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REVIEW

Chimeric antigen receptor T cell therapies for acute myeloid leukemia

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Abstract **Chimeric antigen receptor T cell (CAR T) therapies have achieved unprecedented efficacy in B-cell tumors, prompting scientists and doctors to exploit this strategy to treat other tumor types. Acute myeloid leukemia (AML) is a group of heterogeneous myeloid malignancies. Relapse remains the main cause of treatment failure, especially for patients with intermediate or high risk stratification. Allogeneic hematopoietic stem cell transplantation could be an effective therapy because of the graft-versus-leukemia effect, which unfortunately puts the patient at risk of serious complications, such as graft-versus-host disease. Although the identification of an ideal target antigen for AML is challenging, CAR T therapy remains a highly promising strategy for AML patients, particularly for those who are ineligible to receive a transplantation or have positive minimal residual disease. In this review, we focus on the most recent and promising advances in CAR T therapies for AML.**

Keywords acute myeloid leukemia; CAR T; immunotherapy

Introduction

Acute myeloid leukemia (AML) is the most common type of leukemia showing heterogeneity behavior and is characterized by the clonal expansion of myeloid blasts. Despite recent improvements in treatment, the complete remission (CR) rates for AML are approximately 70% in younger adults and only 40%–60% in older patients (more than 60 years old) [1,2]. Disease recurrence remains the most common cause of treatment failure, and the 5-year survival of AML patients with intermediate and high risk cytogenetics was no more than 41% [3–5].

Allogeneic hematopoietic stem cell transplantation (Allo-HSCT) could be an effective therapy for AML patients through the graft-versus-leukemia effect mediated by donor T lymphocytes. However, it is often accompanied with the risk of life-threatening graft-versus-host disease [6]. The physiological mechanism responsible for the killing effect of cytotoxic T lymphocytes has been well studied. A recognition signal from T cell receptors (TCRs) is the first step; this is complemented by a costimulatory signal to further augment the activation of cytotoxic T lymphocytes (Fig. 1). A TCR could recognize an antigen in the context of major histocompatibility complex (MHC) presentation. In stark contrast, chimeric antigen receptor (CAR) is an MHC-independent model that is commonly composed of an extracellular domain with a single-chain variable fragment (scFv) from a monoclonal antibody, a hinge region, a transmembrane domain, and a TCR-derived CD3ζ domain with or without one or more intracellular costimulatory domains. The design of CAR has developed over the years to boost efficacy and safety in detailed immunological structures (Fig. 2).

One of the most important prerequisites for successful CAR T therapy is the identification of the suitable target antigens [7,8]. Theoretically, an ideal target antigen should be immunogenic and should play a crucial role in the differentiation, survival, and expansion of malignant cells. The antigen expression should be restricted to all malignant cells with high antigen densities [9,10], even including malignant stem cells. A large fraction of patients should be positive for the antigen, which should be on the surface of malignant cells. CD19, which is ubiquitously expressed on the surface of B cell, is a satisfying target for B cell malignancies. Infusion with anti-CD19 CAR T resulted in an unheard-of antitumor effect and long-term remissions in chronic lymphocytic and acute lymphocytic

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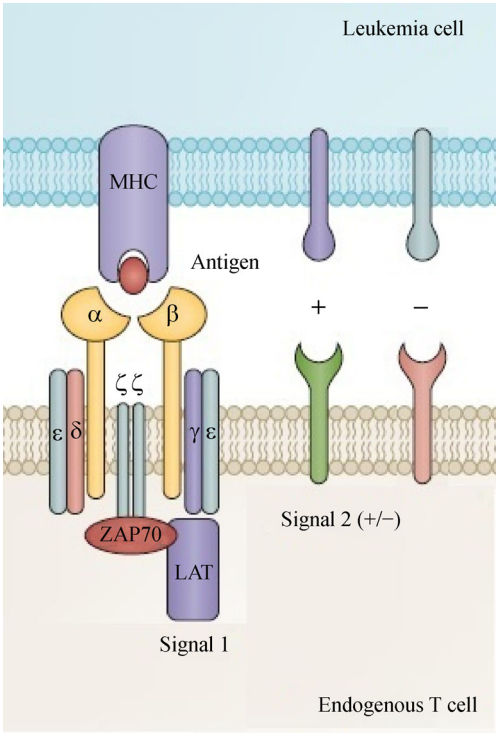

