THERAPEUTIC ADVANCES in
Hematology

Review

Recent advances of CAR-T cells in acute myeloid leukemia

Ther Adv Hematol

2025, Vol. 16: 1–19

DOI: 10.1177/20406207251326802

Huan Deng, Qi Wang, Xiaodong Tong, Zhiwei Cui, Yang Yang and Ying Xiang

***Abstract*:** Acute myeloid leukemia (AML), the most common type of leukemia in adults, is a highly heterogeneous and aggressive hematologic malignancy. Since the 20th century, the combination of cytosine arabinoside and anthracyclines has been the most common chemotherapy drug used to treat patients with AML. Although, new targeted medicines have emerged, such as midostaurin and gilteritinib targeting FMS-like tyrosine kinase 3 (FLT3), ivosidenib (isocitrate dehydrogenase 1 (IDH1) inhibitor) and enasidenib (IDH2 inhibitor) targeting IDH, and gemtuzumab ozogamicin targeting CD33, which have changed the treatment strategies of AML. But, until now, hematopoietic stem cell transplantation remains the best treatment option in most cases. However, treatment resistance and relapse are still the major consequences of disease progression in AML, highlighting the urgent need for novel therapeutic approaches. As an alternative, chimeric antigen receptor (CAR)-T cells are engineered T-cells developed as a breakthrough in cancer therapy in recent years, and explored and used in various tumor types. In particular, it has achieved remarkable efficacy in the field of relapsed and refractory B lymphocyte tumors. This review mainly summarizes and discusses the research progress and the clinical application of CAR-T cell immunotherapy in AML in recent years.

Keywords: acute myeloid leukemia, chimeric antigen receptor T cells, immunotherapy, target antigen

Received: 27 May 2024; revised manuscript accepted: 9 October 2024.

Acute myeloid leukemia (AML) is a malignant tumor characterized by an abnormal proliferation of blast in the bone marrow and peripheral blood, which is prevalent in the middle-aged and older age groups, with a median age of onset at 68 years old, and a 5-year relative survival rate of approximately 31.7%.[1] Due to the insidious onset and critical condition of AML, early detection and early treatment are particularly important. Patients with a precise diagnosis of AML are usually treated with intensive chemotherapy using anthracyclines and cytarabine[2] or hematopoietic stem cell transplantation (HSCT). Although, a large number of novel targeted medicines have subsequently emerged that have been approved for the treatment of AML. Small molecule agents such as the FMS-like tyrosine kinase 3 (FLT3) inhibitors (such as midostaurin and gilteritinib), isocitrate dehydrogenase inhibitors (such as ivosidenib and enasidenib), and B-cell lymphoma-2 (BCL-2) inhibitors (e.g., venetoclax) have significantly broadened the therapeutic options for AML. However, tolerance to small molecules is inevitable with long-term use. Moreover, many patients cannot undergo HSCT due to various clinical factors, including age and comorbidities such as infections or graft-versus-host disease (GVHD). Therefore, there is an urgent need to develop new therapeutic approaches to improve the long-term prognosis of AML.

The chimeric antigen receptor (CAR)-T cells, as a revolutionary new precision-targeted therapeutic technology, have been widely studied in various hematologic malignancies.[3] For example, CAR-T cell immunotherapy targeting CD19

Correspondence to:
Huan Deng
Department of Medical Laboratory, The People's Hospital of Leshan, No. 238, Baita Street, Shizhong District, Leshan, Sichuan 614000, China
hope96112@163.com

Qi Wang
Department of Medical Records and Statistics Room, The People's Hospital of Leshan, Leshan, Sichuan, China

Xiaodong Tong
Zhiwei Cui
Yang Yang
Ying Xiang
Department of Medical Laboratory, The People's Hospital of Leshan, Leshan, Sichuan, China

brings hope for treating patients with B-cell leukemia and lymphoma. Yet, the lack of the specific AML target antigens, immune escape, immune side effects (such as cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS)), heterogeneity of the target antigens, and generation of the inhibitory immune microenvironment pose challenges to research. Nonetheless, many researchers have been actively involved in this field and have reported some promising data. This review summarizes and discusses the progress of research on the structure and mechanism of CAR-T cells, relevant targets for treating AML, and clinical trials currently underway for the treatment of AML.

