

Cluster of differentiation 19 chimeric antigen receptor T-cell therapy in pediatric acute lymphoblastic leukemia (Review)

ZEXIN FU^{1,2}, JINLEI ZHOU^{1,2}, RUI CHEN^{1,2}, YIHUA JIN^{1,2}, TING NI^{1,2}, LINGBO QIAN¹ and CHI XIAO¹

¹School of Basic Medical Sciences and Forensic Medicine and ²Department of Clinical Medicine, Hangzhou Medical College, Hangzhou, Zhejiang 310053, P.R. China

Received January 31, 2020; Accepted June 19, 2020

DOI: 10.3892/ol.2020.11897

Abstract. Chimeric antigen receptor (CAR) T cells have an unprecedented positive curative effect for hematological malignancies. Most notably, cluster of differentiation 19 (CD19) CAR T-cell therapy for pediatric acute lymphoblastic leukemia is associated with a high complete remission rate and has aroused considerable attention in the medical field. However, it also causes a series of adverse reactions and increases the risk of recurrence. The present review examines the results of CD19 CAR T-cell therapy and lists its adverse effects. In addition, some of the mechanisms of recurrence are characterized and applicable strategies to address this challenging problem are proposed.

Contents

1. Introduction
2. CAR T cells
3. Therapeutic effect of CD19-CAR T cells
4. Treatment-related complications
5. Recurrence after CAR T-cell therapy
6. Strategies to prevent recurrence after CAR T-cell therapy
7. Conclusions and perspectives

Correspondence to: Dr Chi Xiao or Professor Lingbo Qian, School of Basic Medical Sciences and Forensic Medicine, Hangzhou Medical College, 481 Binwen Road, Hangzhou, Zhejiang 310053, P.R. China

E-mail: xcier@163.com

E-mail: bioqian@163.com

Abbreviations: ALL, acute lymphoblastic leukemia; CAR T, chimeric antigen receptor T; CD19, cluster of differentiation 19; CRES, CAR T cell-related encephalopathy syndrome; CRS, cytokine-release syndrome; HLH, hemophagocytic lymphohistiocytosis; HSCT, hematopoietic stem cell transplantation; MAS, macrophage-activation syndrome; RR-ALL, relapsed or refractory ALL; TAA, tumor-associated antigen

Key words: ALL, adverse effects, CAR T, CD19, pediatric ALL

1. Introduction

Between 2003 and 2007, in 21 countries representative of four regions of the globe, the incidence rate of acute lymphoblastic leukemia (ALL) ranged from 2.17-3.78/100,000 individuals per year, in children younger than 19 years (1). ALL is the most common form of childhood cancer, with a peak in incidence between 2 and 5 years of age (2,3). The revision of and improvements in drug combinations and the intensity of radiotherapy has improved the outcome of ALL. The 5-year survival rate of children with ALL in most developed countries is 80-85% (4). However, long-term antitumor treatment has a particularly large impact on children and there is still a high possibility of recurrence. In total, 10-15% of pediatric patients will experience a relapse despite the successful induction of chemotherapy (5). Recurrence has been one of the key factors affecting the further enhancement of event-free survival rates and overall survival rates of children with ALL. However, adoptive immunotherapy using genetic engineering to produce chimeric antigen receptor (CAR) T cells has shown unique advantages for the treatment of relapsed or refractory ALL (RR-ALL). Treatment with CAR T-cells can alleviate refractory and secondary relapse of ALL to a certain extent, even in patients who fail to respond to bone marrow stem cell transplants (6,7).

CAR T cells are modified normal T cells that are not bound by the major histocompatibility complex and have the ability to recognize tumor-associated antigens (TAAs) and kill tumor cells (8). CAR T cells are superior to normal antibody-based drugs as they are comprised of autogenous or allogeneic living cells that can proliferate and develop in the body of a patient and remain stable for a considerable period of time. CAR T cells with cluster of differentiation (CD)19 (9,10), CD20 and CD22 (11,12) antigen-binding domains have been tested in humans; since CD19 is an essential biomarker of B cell lineage, CD19-CAR T cells are predominantly used to treat B-cell malignancies, including chronic lymphocytic leukemia, B-cell non-Hodgkin's lymphoma and B-ALL (7,8). In 2017, tisagenlecleucel, which is mainly used to treat patients <25 years of age with B-cell precursor ALL that is refractory or in secondary or later relapse stages, became the first CAR T-cell therapy approved by the US Food and Drug Administration (FDA) (13). In China, certain clinical trials being performed for RR-ALL also include this cellular

immunotherapy (14-19). Although CAR T-cell therapy has been recognized by major cancer medical centers, it still has serious side effects and toxicity, which can rapidly lead to fatal cytokine-release syndrome (CRS) (20,21) and neurotoxicity [CAR T cell-related encephalopathy syndrome (CRES)] (22). Long-term side effects include a risk of chronic B-cell deficiency (14). Hence, in the present review, the research progress and improvements in CAR T-cell treatment for ALL in children are assessed, and the relapse-related experiences and future prospects are analyzed.

2. CAR T cells

The main structure of the CAR protein comprises an intracellular signaling domain, a transmembrane domain, and an extracellular antigen-binding domain, usually derived from the single-chain variable fragment (scFv) targeted to human TAAs such as CD19 (23,24). The intracellular signal domain is dominated by the T cell receptor CD3- ζ , as well as one (second-generation CAR) or two (third-generation CAR) connected co-stimulatory molecules, or alternatively, no co-stimulatory molecules (first-generation CAR) (Fig. 1). Presently, second-generation CAR T cells are widely used in clinical practice, and the stability and killing effect of these cells are much greater than those of first-generation agents (25). However, current clinical results show that the third-generation agents possess no benefits above second-generation drugs (3,26). Some scholars have developed the concept of T cells redirected for universal cytokine-mediated killing (TRUCKs), fourth-generation CARs (27). TRUCKs mediate CAR T-cell delivery of transgenic products [such as interleukin (IL)-12] to targeted tumor tissue to modulate the T cell responses (Fig. 2). Fifth-generation CAR T cell products incorporate a novel co-stimulatory domain to activate other signaling pathways, such as the IL-2-2R β intracellular binding domain of signal transducer and activator of transcription 3/5 (25).

The selection of the target antigen is a key determinant of the specificity and effectiveness of the CAR and the safety of the genetically modified T cells. CD19 is specifically expressed by B-lymphocyte lineage cells at different stages of differentiation, and >95% of B-lymphoblastic leukemia cases express the CD19 antigen (28). To date, the CD19 antigen is the most widely used CD for CAR T-cell production. CAR T cells designed using genetic engineering have been amplified *in vitro* and then transferred back into the patient without graft-versus-host disease. The CAR protein gives T cells the ability to recognize tumor antigens in a human leukocyte antigen-independent manner (29). Therefore, cytotoxic T cells can be activated in a short time and cytokines can be released to kill malignant cells.