Fig. 1 Endogenous T cell receptor (TCR) is comprised of paired α and β chains associated with δ, ε, and γ chains, and ζ chains. The antigen is presented by either major histocompatibility complex (MHC) class I or MHC class II. The specificity signal delivered by the TCR is commonly defined as signal 1, which is the recognition signal. T cell activation also requires a co-stimulatory signal, referred to as signal 2. Activating co-stimulatory receptors include CD28, 4-1BB, and others.

leukemia [11–13]. Unlike B cell malignancies, different antigens are expressed on distinct subtype AML cells, which means that we cannot treat all AML patients with CAR T targeting the same antigen. In the following paragraphs we will focus on CAR T therapies in AML.

CAR T therapies for AML

As of this writing, there is no licensing authority approving CAR T therapy for AML in contrast to B cell malignancies, but several antigens have been proposed as potential CAR T targets against AML (Table 1). The greatest challenge for the successful application of CAR T for patients with AML is the selection of effective and safe antigen targets. AML is a heterogeneous clonal malignancy, and the subclones may evolve over time, thereby possibly leading to the genetic and phenotypic heterogeneity of the leukemia cells in one patient [14]. Phenotypic heterogeneity is characterized by differential antigen expression on the leukemia cell surface, especially in patients suffering from leukemia relapse. During the relapse stage, for B-ALL, leukemia cells lose the target antigen, generate antigen-negative blast cells, or exhaust CAR T persistence [15–19]. However, the study on anti-LeY CAR T for five AML patients showed that the AML blasts of three patients present at relapse continued to express the LeY antigen, indicating that progression was not due to the antigenic change in these AML cases [20]. It might be necessary to target more than one antigen to optimize the anti-leukemia effect of CAR T. Perna *et al*. developed the combinatorial

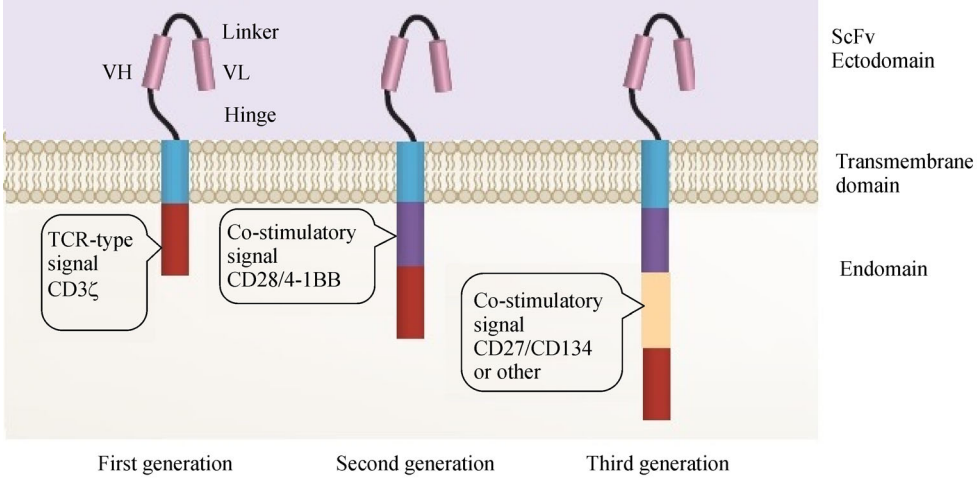

Fig. 2 Basic structures of chimeric antigen receptors. Chimeric antigen receptors (CARs) recognize cell-surface antigens in a major histocompatibility complex-independent manner and are composed of an extracellular binding domain, a hinge, a transmembrane domain, and intracellular signal domains. The first-generation CAR has a single T cell receptor signal domain comprising a CD3ζ chain. The second-generation CARs incorporating CD28/4-1BB as a co-stimulatory domain are developed. CARs incorporating three or more signal domains, the so-called third- and fourth-generation CARs, have also been developed and have started to be tested in clinical trials.