Structure of the CAR

CAR is an artificial synthetic receptor that combines multiple specific domains to recognize specific antigens, and mainly consists of four regions: the extracellular domain, the hinge domain, the transmembrane domain, and the intracellular signaling domain. The extracellular domain is a single-chain variable fragment (scFv) consisting of the heavy-chain variable region (VH) and the light-chain variable region (VL) of the monoclonal antibody (mAb) connected by a flexible junction, which is responsible for recognizing the relevant antigens on the surface of tumor cells.[4] The hinge domain (also known as spacer regions) is the region that extends from the transmembrane domain to the extracellular domain and provides flexibility to overcome spatial site resistance.[5] Differences in the length and composition of the hinge domain affect CAR flexibility, epitope recognition, and signaling, thereby affecting CAR function.[6] The transmembrane domain is a membrane protein that anchors CAR to the T-cell membrane, mainly CD3ζ, CD4, CD8α, and CD28.[5] The intracellular signaling domain mainly consists of the CD3ζ signaling domain and the co-stimulatory domain, which provides the persistence, quality, and strength of the T-cell response to cancer cell-specific antigens in order to enhance the anticancer efficacy of CAR-T cells.[7] Currently, five generations of CARs have been developed, and the main difference lies in the intracellular signaling domains.[1,8–10] Its structure is shown in Figure 1.

Mechanism of action of CAR-T cells

On binding of scFv on the surface of CAR-T cells to antigens on the surface of tumor cells, immunoreceptor tyrosine-based activation motifs (ITAM) in the CD3ζ signaling domain of the intracellular domain is phosphorylated by the lymphocyte-specific protein tyrosine kinase (Lck). The phosphorylated ITAM binds to ζ-associated protein 70 kDa (ZAP70), leading to its phosphorylation by LcK as well, which activates the protein.[11,12] Activated ZAP70 mediates downstream signaling and ultimately activates the effector function of CAR-T cells, causing them to secrete things such as perforin and granzyme to directly lyse cancer cells or to recruit endogenous immune cells to kill cancer cells by releasing cytokines.[11–13] The signaling of activated CAR-T cells is achieved by the CD3ζ signaling domain and co-stimulatory domains, which conduct various co-stimulatory signals depending on the different co-stimulatory domains used. For example, first-generation CAR-T cells with only the CD3ζ signaling domain were eliminated due to insufficient proliferation, short lifespan, and lack of cytokine secretion, among other drawbacks.[7] Based on the first generation of CAR-T cells, CAR-T cells with the addition of the co-stimulatory domain not only improve proliferation and cytotoxicity response but also prolong the lifespan of CAR-T cells.[5] Among them, the fourth generation of TRUCK cell, containing an nuclear factor of activated T-cells, enables CAR-T cells to secrete specific cytokines (e.g., interleukin-12 (IL-12)) to recruit and activate immune cells to destroy tumor cells.[14] As shown in Figure 2.

Targets for clinical application of CAR-T cell therapy for AML

CD33

CD33 is a glycoprotein belonging to the sialic acid-binding Ig-like lectin family. Since CD33 is expressed in approximately 90% of cancer cells in AML,[15] CD33 is an attractive target to become an immunotherapy for AML. The first report in the article about the use of anti-CD33 CAR-T cells for the treatment of AML patients was presented by Wang et al.[16] This clinical trial (NCT01864902) included one patient with relapsed/refractory (R/R) AML who was treated with an infusion of 1.12×10^9 autologous anti-CD33 CAR-T cells (carrying the 4-1BB co-stimulatory domain). Two weeks later, examination revealed a decrease in blasts from >50% before treatment to <6%, and the patient eventually died as a result of poor treatment. In another