3. Therapeutic effect of CD19-CAR T cells

Recently, CAR T cells that recognize and eliminate specific cancer cells have increased the recognition of their therapeutic usefulness, especially for hematological tumors. Table I summarizes some clinical trials (14,30-33) in which the rate of complete remission was unexpected positive for patients with ALL or RR-ALL. CAR T-cell therapy is a good strategy to completely alleviate ALL and may be a novel strategy for

RR-ALL. Previously, the available options were to increase the chemotherapy dose or change to different chemotherapy agents and regimens, which could put patients into remission; however, this was associated with a high recurrence rate (34,35), even with allogeneic hematopoietic stem cell transplantation (alloHSCT), which is also limited by the availability of suitable matched donors and potential immunologic complications (36). Therefore, CAR T-cell therapy appears to be an extremely effective adoptive therapy that serves as a feasible, safe and efficacious approach to treat ALL, and particularly RR-ALL.

Furthermore, cytokines mediated by CAR T cells also play a prominent role in activating their antitumor effects; however, excessive cytokine levels can be life threatening (37). Patients receiving treatment should undergo continuous vital sign monitoring, as well as assessment of cytokine, tumor necrosis factor (TNF)- α , IL-6 and C-reactive protein (CRP) levels (6). However, TNF- α and IL-6 are not typically monitored in the normal clinical setting. CRS should be highly suspected when the following signs occur (38), especially in the CRS-risk period after CAR T-cell infusion: i) Fever $\geq 38^{\circ}\text{C}$; ii) hypotension ≤ 90 mmHg for patients >10 years old; iii) hypoxia with an arterial oxygen saturation of <90% when breathing room air; and iv) evidence of organ toxicity by the most recent Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0) grading system (38). A clearer understanding of CRS has led to the publication of more detailed and standardized classifications and treatment management systems (21,38).

Although these experimental data are encouraging, there are obviously significant differences in pretreatment schemes, cellular injection doses (using various products) and even receptor composition among various medical centers. It is thus important to reasonably summarize multi-center clinical trials and evaluate the most comprehensive treatment scheme statistically. The latest management guidelines of 2018, established by the MD Anderson Cancer Center and the HSCT Subgroup of the Pediatric Acute Lung Injury and Sepsis Investigators Network, comprehensively recommend that pediatric patients with B-cell precursor leukemias that are treatment refractory or in secondary or later relapse stages should receive CAR T-cell treatment, including nursing management, patient evaluation, examination before cell collection, attention to critical procedures when injecting cells and rescue measures, CRS monitoring and monitoring of CRES (38). The guidelines also explicitly state that CAR T-cell therapy is a bridge to HSCT as an interim therapy rather than a cure for ALL; therefore, the expectations might still be limited. Improvement in novel CAR T-cell therapy with enhanced efficacy and safety can potentially make it a mainstream cancer therapeutic.

4. Treatment-related complications

CAR T-cell therapy is associated with a series of side effects. Complications that require special attention in clinical practice include CRS, CRES, B-cell aplasia and palindromia. These adverse effects are some of the obstacles restricting the clinical application of CAR T-cell therapy and affecting its curative effect (39). For example, while we use glucocorticoids with inhibitory effect to inflammatory responses to reverse CRS symptoms rapidly, glucocorticoids that are lymphocytotoxic

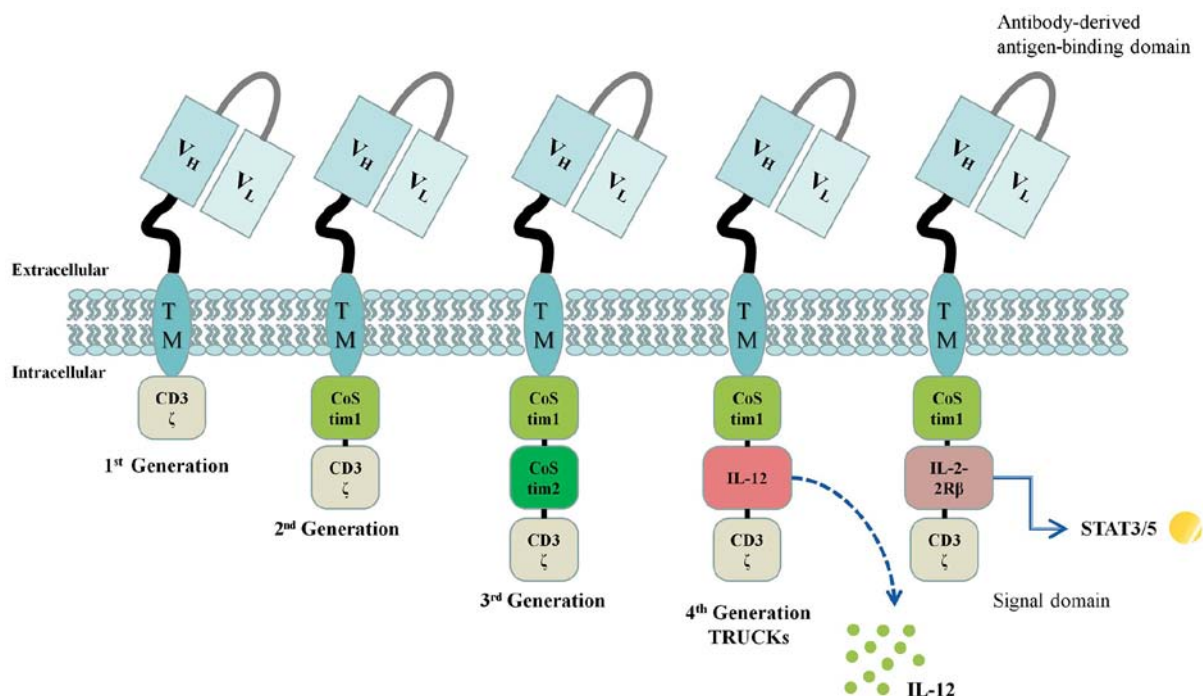


Figure 1. Five generations of CAR T-cell structure. CARs of basic design can be divided into three regions as follows: i) Antibody-derived antigen-binding domain for antigen recognition binding; it usually contains a single-stranded variable fragment derived from an antibody; ii) transmembrane domain for anchor support to the plasma membrane; and iii) signaling domain for T-cell activation. The signaling domain of first-generation CARs only has a CD3 ζ -derived signaling module. Second-generation CARs also contain a co-stimulatory domain. Third-generation CARs contain two co-stimulatory domains. Co-stimulatory molecules include CD28, 4-1BB (CD137), CD27 and OX40 (CD134). Fourth-generation CAR T cells are also referred to as TRUCKs, which inducibly express chemokines such as IL-12. Fifth-generation CARs consist of a novel co-stimulatory domain to activate some specific signaling pathways. CAR T, chimeric antigen receptor T; TM, transmembrane domain; VL + VH, antibody-derived antigen-binding domain; TRUCK, T cell redirected for universal cytokine-mediated killing; CoStim, co-stimulatory domain; IL, interleukin; CD, cluster of differentiation; IL-2R β , IL-2 receptor β ; STAT3/5, signal transducer and activator of transcription 3/5.

will dampen expansion of the CAR T-cells *in vivo* and will affect therapeutic outcomes (40).