Table 1 Chimeric antigen receptor (CAR) T cell therapy trails for acute myeloid leukemia (AML)

Antigen target	Trail number	Trail phase	Disease	First posted	Recruitment status	Country	Sponsor
CD33	NCT03126864	I	RR-AML	April 24, 2017	Recruiting	USA	M.D. Anderson Cancer Center
CD33	NCT02799680	I	RR-AML	June 15, 2016	Unknown	China	The Affiliated Hospital of the Chinese Academy of Military Medical Sciences
CD33	NCT01864902	I/II	RR-AML	May 30, 2013	Unknown	China	Chinese PLA General Hospital
CD33, CD38, CD56, CD117, CD123, CD34, and Mucl	NCT03291444	I	RR-AML and MDS	September 25, 2017	Recruiting	China	Zhujiang Hospital
CD33, CD38, CD56, CD123, CD117, CD133, CD34 or Mucl	NCT03473457	Not applicable	RR-AML	March 22, 2018	Recruiting	China	Zhujiang Hospital
CD33, CD38, CD123, CD56, Mucl, and CLL1	NCT03222674	I/II	AML	July 19, 2017	Recruiting	China	Shenzhen Geno-Immune Medical Institute
CD123	NCT03585517	I	AML	July 13, 2018	Recruiting	China	Beijing Immunochina Medical Science & Technology Co., Ltd.
CD123	NCT03114670	I	Relapse AML after Allo-HSCT	April 14, 2017	Recruiting	China	Affiliated Hospital to Academy of Military Medical Sciences
CD123	NCT03556982	I	RR-AML	June 14, 2018	Recruiting	China	The Affiliated Hospital of the Chinese Academy of Military Medical Sciences
CD123	NCT02159495	I	RR-AML and persistent/recurrent blastic plasmacytoid dendritic cell neoplasm	June 10, 2014	Recruiting	USA	City of Hope Medical Center
CD123	NCT03672851	I	RR-AML	September 17, 2018	Enrolling by invitation	China	Second Affiliated Hospital of Xi'an Jiaotong University
CD123	NCT03766126	I	RR-AML	December 5, 2018	Recruiting	USA	University of Pennsylvania
CD123	NCT03190278	I	RR-AML	June 16, 2017	Recruiting	USA	Cellectis S.A.
CD123/CLL1	NCT03631576	II/III	RR-AML	August 15, 2018	Recruiting	China	Fujian Medical University
CD123	NCT03796390	I	AML	January 8, 2019	Recruiting	China	Hebei Senlang Biotechnology Inc., Ltd.
CD123	NCT02937103	I	Myeloid malignancies	October 18, 2016	Recruiting	China	Southwest Hospital, China
CD123	NCT02623582	I	AML	December 7, 2015	Terminated	USA	University of Pennsylvania
NKG2D	NCT02203825	I	AML, MM	July 30, 2014	Completed	USA	Celyad
NKG2D	NCT03018405	I/II	AML, MM	January 12, 2017	Recruiting	USA	Celyad
Lewis Y	NCT01716364	I	AML	October 29, 2012	Active, not recruiting	Australia	Peter MacCallum Cancer Centre

CAR therapy for AML with the aid of high-throughput surfaceome expression data. The ideal antigen pair should be at a very low level of expression in normal tissues and $CD34^{+}CD38^{-}$ hematopoietic stem cells to minimize the toxic side effect; the combination expressions need to be in all tumor cells (including leukemia stem cells) to overcome clonal heterogeneity and minimize the risk of antigen escape [21] (Fig. 3A and 3B).