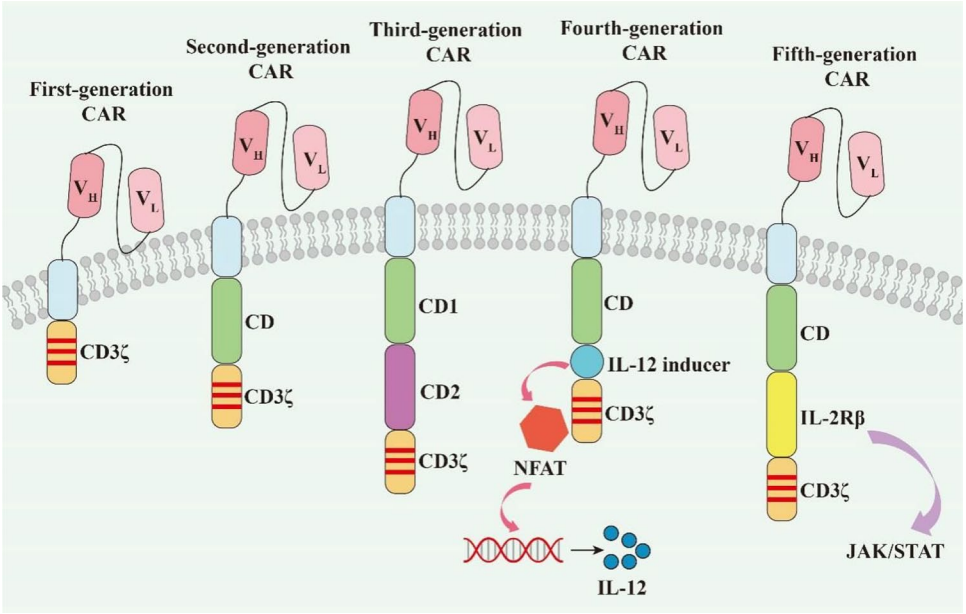

Figure 1. Different generations of CARs: The intracellular signaling domain of the first generation of CARs contains only the CD3ζ signaling domain with three ITAMs; the second-generation CAR has added a co-stimulatory domain (CD28 or 4-1BB, etc.) on the basis of the first generation to prolong the survival time of CAR-T cells and improve the antitumor ability of CAR-T cells; two co-stimulatory domains (e.g., CD28 and 4-1BB) have been added to the third-generation CAR to enhance cytokine production and killing; the fourth-generation CAR has introduced a transcription factor capable of triggering signals induced by cytokines or blocking some signaling pathways affecting the function of CAR-T cells. For example, TRUCK cell; and the fifth-generation CAR has added IL-2Rβ, which activates the JAK/STAT pathway.
CAR, chimeric antigen receptor; CD, co-stimulatory domain; IL, interleukin; ITAM, immunoreceptor tyrosine-based activation motif; JAK/STAT, janus kinase/signal transducer and activator of transcription; NFAT, nuclear factor of activated T-cells; TRUCK, T-cells redirected for universal cytokine-mediated killing.

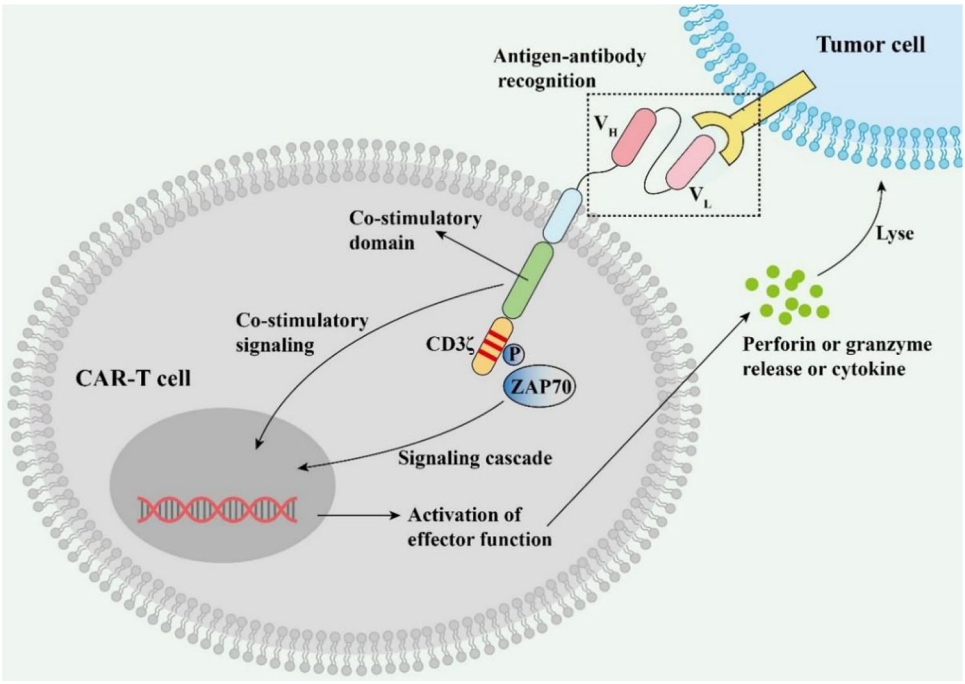

Figure 2. Mechanism diagram of CAR-T cells.
CAR, chimeric antigen receptor.