CRS. CRS occurs when cytokines are suddenly released in large quantities, leading to systemic inflammatory responses, including a high fever, increased levels of acute-phase proteins, and visceral and vascular endothelial damage, and eventually death from respiratory distress and heart failure (40,41). As shown in Table I, numerous young adult and pediatric patients develop CRS after treatment with CD19-CAR T cells. Maude *et al* (31) conducted a global study on a cohort of tisagenlecleucel-treated pediatric and young adult patients with CD19⁺ B-cell RR-ALL. It was found that 77% of the patients in >25 medical centers involved in the trial developed CRS after tisagenlecleucel infusion, and almost half of these cases were life threatening, requiring intensive care (grades 3-4 CRS) (38,42). Glucocorticoids that affect the proliferation and function of CAR T cells or anti-IL-6 therapy (e.g., tocilizumab; brand name, ACTEMRA; Genentech Inc.; Roche Diagnostics) can relieve CRS symptoms (21). More than half of patients with severe or life-threatening CRS exhibit resolution within 2 weeks of one or two doses of tocilizumab. However, it has been demonstrated that patients with severe CRS are prone to early recurrence owing to the application of glucocorticoids (40). Therefore, in such patients, premature interventions after CAR T cells' therapy may reduce the endurance/efficacy of T cells and decrease its therapeutic potential. Ultimately, the administration of timely and effective

treatments to patients with severe CRS should be based on the rational/objective assessment of their clinical symptoms (such as high fever), and the timely monitoring of their biochemical indicators (such as CRP) and cytokine responses.

CRES. The serious neurotoxic symptoms associated with CAR T-cell therapy, known as CRES, usually present as headaches, emesis, tremors, delirium and seizures or cerebral edema (21,43). CRES is often associated with CRS or occurs after the fever and other CRS symptoms subside (42). After CRS improves, neurotoxic encephalopathy can also improve. Although there is no exact pathophysiological explanation, evidence shows that this phenomenon is related to increased cytokines in the cerebrospinal fluid (21,44). Hu *et al* (43) first reported the case of a patient with CRS and neurotoxic symptoms (CRES) who improved after the reduction of intracranial pressure and glucocorticoid treatment, suggesting that the CRS-induced release of cytokines with a resultant overload might be one of main causes of neurotoxicity. Moreover, the use of anti-IL-6 therapy seems to be more effective for CRES that occurs concurrently with CRS (42). Notably, soon after CRES onset, adult patients often have diminished attention, stuttering or writing dysfunction (42). These signs may help us identify CRES patients as early as possible; therefore, the CARTOX 10-point neurological assessment tool or the Immune Effector Cell-Associated Encephalopathy (ICE) scoring system should be used, to evaluate potential acute neurological deficits due to CAR-T cell therapy in these adult

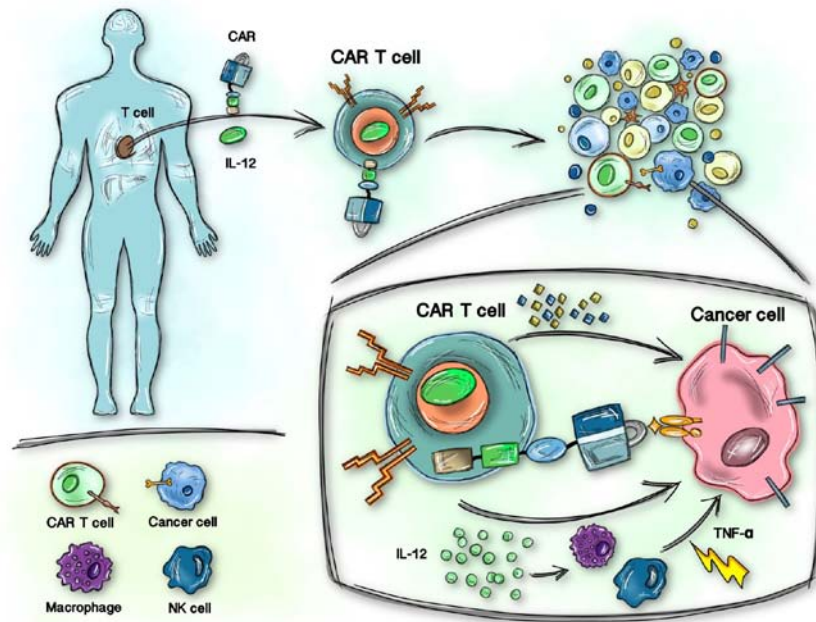


Figure 2. Fourth-generation CAR T cells. T cells redirected for universal cytokine-mediated killing not only have a basic CAR T-cell structure, but also a constitutive expression cassette for transgenic IL-12 or cytokines. Such CAR T cells have a dual anticancer effect against malignant cells aided by inducible IL-12. IL-12 promotes CAR T-cell activity in an autocrine manner and activates intrinsic immune cells, such as NK cells and macrophages, to lyse those antigen-negative cancer cells. CAR T, chimeric antigen receptor T; IL, interleukin; NK, natural killer; TNF- α , tumor necrosis factor α .

patients (42,45-47). However, symptoms in pediatric patients are subtle and completely different from those in adults, and the symptoms of early CRES are difficult to detect and diagnose in a timely manner in this population. The Cornell Assessment of Pediatric Delirium (CAPD) is an indispensable screening tool for the recognition of early CRES among young children and juveniles (<21 years of age, especially for patients less than 12 years old, the sensitivity and specificity are relatively high), as a CAPD score >8 represents delirium. Furthermore, CAPD can also be used as a marker of CRES severity. Grade 2 CNS toxicities, including somnolence, confusion, encephalopathy, dysphasia, seizure (brief generalized seizure), and/or tremor, neurological assessment scores of 3-6 and a CAPD score <9 is indicative of grade 2 CRES. Conversely, a CAPD score >9 indicates grade 3-4 CRES. If there is a risk of neurological symptoms, patients should be closely monitored for signs and symptoms of cerebral edema, for example, by specialized screening for papilloedema (38,47-49).