Anti-CD33 CAR T

CD33 is a transmembrane receptor that binds to sialic acid and is expressed on about 85%–90% of AML blast cells. It is also present in early multilineage hematopoietic progenitors, bone marrow mononuclear cell precursors, and hepatocytes, thereby possibly causing the toxicity of veno-occlusive liver disease and limiting the use of CD33-directed immunotherapies [22]. Preclinical studies provide data on the effectiveness of an anti-CD33 CAR T therapy for AML in mice and support its development as a clinical therapeutic approach [23,24]. Considering the potential toxicity associated with targeting CD33 in patients, Rafiq *et al*. created the EGFRt/HuM195-28z/IL-12 CAR T, in which an elimination gene was included to allow CAR T clearance after disease remission (Fig. 3C), and tested anti-tumor efficacy in two preclinical mouse models of AML *in vivo* [25]. Wang *et al*. reported that a 41-year-old male patient with AML was administered a total of 1.12×10^{9} autologous anti-CD33 CAR T, of which ~38% were transduced with CAR. After 2 weeks of tolerable side effects, including fever and jaundice, the patient had a dramatic decrease of blasts in the bone marrow, but the leukemia cells gradually increased 9 weeks after therapy [26]. Based on these inspiring preliminary results, some ongoing clinical trials on anti-CD33 CAR T therapy were conducted (Table 1). To accurately target AML cells without affecting normal hematopoiesis, Kim *et al.* produced CD33 knockout human hematopoietic stem cells and progenitor cells (HSPCs) and demonstrated normal implantation and differentiation in immunodeficient mice. Human HSPCs lacking CD33 could obviate the attack of anti-CD33 CAR T, which would efficaciously eliminate leukemia cells without marrow toxicity [27]. To achieve this same goal, Humbert *et al*. definitively eliminated CD33 exon2 by CRISPR/Cas9 technology, thereby expressing a shorter isoform of CD33 but not the full-type CD33. They also evaluated the genome-edited HSPCs *in vitro* and in immunodeficient mice to reserve the function of engraftment and avoid the non-leukemic cytotoxicity [28]. Borot *et al.* also used CRISPR/Cas9 to ablate CD33 antigen in HSPCs and demonstrated that the infusion of CD33-deleted HSPCs and anti-CD33 CAR T accomplished the clearance of blast cells without myelosuppression [29].

Anti-CD123 CAR T

CD123, a transmembrane α subunit of the IL-3 receptor, which is highly expressed on AML blasts and leukemia stem cells, represents another attractive target for immunotherapy [30–32]. Du *et al*. demonstrated the role of CD123 epitope selection in immunotoxin action and

