory B-cell acute lymphoblastic leukemia: an open-label pragmatic clinical trial. Am J Hematol 2019;94:1113–1122. doi: 10.1002/ajh.25582.
95. Hu Y, Zhou Y, Zhang M, Ge W, Li Y, Yang L, *et al*. CRISPR/Cas9-engineered universal CD19/CD22 dual-targeted CAR-T cell therapy for relapsed/refractory B-cell acute lymphoblastic leukemia. Clin Cancer Res 2021;27:2764–2772. doi: 10.1158/1078-0432.Ccr-20-3863.
96. Jackson Z, Roe A, Sharma AA, Lopes FBTP, Talla A, Kleinsorge-Block S, *et al*. Automated manufacture of autologous CD19 CAR-T cells for treatment of non-Hodgkin lymphoma. Front Immunol 2020;11:1941. doi: 10.3389/fimmu.2020.01941.
97. Joedicke JJ, Großkinsky U, Gerlach K, Künkele A, Höpken UE, Rehm A. Accelerating clinical-scale production of BCMA CAR T cells with defined maturation stages. Mol Ther Methods Clin Dev 2022;24:181–198. doi: 10.1016/j.omtm.2021.12.005.

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