B-cell aplasia. scFv-based CARs only attack target specific antigens (such as CD19), which can be expressed by both tumor and normal cells, but in different amounts. Thus, on-target/on-tumor activity leads to a therapeutic effect, whereas an on-target/off-tumor effect induces a toxic reaction (50). During long-term follow-up of patients receiving CAR T-cell therapy, one common adverse reaction is that CAR T cells attack normal B cells leading to B-cell lysis with a reduced immune response (38,51). In a clinical trial (52) using CAR T-cell therapy to treat refractory relapsed chronic lymphocytic leukemia, it was reported that all patients achieving complete remission developed B-cell aplasia, which lasted for up to 4 years in some patients due to the persistence

of CAR T cells in their bodies. Maude *et al* (6) observed that of 25 children with relapsed ALL, each had B cell aplasia, but none had serious infections due to B-cell aplasia. It is worth noting that by monitoring patients for the development of B-cell aplasia, the function of CAR T-cell therapy can be measured. Such patients are managed with intravenous immunoglobulin repletion, which is only a temporary alternative treatment, until the B cells are rejuvenated. Selecting appropriate tumor-specific antigens and gradually increasing the injection dose could reduce the risk of missing the target (6,53,54). It has also been reported that the introduction of suicide genes (55) and multi-antigen chimeric receptors (56) is an effective strategy. The most well-known approach is the fusion of the pro-apoptotic protein caspase-9 with the iCasp9 domain. When dimers of small molecules such as AP 1903 are introduced, the FKBP12 domain of iCasp9 becomes dimerized, resulting in the rapid apoptotic death of T cells (57).

Other complications. Acute tumor lysis syndrome is caused by the massive degradation of tumor cells with a rapid release of intracellular substances (such as potassium ions, phosphorus and nucleic acids). When the level of these intracellular substances exceeds the threshold of liver and kidney metabolic capacity, abnormal metabolism and electrolyte disorders of the body occur, leading to life-threatening severe arrhythmia and renal failure (58). Allopurinol has been the main treatment for tumor lysis syndrome, and rasburicase is administered to manage serum uric acid levels (55). Moreover, hemophagocytic lymphohistiocytosis (HLH) is a serious and potentially fatal complication caused by the excessive secretion of inflammatory factors through the non-malignant proliferation of lymphocytes and histiocytes (59). Secondary

Table I. Summary of reported therapy in trials of CAR T cells for children with ALL.

First author, year	Patients	CAR T cells/kg	Therapeutic effect	CRS and CRES	Reported relapse	(Refs.)
Lee <i>et al</i> , 2015	21 children and young adults (aged 1-30 years), CD19 ⁺ B-ALL (n=17) or NHL (n=4)	0.028-3x10 ⁶ cells	CR: 71% (12/17)	CRS (16/21; 76%), neurotoxicity (6/21; 29%)	Unknown	(30)
Maude <i>et al</i> , 2018	75 children and young adults (aged 3-23 years) CD19 ⁺ ALL	0.2-5.4x10 ⁶ cells	CR: 81% (61/75)	CRS (58/75; 77%), neurological event (30/75; 40%)	36% (22/61) relapsed (15 CD19- recurrence)	(31)
Gardner <i>et al</i> , 2017	45 children and young adults (aged 1.3-25.4 years), RR-CD19 ⁺ ALL	0.5/1.5/10x10 ⁶ cells	CR: 93% (40/43)	45% (18/40) relapse	(30)	
Zuo <i>et al</i> , 2019	48 children (aged 3-17 years), RR-CD19 ⁺ ALL	(0.61-10.94)x10 ⁶ cells	CR: 77% (37/48)	CRS (34/48; 71%), CRES (10/48; 21%)	68% (25/37) relapse	(33)
Ma <i>et al</i> , 2019	10 pediatric patients with CD19 + RR B-ALL	(0.3-1.58)x10 ⁶ cells	CR: 80% (8/10)	CRS (4/10; 40%), CRES (3/10; 30%)	50% (4/8) relapse (3 CD19- recurrence)	(14)

CAR T, chimeric antigen receptor T; CRS, cytokine-release syndrome; CRES, CAR T cell-related encephalopathy syndrome; NHL, non-Hodgkin lymphoma; CR, complete remission; RR, relapsed or refractory; CD, cluster of differentiation; ALL, acute lymphoblastic leukemia.

HLH is often referred to as macrophage-activation syndrome (MAS) (60). Given the overlapping symptoms of the inflammatory state, the clinical diagnosis of CAR T cell-related HLH (or MAS) and CRS-CRES is a great challenge (21,42). The recommended diagnostic criteria for this rare situation have, however, been provided in the management guidelines for pediatric patients receiving CAR T-cell therapy, as described by Mahadeo *et al* (38).

5. Recurrence after CAR T-cell therapy

Although the remission rate of ALL in children is increasing gradually due to improved treatment strategies, the recurrence rate is still extremely high. Factors such as the short maintenance time of CAR T cells, insufficient copy number and antigen escape contribute to the recurrence of ALL after CAR T-cell therapy (61-63).

The detection or absence of the B-cell marker CD19 can be used to categorize recurrence as CD19-positive and CD19-negative leukemia, with the latter being more challenging to address. Antigen deletion is a notable phenomenon wherein tumor cells mutate the antigen gene to inhibit its expression (64) or induce defects in transcription factors involved in its synthesis to avoid death. Ruella *et al* (65) encountered a case in which CAR, originally modified T cells, were bound to leukemia cells and proliferated in the body, eventually causing a CD19-negative relapse. CAR genes reversely bind to leukemia cells covering CD19 epitopes when CAR T cells are produced, allowing leukemia cells to mask CD19 proteins and develop CAR T-cell resistance to evade cellular immunity (65). There is also the perspective that patients with RR-ALL express CD19-positive and -negative malignant cells. When CD19-CAR T cells kill malignant cells with the CD19 antigen, a few previously existing CD19-negative cells begin to take advantage of the cloning (9,66). Furthermore, CD19-negative relapse is resistant to CD19-CAR T-cell therapy, which can only be discontinued or switched to other clinical protocols (67,68).

Moreover, some patients relapse without antigen deletion. Monitoring the quantity of CAR T cells in patients reveals no detectable CAR T cells in the peripheral blood before relapse, which may be related to the insufficient copy number of CAR T cells and their inability to persist in the body for a long time (66,69). Thus, the attending physician tends to administer a second injection of CAR T cells or an increased dose of the cells to prevent recurrence. However, the actual effect of this is minimal and could even lead to a poor prognosis (70). Hamieh *et al* (71) found that the CD19 antigen is transferred to CAR T-cells from malignant cells by trogocytosis, which not only leads to escape of the CD19 antigen, but also causes CAR T cells to kill each other and accelerates the depletion of T cells. To weaken mutual killing, CAR T cells gradually express and activate T cell-inhibiting molecules such as programmed cell death protein-1, lymphocyte activation gene-3, and T-cell immunoglobulin and mucin domain 3, leading to the immune escape of tumors (71-73). Similar experiments performed on CD22-CAR T cells, co-cultured with various cell lines such as SUP-B15 and Raji, and primary tumor cells from patients, also showed similar results that is CAR-induced trogocytosis was observed (71,74,75). This indicates that trogocytosis leads to

tumor antigen deletion and metastasis via a universal mechanism that causes tumor surface antigen density to decrease after CAR T-cell therapy.