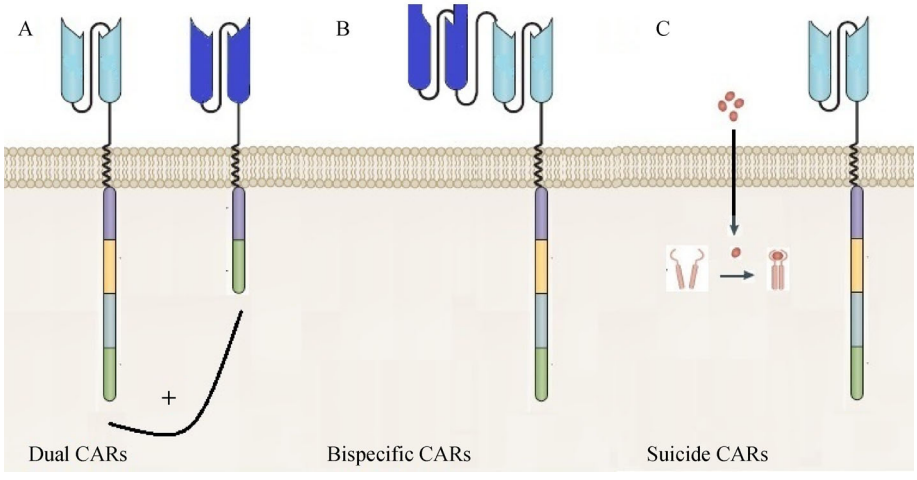

Fig. 3 (A) Dual-targeting CARs express two different antigen-specific CARs. (B) Bispecific CARs combine two linked scFV within one CAR construct. (C) Suicide CARs were designed to contain the suicide gene to serve as control mechanisms for toxicity management. One example is the inducible caspase 9 (iCasp9). When the small molecule AP1903 is administered, iCasp9 domains dimerize and activate apoptosis independent of CAR activation.

further found that 26292(Fv)-PE38-KDEL has good cytotoxic activity against CD123 positive cell lines [33]. The use of CD123-targeted T cells could be an encouraging strategy for the potential clearance of AML cells [34,35]. Gills *et al*. showed that the donor-derived anti-CD123-41BB CAR T had graft-versus-leukemia (GVL) effect after infusion in an acute myeloid leukemia xenograft model with NSG mice [35], as well as also other CD123 positive malignancies [36]. Based on those preclinical results, many clinical trials have been launched to evaluate the therapeutic efficacy of anti-CD123 CAR T in AML patients (Table 1). Meanwhile, CD123 is infrequently expressed on HSPCs [37]. The potential influence on hematopoiesis that anti-CD123 CAR T may induce needs to be recognized. For purpose of controlling harmful off-target toxicities, Wang *et al.* had included EGFRt in their lentiviral construct to provide a target for the elimination of CAR T *in vivo* [38]. Straathof *et al.* showed a late-stage apoptosis pathway molecule, caspase-9, which can be stably expressed in T lymphocytes while retaining their phenotype and function to regulate CAR T abilities through inducible caspase-9 apoptosis switch [39].

Anti-NKG2D CAR T

NKG2D, which is an activating receptor on NK cells, invariant NKT cells, γδ T cells, $CD8^+$ T cells, and a small fraction of $CD4^+$ T cells, provides a costimulatory signal to T cells in its native form. NKG2D ligands are expressed on some solid tumors and hematologic malignancies, including AML and MM, but are generally not on healthy tissues [40]. NKG2D ligand recognition by the anti-NKG2D CAR T mediates T cell activation. Therefore, anti-NKG2D CAR T has the potential to treat these malignancies. Hilpert *et al*. demonstrated that anti-NKG2D CAR T was effective in eradicating established multiple myeloma (MM), lymphoma, and ovarian cancers in murine studies, and it can induce autologous immunity against tumor even when anti-NKG2D CAR T can no longer be detected [41]. Human anti-NKG2D CAR T does not attack autologous peripheral blood mononuclear cells or bone marrow cells from healthy donors *in vitro*. Baumeister *et al.* conducted a phase I dose-escalation study to evaluate the safety and feasibility of anti-NKG2D CAR T for AML/myelodysplastic syndrome and relapsed/refractory MM. Twelve patients (including 7 AML, 5 MM) were infused with anti-NKG2D CAR T, and the dosages were evaluated in four levels (1×10^6–3×10^7 total viable T cells) [42]. There were no adverse events more than grade 3 or significant autoimmune reactions attributable to anti-NKG2D CAR T infusion, although no clinical leukemic responses were obtained up to 28 days after infusion. Further studies investigating the efficacy of multiple anti-NKG2D CAR T infusions are currently underway.