Even considering the advances in the field, such as the use of intensive chemotherapy, new targeted drugs and allogeneic hematopoietic stem cell transplantation, the remission rate of R/R ALL is still not negligible. Although CAR T-cell treatments lead to relatively controllable adverse events in R/R ALL patients, these patients still have a high risk of relapse (6,7). In such patients, CAR T-cell therapy could be a bridge for HSCT, providing temporary molecular remission for transplantation and improving the prognosis (76).

6. Strategies to prevent recurrence after CAR T-cell therapy

Dual antigen-expression construct. To prevent CD19-negative relapse, designing and using CAR T cells with bispecific antigen targets is a good strategy. Zah *et al* (56) designed a CD19/CD20 bispecific CAR capable of OR-gate signal processing, which triggers robust T-cell responses as long as the target cells express either CD19 or CD20. The optimized tandem-CARs control CD19-negative mutants, improving the killing ability of B-lineage malignant cells and reducing the recurrence rate. An increased disease burden is more likely to cause severe CRS and treatment has to be terminated, leading to recurrence. It has been reported that this tandem CAR produces fewer cytokines than CD20-CAR, but is similar to that with CD19-CARs (56). Schneider *et al* (77) opined that tandem CARs are equally effective in standard disease models against single antigen-specific CARs, and might be more effective and less toxic with a high disease burden. This could be attributable to the optimized cell killing with more moderate cytokine production. Furthermore, a dual CAR-expressing construct that combined CD19- and CD123-mediated CAR T cells demonstrated superior activity against B-ALL compared to that with single-expressing CAR T cells *in vivo* (78).

Co-stimulatory molecules. An insufficient copy number of CD19-CAR T cells would greatly curb the duration of action and thereby affect the therapeutic effects. Modification of the co-stimulatory molecule in the CAR signal domain structure could promote the expansion of CAR T cells and prolong their duration *in vivo* (68). The second- and third-generation CAR T cells are designed to contain two different co-stimulatory domain receptors; one is the CAR that provides the T-cell activation signal and the other is the chimeric co-stimulatory receptor. CD28 or 4-1BB (CD137) domain usually provide a costimulatory signal (30,40).

Lymphodepletion chemotherapy. CRS is known to severely affect CAR T cells with respect to the elimination of malignant cells. Ruella *et al* (79) found that the kinase inhibitor ibrutinib reduces CD19-CAR T-mediated CRS by inhibiting the production of inflammatory cytokines, without impairing the proliferation of T cells. In addition, 5-aza-2'-deoxycytidine (80), fludarabine (81), cyclophosphamide (10) and other drugs are also believed to improve the expansion and persistence of adoptive CAR T cells to enhance their anticancer efficacy. Various combinations of these drugs constitute

the lymphodepletion regimen. Most pediatric patients who participated in the tisagenlecleucel trial were initiated on a lymphodepletion regimen prior to CAR T-cell injection (31).

Other strategies. In early trials, most CAR T cells were made using mouse scFv, which may result in a decrease in T cells. Some experts believe that human scFv can reduce the immunogenicity of CAR T cells, thereby increasing their persistence *in vivo* (10). There are also a number of factors that inhibit T-cell function in the tumor microenvironment, such as hypoxia, an acidic environment and metabolites (37,82). For example, indoleamine 2,3-dioxygenase is an intracellular enzyme that regulates tryptophan metabolism to inhibit the expansion of CAR T cells (83). Antagonizing this enzyme may benefit CAR T-cell therapy.

7. Conclusions and perspectives

After several decades of development, CAR T-cell design has become more sophisticated and its clinical application has become more extensive, even for the treatment of solid tumors. Moreover, a breakthrough occurred when the FDA approved tisagenlecleucel for the treatment of B-ALL, which has been gradually recognized and recommended by the global medical community, and this avant-garde technology could become the mainstream treatment for patients with hematologic tumors in the near future. Professional guidelines for the management of CAR T-cell therapy in pediatric patients have also been issued. The multi-disciplinary nature of the treatment team ensures a complete understanding of relevant issues, and timely response strategies are instituted in the event of corresponding adverse reactions.

Problems encountered in the clinic currently suggest that improving the efficacy and safety of CAR T cells is an important starting point for CAR design. Dual-targeted CAR potentially reduces the risk of off-target effects, co-stimulatory molecules can stimulate the proliferation of T cells *in vivo* and suicide gene intervention is a powerful strategy to control adverse reactions. However, a number of issues remain unresolved. First, the present review did not specify the range of doses of CAR T cells to be administered, given that a core strategy to minimize adverse reactions is required to obtain the maximum value with the minimum individual dose. Second, in the search for specific antigens for individual patients, there are still unresolved issues associated with relapse after CD19-CAR T-cell treatment. Furthermore, it is debatable whether bone marrow transplantation should be continued or if CAR T-cell therapy should be repeated after the first CAR T-cell therapy. Finally, the exorbitant cost of tisagenlecleucel (with a one-time infusion cost of US\$475,000) is also an economic challenge that has to be addressed to make this clinical treatment popular.

Acknowledgements

Not applicable.

Funding

This study was supported by the Program of Cultivating Zhejiang Provincial High-Level Personnel in Health (Excellent Medical Younger in 2017) and the Chinese Medicine Research Program of Zhejiang Province (grant no. 2020ZB148).

Availability of data and materials

Not applicable.

Authors' contributions

CX conceived, designed and revised the review. LQ designed the table and revised the review. ZF drafted the initial manuscript. JZ, RC, YJ and TN helped draft the initial manuscript and analyze the data. All authors reviewed and approved the submitted version of the paper.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Katz AJ, Chia VM, Schoonen WM and Kelsh MA: Acute lymphoblastic leukemia: an assessment of international incidence, survival, and disease burden. *Cancer Causes Control* 26: 1627-1642, 2015.
- Noone AM HN, Krapcho M, *et al*: SEER Cancer Statistics Review, 1975-2015, National Cancer Institute. Bethesda, MD, https://seer.cancer.gov/csr/1975_2015/, based on November 2017 SEER data submission, posted to the SEER web site, April 2018.
- Inaba H, Greaves M and Mullighan CG: Acute lymphoblastic leukaemia. *Lancet* 381: 1943-1955, 2013.
- Pui CH, Nichols KE and Yang JJ: Somatic and germline genomics in paediatric acute lymphoblastic leukaemia. *Nat Rev Clin Oncol* 16: 227-240, 2019.
- Hunger SP and Mullighan CG: Acute lymphoblastic leukemia in children. *N Engl J Med* 373: 1541-1552, 2015.
- Maude SL, Noelle F, Shaw PA, Richard A, Barrett DM, Bunin NJ, Anne C, Gonzalez VE, Zhaohui Z, Lacey SF, *et al*: Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med* 371: 1507-1517, 2014.
- Brudno JN, Somerville RP, Shi V, Rose JJ, Halverson DC, Fowler DH, Geabanacloche JC, Pavletic SZ, Hickstein DD, Lu TL, *et al*: Allogeneic T cells that express an anti-CD19 chimeric antigen receptor induce remissions of B-cell malignancies that progress after allogeneic hematopoietic stem-cell transplantation without causing graft-versus-host disease. *J Clin Oncol* 34: 1112-1121, 2016.
- Kebric P, Singh H, Huls MH, Figliola MJ, Bassett R, Olivares S, Jena B, Dawson MJ, Kumaresan PR, Su S, *et al*: Phase I trials using Sleeping Beauty to generate CD19-specific CAR T cells. *J Clin Invest* 126: 3363-3376, 2016.
- Gardner R, Wu D, Cherian S, Fang M, Hanafi L, Finney O, Smithers H, Jensen MC, Riddell SR, Maloney DG and Turtle CJ: Acquisition of a CD19 negative myeloid phenotype allows immune escape of MLL-rearranged B-ALL from CD19 CAR-T cell therapy. *Blood* 127: 2406-2410, 2016.
- Turtle CJ, Hanafi LA, Berger C, Gooley TA, Cherian S, Hudecek M, Sommermeyer D, Melville K, Pender B, Budiarto TM, *et al*: CD19 CAR-T cells of defined CD4⁺:CD8⁺ composition in adult B cell ALL patients. *J Clin Invest* 126: 2123-2138, 2016.
- Fry TJ, Shah NN, Orentas RJ, Stetler-Stevenson M, Yuan CM, Ramakrishna S, Wolters P, Martin S, Delbrook C, Yates B, *et al*: CD22-targeted CAR T cells induce remission in B-ALL that is naive or resistant to CD19-targeted CAR immunotherapy. *Nat Med* 24: 20-28, 2017.
- Fuerst ML: CD22 CAR T-Cell therapy induces remissions in young ALL patients. *Oncol Times* 39: 35, 2017.
- Prasad V: Immunotherapy: Tisagenlecleucel-the first approved CAR-T-cell therapy: Implications for payers and policy makers. *Nat Rev Clin Oncol* 15: 11-12, 2018.
- Ma F, Ho JY, Du H, Xuan F, Wu X, Wang Q, Wang L, Liu Y, Ba M, Wang Y, *et al*: Evidence of long-lasting anti-CD19 activity of engrafted CD19 chimeric antigen receptor-modified T cells in a phase I study targeting pediatric acute lymphoblastic leukemia. *Hematol Oncol* 37: 601-608, 2019.
- Cai B, Guo M, Wang Y, Zhang Y, Yang J, Guo Y, Dai H, Yu C, Sun Q, Qiao J, *et al*: Co-infusion of haplo-identical CD19-chimeric antigen receptor T cells and stem cells achieved full donor engraftment in refractory acute lymphoblastic leukemia. *J Hematol Oncol* 9: 131, 2016.
- Li S, Zhang J, Wang M, Fu G, Li Y, Pei L, Xiong Z, Qin D, Zhang R, Tian X, *et al*: Treatment of acute lymphoblastic leukaemia with the second generation of CD19 CAR-T containing either CD28 or 4-1BB. *Br J Haematol* 181: 360-371, 2018.
- Weng J, Lai P, Qin L, Lai Y, Jiang Z, Luo C, Huang X, Wu S, Shao D, Deng C, *et al*: A novel generation 1928zT2 CAR T cells induce remission in extramedullary relapse of acute lymphoblastic leukemia. *J Hematol Oncol* 11: 25, 2018.
- Xiao X, Yuan T, Meng JX, Jiang YY, Cao YQ, Li Q, Sun R and Zhao MF: Analysis on poor efficacy factors in the treatment of recurrent/refractory B-cell lymphoma with CD19 CAR-T cells. *Zhonghua Yi Xue Za Zhi* 100: 593-598, 2020 (In Chinese).
- Zhang J, Yang F, Qiu HY, Wu Q, Kong DQ, Zhou J, Han Y and Wu DP: Anti-CD19 chimeric antigen receptors T cells for the treatment of relapsed or refractory E2A-PBX1 positive acute lymphoblastic leukemia: Report of three cases. *Leuk Lymphoma* 60: 1454-1461, 2019.
- Kochenderfer JN, Dudley ME, Feldman SA, Wilson WH, Spaner DE, Irlin M, Maryalice SS, Phan GQ, Hughes MS, Sherry RM, *et al*: B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. *Blood* 119: 2709-2720, 2012.
- Lee DW, Gardner R, Porter DL, Louis CU, Ahmed N, Jensen M, Grupp SA and Mackall CL: Current concepts in the diagnosis and management of cytokine release syndrome. *Blood* 124: 188-195, 2014.
- Richman SA, Nunez-Cruz S, Moghimi B, Li LZ, Gershenson ZT, Mourelatos Z, Barrett DM, Grupp SA and Milone MC: High-affinity GD2-specific CAR T cells induce fatal encephalitis in a preclinical neuroblastoma model. *Cancer Immunol Res* 6: 36-46, 2018.
- Curran KJ, Pegram HJ and Brentjens RJ: Chimeric antigen receptors for T cell immunotherapy: Current understanding and future directions. *J Gene Med* 14: 405-415, 2012.
- Dai H, Wang Y, Lu X and Han W: Chimeric antigen receptors modified T-cells for cancer therapy. *J Natl Cancer Inst* 108: djv439, 2016.
- Tokarew N, Ogonek J, Endres S, von Bergwelt-Baildon M and Kobold S: Teaching an old dog new tricks: Next-generation CAR T cells. *B J Cancer* 120: 26-37, 2019.
- Kenderian SS, Ruella M, Gill S and Kalos M: Chimeric antigen receptor T-cell therapy to target hematologic malignancies. *Cancer Res* 74: 6383-6389, 2014.
- Chmielewski M, Kopecky C, Hombach AA and Abken H: IL-12 release by engineered T cells expressing chimeric antigen receptors can effectively Muster an antigen-independent macrophage response on tumor cells that have shut down tumor antigen expression. *Cancer Res* 71: 5697-5706, 2011.
- Scheuermann RH and Racila E: CD19 Antigen in Leukemia and Lymphoma Diagnosis and Immunotherapy. *Leuk Lymphoma* 18: 385-397, 1995.
- Abate-Daga D and Davila ML: CAR models: Next-generation CAR modifications for enhanced T-cell function. *Mol Ther Oncolytics* 3: 16014, 2016.
- Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, Fry TJ, Orentas R, Sabatino M, Shah NN, *et al*: T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: A phase I dose-escalation trial. *Lancet* 385: 517-528, 2015.
- Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, Bader P, Verneris MR, Stefanski HE, Myers GD, *et al*: Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N Engl J Med* 378: 439-448, 2018.
- Gardner RA, Finney O, Annesley C, Brakke H, Summers C, Leger K, Bleakley M, Brown C, Mgebroff S, Kelly-Spratt KS, *et al*: Intent-to-treat leukemia remission by CD19 CAR T cells of defined formulation and dose in children and young adults. *Blood* 129: 3322-3331, 2017.