Anti-Lewis Y CAR T

Lewis Y (LeY) is a difucosylated carbohydrate antigen expressed on many malignancies including AML, but it is limited to normal tissue [43–45]. Peinert *et al.* demonstrated that anti-LeY CAR T produced varying amounts of IFN-γ on exposure to AML cells and displayed apparent cytolytic activity in a preclinical study [45]. Ritchie *et al.* examined the safety and efficacy of second generation CAR T against the LeY antigen in AML in a phase I study [20]. Out of four evaluated patients, one achieved cytogenetic remission for 5 months, whereas another with active leukemia showed a decrease in peripheral blood blasts, and another showed stable disease for 23 months. No grade 3 or 4 adverse events or CRS were observed. Although all the patients eventually relapsed, serial PCR for detection of the LeY transgene demonstrated that infused CAR T could persist for up to 10 months.

Anti-CD19 CAR T

A fraction of AML patients could relatively highly express the antigen of CD19, which can be marked with the anti-CD19 CAR T regardless of cell origin. Ma *et al.* identified 527 AML cases from 1/1/2012 to 10/20/2017 at Stony Brook University Hospital and found that 17 out of 527 (3.2%) AML patients expressed CD19 [46]. Even at a low effector:target cell ratio of 2:1, anti-CD19 CAR T was able to effectively extinguish AML blast cells expressing CD19 within 6 h, suggesting that anti-CD19 CAR T therapy may be potentially applied for $CD19^+$ AML. These $CD19^+$ AML patients are distinguished from mixed phenotype acute leukemia according to the World Health Organization classification [47].

Promising target antigens for AML

FMS-like tyrosine kinase 3 (FLT3), also known as CD135, is a transmembrane protein expressed on malignant blasts in AML and retained on normal hematopoietic stem and progenitor cells. In the preclinical research, Jetani *et al.* reported that anti-FLT3 CAR T demonstrated potent reactivity against AML cell lines and primary AML blasts, which expressed either wild-type FLT3 or FLT3 with internal tandem duplication (FLT3-ITD) [48]. In addition, they showed that the FLT3-inhibitor Crenolanib could further increase the expression of FLT3 particularly on $FLT3\text{-}ITD^+$ AML blast cells, which rendered the AML cells more susceptible to attack by anti-FLT3 CAR T *in vitro* and *in vivo*. Unfortunately, anti-FLT3 CAR T could also recognize normal hematopoietic stem cells and impair normal hematopoiesis *in vitro* and *in vivo*, indicating that

anti-FLT3 CAR T therapy will require subsequent CAR T depletion and Allo-HSCT to reconstitute the hematopoietic system. Notably, the specific cytotoxicity of anti-FLT3 CAR T against FLT3$^+$ leukemia cell lines and primary AML cells was also demonstrated *in vitro* and in xenograft mouse models in other studies [49,50].

CD7 is expressed in more than 90% of lymphoblastic T cell leukemia and lymphoma and in approximately 30% of AML patients [51–54], but it is absent in normal erythroid and myeloid cells. CD7 expression of AML blasts is associated with poor prognosis. Thus, targeting CD7 could be beneficial for these AML patients. Gomes-Silva *et al.* showed that CD7-directed CART from CD7 gene-edited (CD7^{KO}) T cells was capable of decimating CD7$^+$ AML cell lines while sparing myeloid and erythroid progenitor cells [55], thereby supporting the feasibility of using anti-CD7 CAR T for the treatment of CD7$^+$ AML.

C-type lectin-like molecule 1 (CLL1), also known as CD371, is a type II transmembrane glycoprotein highly expressed on the blast cells of AML, but it is also on normal myeloid cells. CLL1 is lowly expressed on normal hematopoietic stem cells [56]. CLL1 is considered as a promising CAR T target. There are several preclinical studies on anti-CLL1 CAR T. Wang *et al*. generated CLL-1-redirected CAR T carrying a CAR composed of a CLL1 specific single chain variable fragment in combination with CD28/4-1BB costimulatory domains, and CD3ζ signaling domain [57]; this CAR T specifically lysed CLL-1$^+$ cell lines and patient-derived AML cells *in vitro* and showed strong anti-leukemic activity in the xenograft model of disseminated AML. In agreement with this finding, several other groups also demonstrated the potent activity of anti-CLL-1 CAR T against CLL1$^+$ AML cell lines *in vitro* and in xenograft mouse models [58–60].