33. Zuo YX, Jia YP, Wu J, Wang JB, Lu AD, Dong LJ, Chang LJ and Zhang LP: Chimeric antigen receptors T cells for treatment of 48 relapsed or refractory acute lymphoblastic leukemia children: Long term follow-up outcomes. *Zhonghua Xue Ye Xue Za Zhi* 40: 270-275, 2019 (In Chinese).
34. Barba P, Sampol A, Calbacho M, Gonzalez J, Serrano J, Martínez-Sánchez P, Fernández P, García-Boyeró R, Bueno J and Ribera JM: Clofarabine-based chemotherapy for relapsed/refractory adult acute lymphoblastic leukemia and lymphoblastic lymphoma. The Spanish experience. *Am J Hematol* 87: 631-634, 2012.
35. Zhang C, Zhang X and Chen XH: Interleukin-2 priming chemotherapy: A strategy to improve the remission of refractory/relapsed T cell acute lymphoblastic leukemia. *Med Hypotheses* 81: 878-880, 2013.
36. Morgan RA, Gray D, Lomova A and Kohn DB: Hematopoietic stem cell gene therapy: Progress and lessons learned. *Cell Stem Cell* 21: 574-590, 2017.
37. Maus MV, Grupp SA, Porter DL and June CH: Antibody-modified T cells: CARs take the front seat for hematologic malignancies. *Blood* 123: 2625-2635, 2014.
38. Mahadeo KM, Khazal SJ, Abdel-Azim H, Fitzgerald JC, Taraseviciute A, Bollard CM, Tewari P, Duncan C, Traube C, McCall D, *et al*: Management guidelines for paediatric patients receiving chimeric antigen receptor T cell therapy. *Nat Rev Clin Oncol* 16: 45-63, 2019.
39. Sermer D and Brentjens R: CAR T-cell therapy: Full speed ahead. *Hematol Oncol* 37 (Suppl 1): S95-S100, 2019.
40. Davila ML, Riviere I, Wang X, Bartido S, Park J, Curran K, Chung SS, Stefanski J, Borquez-Ojeda O, Olszewska M, *et al*: Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci Transl Med* 6: 224ra225, 2014.
41. Norelli M, Camisa B, Barbiera G, Falcone L, Purevdorj A, Genua M, Sanvito F, Ponzoni M, Doglioni C, Cristofori P, *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* 24: 739-748, 2018.
42. Neelapu SS, Tummala S, Kebriaei P, Wierda W, Gutierrez C, Locke FL, Komanduri KV, Lin Y, Jain N, Daver N, *et al*: Chimeric antigen receptor T-cell therapy-assessment and management of toxicities. *Nat Rev Clin Oncol* 15: 47-62, 2018.
43. Hu Y, Sun J, Wu Z, Yu J, Cui Q, Pu C, Liang B, Luo Y, Shi J, Jin A, *et al*: Predominant cerebral cytokine release syndrome in CD19-directed chimeric antigen receptor-modified T cell therapy. *J Hematol Oncol* 9: 70, 2016.
44. Gust J, Hay KA, Hanafi LA, Li D, Myerson D, Gonzalez-Cuyar LF, Yeung C, Liles WC, Wurfel M, Lopez JA, *et al*: Endothelial activation and blood-brain barrier disruption in neurotoxicity after adoptive immunotherapy with CD19 CAR-T cells. *Cancer Discov* 7: 1404-1419, 2017.
45. Acharya UH, Dhawale T, Yun S, Jacobson CA, Chavez JC, Ramos JD, Appelbaum J and Maloney DG: Management of cytokine release syndrome and neurotoxicity in chimeric antigen receptor (CAR) T cell therapy. *Expert Rev Hematol* 12: 195-205, 2019.
46. Chavez JC, Jain MD and Kharfan-Dabaja MA: Cytokine release syndrome and neurologic toxicities associated with chimeric antigen receptor T-cell therapy: A comprehensive review of emerging grading models. *Hematol Oncol Stem Cell Ther* 13: 1-6, 2020.
47. Lee DW, Santomasso BD, Locke FL, Ghobadi A, Turtle CJ, Brudno JN, Maus MV, Park JH, Mead E, Pavletic S, *et al*: ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. *Biol Blood Marrow Transplant* 25: 625-638, 2019.
48. Traube C, Silver G, Kearney J, Patel A, Atkinson TM, Yoon MJ, Halpert S, Augenstein J, Sickles LE, Li C and Greenwald B: Cornell Assessment of Pediatric Delirium: A valid, rapid, observational tool for screening delirium in the PICU*. *Crit Care Med* 42: 656-663, 2014.
49. Silver G, Kearney J, Traube C and Hertzog M: Delirium screening anchored in child development: The Cornell Assessment for Pediatric Delirium. *Palliat Support Care* 13: 1005-1011, 2015.
50. Hombach AA and Abken H: Shared target antigens on cancer cells and tissue stem cells: go or no-go for CAR T cells? *Expert Rev Clin Immunol* 13: 151-155, 2016.
51. Hucks G and Rheingold SR: The journey to CAR T cell therapy: The pediatric and young adult experience with relapsed or refractory B-ALL. *Blood Cancer J* 9: 10, 2019.
52. Porter DL, Hwang WT, Frey NV, Lacey SF, Shaw PA, Loren AW, Bagg A, Marcucci KT, Shen A, Gonzalez V, *et al*: Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Sci Transl Med* 7: 303ra139, 2015.
53. Perez EE, Orange JS, Bonilla F, Chinen J, Chinn IK, Dorsey M, El-Gamal Y, Harville TO, Hossny E, Mazer B, *et al*: Update on the use of immunoglobulin in human disease: A review of evidence. *J Allergy Clin Immunol* 139: S1-S46, 2017.
54. Finney OC, Brakke HM, Rawlings-Rhea S, Hicks R, Doolittle D, Lopez M, Futrell RB, Orentas RJ, Li D, Gardner RA and Jensen MC: CD19 CAR T cell product and disease attributes predict leukemia remission durability. *J Clin Invest* 129: 2123-2132, 2019.
55. Bonifant CL, Jackson HJ, Brentjens RJ and Curran KJ: Toxicity and management in CAR T-cell therapy. *Mol Ther Oncolytics* 3: 16011, 2016.
56. Zah E, Lin MY, Silva-Benedict A, Jensen MC and Chen YY: T cells expressing CD19/CD20 bispecific chimeric antigen receptors prevent antigen escape by malignant B Cells. *Cancer Immunol Res* 4: 498-508, 2016.
57. Di Stasi A, Tey SK, Dotti G, Fujita Y, Kennedy-Nasser A, Martinez C, Straathof K, Liu E, Durett AG, Grilley B, *et al*: Inducible apoptosis as a safety switch for adoptive cell therapy. *N Engl J Med* 365: 1673-1683, 2011.
58. Howard SC, Jones DP and Pui CH: The tumor lysis syndrome. *N Engl J Med* 364: 1844-1854, 2011.
59. Minoia F, Bovis F, Davi S, Insalaco A, Lehmborg K, Shenoi S, Weitzman S, Espada G, Gao YJ, Anton J, *et al*: Development and initial validation of the macrophage activation syndrome/primary hemophagocytic lymphohistiocytosis score, a diagnostic tool that differentiates primary hemophagocytic lymphohistiocytosis from macrophage activation syndrome. *J Pediatr* 189: 72-78.e3, 2017.
60. Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S, Ladisch S, McClain K, Webb D, Winiarski J and Janka G: HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer* 48: 124-131, 2007.
61. Wang Z, Wu Z, Liu Y and Han W: New development in CAR-T cell therapy. *J Hematol Oncol* 10: 53, 2017.
62. Ruella M and Maus MV: Catch me if you can: Leukemia escape after CD19-directed T cell immunotherapies. *Comput Struct Biotechnol J* 14: 357-362, 2016.
63. Pan J, Yang JF, Deng BP, Zhao XJ, Zhang X, Lin YH, Wu YN, Deng ZL, Zhang YL, Liu SH, *et al*: High efficacy and safety of low-dose CD19-directed CAR-T cell therapy in 51 refractory or relapsed B acute lymphoblastic leukemia patients. *Leukemia* 31: 2587-2593, 2017.
64. Sotillo E, Barrett DM, Black KL, Bagashev A, Oldridge D, Wu G, Sussman R, Lanauze C, Ruella M, Gazzara MR, *et al*: Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy. *Cancer Discov* 5: 1282-1295, 2015.
65. Ruella M, Xu J, Barrett DM, Fraietta JA, Reich TJ, Ambrose DE, Klichinsky M, Shestova O, Patel PR, Kulikovskaya I, *et al*: Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell. *Nat Med* 24: 1499-1503, 2018.
66. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, Teachey DT, Chew A, Hauck B, Wright JF, *et al*: Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med* 368: 1509-1518, 2013.
67. Mejstříková E, Hrusak O, Borowitz MJ, Whitlock JA, Brethon B, Trippett TM, Zugmaier G, Gore L, von Stackelberg A and Locatelli F: CD19-negative relapse of pediatric B-cell precursor acute lymphoblastic leukemia following blinatumomab treatment. *Blood Cancer J* 7: 659, 2017.
68. Xu X, Sun Q, Liang X, Chen Z, Zhang X, Zhou X, Li M, Tu H, Liu Y, Tu S and Li Y: Mechanisms of relapse after CD19 CAR T-cell therapy for acute lymphoblastic leukemia and its prevention and treatment strategies. *Front Immunol* 10: 2664, 2019.
69. Maude SL, Teachey DT, Rheingold SR, Shaw PA, Aplenc R, Barrett DM, Barker CS, Callahan C, Frey NV, Nazimuddin F, *et al*: Sustained remissions with CD19-specific chimeric antigen receptor (CAR)-modified T cells in children with relapsed/refractory ALL. *J Clin Oncol* 34: 3011, 2016.
70. Orlando EJ, Han X, Tribouley C, Wood PA, Leary RJ, Riester M, Levine JE, Qayed M, Grupp SA, Boyer M, *et al*: Genetic mechanisms of target antigen loss in CAR19 therapy of acute lymphoblastic leukemia. *Nat Med* 24: 1504-1506, 2018.