CD44v6, the isoform variant 6 of the hyaluronic acid receptor CD44, is a class I membrane glycoprotein and is expressed in hematologic malignancies such as AML [61]. CD44v6 is absent on hematopoietic stem cells and only shows a low level of expression on normal cells, including monocytes, activated T cells, and keratinocytes [61,62]. Casucci *et al*. constructed a second generation anti-CD44v6 CAR T targeting AML cells while sparing normal HSPCs [62], and they also demonstrated the feasibility of incorporation of a suicide gene in the CAR structure to improve the safety of anti-CD44v6 CAR T given that anti-CD44v6 CAR T could potentially damage normal monocytes and keratinocytes.

Folate receptor β (FRβ) is expressed on ~70% of primary AML patient tumors, and its expression can be raised on AML blasts by all-trans retinoic acid (ATRA) [63,64]. In preclinical models, the effect of folate-conjugated drug therapy against FRβ-positive AML was improved when combined with ATRA [64]. Lynn *et al*. displayed the efficacy of anti-FRβ CART and the better efficacy of high-affinity anti-FRβ CART against AML cells *in vitro* and *in vivo* without toxicity on normal hematopoietic stem cells [65,66].

The main challenge in CAR T therapy for AML is the discovery of targets as favorable as CD19 for ALL. Perna *et al*. outlined a framework describing the ideal characteristics of CAR targets and established a methodological analysis for mining composite high-throughput surfaceome expression data [21]. They optimized combinative target selection based on expression profiles in malignant and normal tissues. This approach provided the foundation for intellectual design of CAR therapies for AML and a guide for combinatorial targeting, and they screened out four promising targets, namely, ADGRE2, CCR1, CD70, and LILRB2. To enhance the efficiency of targeting antigens while mitigating toxicity, the combinatorial strategy of dual CAR was projected.

CAR T therapy plus Allo-HSCT

Historically, Allo-HSCT is recommended for patients with refractory/relapsed acute leukemia during the CR period, and the minimal residual disease (MRD) level before transplantation was considered as an independent prognostic factor. Recently, the encouraging efficacy of CD19-targeted CAR T therapy has begun to challenge this algorithm for B cell malignancies. However, up to this date, no available clinical trial data on AML can be used to make a definitive Allo-HSCT recommendation. It remains uncertain whether patients in remission post-CAR T therapy should be administered with Allo-HSCT and whether CAR T therapy is sufficient for AML patients. According to these unsatisfactory long-term data on CD19 CAR T therapy for relapsed/refractory B-ALL [67–70] and the results of early clinical studies on anti-LeY CAR T for patients with relapsed AML [20], we speculate that CAR T therapy for AML should be considered as a "bridge" to Allo-HSCT rather than a replacement. CAR T therapy could strive for an opportunity for disease remission and induce a deeper MRD-level prior to Allo-HSCT. However, CAR T therapy could be used as a regimen for patients with relapsed disease post-transplantation. We believe that the consolidative Allo-HSCT following CAR T therapy in eligible AML patients could represent a very promising therapeutic strategy that has the potential to decrease the risk of relapse, although this idea warrants further investigation.

Summary and perspective

The value of CAR T therapy for AML remains to be determined. As the general background of CAR T technology evolves, CAR T therapies for AML will improve. The design of CAR with optimized antigen

recognition, different costimulatory, hinge, and transmembrane domains will improve the affinity of the CAR T and minimize toxicity. Further studies involving the optimization of *ex vivo* culture conditions and genetic manipulation of CAR structure are needed. Combination therapies may be necessary to achieve a better outcome.

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Compliance with ethics guidelines

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