71. Hamieh M, Dobrin A, Cabriolu A, van der Stegen SJC, Giavridis T, Mansilla-Soto J, Eyquem J, Zhao Z, Whitlock BM, Miele MM, *et al*: CAR T cell trogocytosis and cooperative killing regulate tumour antigen escape. *Nature* 568: 112-116, 2019.
72. Zolov SN, Rietberg SP and Bonifant CL: Programmed cell death protein 1 activation preferentially inhibits CD28.CAR-T cells. *Cytotherapy* 20: 1259-1266, 2018.
73. Zou F, Lu L, Liu J, Xia B, Zhang W, Hu Q, Liu W, Zhang Y, Lin Y, Jing S, *et al*: Engineered triple inhibitory receptor resistance improves anti-tumor CAR-T cell performance via CD56. *Nat Commun* 10: 4109, 2019.
74. Zheng S, Asnani M and Thomas-Tikhonenko A: Escape from all-CARTaz: Leukemia immunoediting in the age of chimeric antigen receptors. *Cancer J* 25: 217-222, 2019.
75. Cho FN, Chang TH, Shu CW, Ko MC, Liao SK, Wu KH, Yu MS, Lin SJ, Hong YC, Chen CH, *et al*: Enhanced cytotoxicity of natural killer cells following the acquisition of chimeric antigen receptors through trogocytosis. *PLoS One* 9: e109352, 2014.
76. Jiang H, Li C, Yin P, Guo T, Liu L, Xia L, Wu Y, Zhou F, Ai L, Shi W, *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* 94: 1113-1122, 2019.
77. Schneider D, Xiong Y, Wu D, Nille V, Schmitz S, Haso W, Kaiser A, Dropulic B and Orentas RJ: A tandem CD19/CD20 CAR lentiviral vector drives on-target and off-target antigen modulation in leukemia cell lines. *J Immunother Cancer* 5: 42, 2017.
78. Ruella M, Barrett DM, Kenderian SS, Shestova O, Hofmann TJ, Perazzelli J, Klichinsky M, Aikawa V, Nazimuddin F, Kozlowski M, *et al*: Dual CD19 and CD123 targeting prevents antigen-loss relapses after CD19-directed immunotherapies. *J Clin Invest* 126: 3814-3826, 2016.
79. Ruella M, Kenderian SS, Shestova O, Klichinsky M, Melenhorst JJ, Wasik MA, Lacey SF, June CH and Gill S: Kinase inhibitor ibrutinib to prevent cytokine-release syndrome after anti-CD19 chimeric antigen receptor T cells for B-cell neoplasms. *Leukemia* 31: 246-248, 2017.
80. Dolnikov A, Shen S, Klamer G, Joshi S, Xu N, Yang L, Micklethwaite K and O'Brien TA: Antileukemic potency of CD19-specific T cells against chemoresistant pediatric acute lymphoblastic leukemia. *Exp Hematol* 43: 1001-1014.e5, 2015.
81. Lowe KL, Mackall CL, Norry E, Amado R, Jakobsen BK and Binder G: Fludarabine and neurotoxicity in engineered T-cell therapy. *Gene Ther* 25: 176-191, 2018.
82. Wang Y, Jiang H, Luo H, Sun Y, Shi B, Sun R and Li Z: An IL-4/21 inverted cytokine receptor improving CAR-T cell potency in immunosuppressive solid-tumor microenvironment. *Front Immunol* 10: 1691, 2019.
83. Ninomiya S, Narala N, Huye L, Yagyu S, Savoldo B, Dotti G, Heslop HE, Brenner MK, Rooney CM and Ramos CA: Tumor indoleamine 2,3-dioxygenase (IDO) inhibits CD19-CAR T cells and is downregulated by lymphodepleting drugs. *Blood* 125: 3905-3916, 2015.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.