phase I clinical trial (NCT03126864), in order to evaluate the feasibility and safety of autologous anti-CD33 CAR-T cells, Tambaro et al.[17] created CAR constructs with 4-1BB and CD3ζ intracellular domains and enrolled three AML patients for treatment. Two of these patients developed CRS after infusion of anti-CD33 CAR-T cells, and one patient suffered from ICANS, and all of them eventually died due to disease progression. As mentioned above, CAR-T cell immunotherapy may contribute to severe and even life-threatening side effects for patients, such as CRS, ICANS, tumor lysis syndrome.[18]

In 2016, Cartellieri et al.[19] developed a novel modular technology called UniCAR, consisting of a universal CAR for T-cell inert manipulation and a tumor-specific targeting module (TM). The technology divides the signaling and antigen-binding domains of the traditional CAR into two separate components: the intracellular signaling domain with CD28/CD3ζ and the extracellular domain consisting of an scFv binding domain. The CD28 acts as the intracellular domain to mediate downstream signaling, enabling engineered T-cells to synthesize and secrete IL-2. Furthermore, the CD28 signaling prevents the death of activated T-cells and enhances the persistence of CAR-T cells. The scFv in the universal CAR does not recognize cell surface antigens, but rather identifies the peptide motifs of the human nuclear autoantigen La/SS-B. Therefore, engineered UniCAR T-cells remain inactive after reinfusion, and the only way to activate the toxicity mechanism of UniCAR T-cells is via TM. This study confirmed that UniCAR-T cells using anti-CD33 TM and anti-CD123 TM not only lyse AML cells but also that UniCAR remains inert in vivo without side effects when TM was not used. Liu et al.[15] developed a novel third-generation anti-CD33 CAR-T cell (3G.CAR33-T) with CD28 and 4-1BB co-stimulatory domains. They not only showed higher proliferation and potent toxicity against AML cells but also reduced the side effects on hematopoietic stem and progenitor cells (HSPCs) with CD33 knockout (KO), confirming that CD33 deletion in HSPC decreases the off-target utility of CAR-T cells immunotherapy, while preserving the tumor-killing ability. It is an effective and feasible approach.

CD123

CD123 is the alpha chain of the IL-3 receptor.[20] CD123 is one of the targets for the treatment of AML due to its overexpression on the surface of AML blasts and leukemia stem cells (LSCs).[20,21] In 2014, Gill et al.[22] published one of the first research results on anti-CD123 CAR-T cell therapy for AML. This study confirmed that anti-CD123 CAR-T cells (carrying 4-1BB and CD3ζ signaling domains) not only have multiple antigen-specific effector functions (such as cytokine production, degranulation) and a good anti-leukemic effect but also establish the body's immunological memory and a recall response to specific antigens. To eliminate the serious toxic side effects associated with anti-CD123 CAR-T cell immunotherapy, Tasian et al.[23] proposed three methods to improve the safety of anti-CD123 CAR-T cell therapy for AML, namely CAR-T cell depletion after leukemia attained remission. This study further demonstrated that CAR-T cell elimination does not affect following HSCT, adding to the feasibility of subsequently advancing the use of anti-CD123 CAR-T cells. In a phase I clinical trial (NCT04230265), Wermke et al.[24] used UniCAR-T-CD123 cells (containing CD28 co-stimulatory domain) to treat patients suffering from R/R AML, three patients successfully received treatment. Patient 1 showed partial response (PR) with a decrease in bone marrow blasts from 26% before treatment to 13%. Patient 2 had a reduction of bone marrow blasts from 20% pretreatment to 0%, demonstrating complete remission with incomplete hematologic recovery (CRi). Patient 3 had a decline in bone marrow blasts from 30% pretreatment to 2%, and CRi was shown.

FMS-like tyrosine kinase 3

FLT3 (CD135) is a membrane-bound glycosylated protein involved in normal hematopoiesis by controlling cell survival, proliferation, and differentiation.[25,26] However, the mutation of the *FLT3* gene leads to sustained activation of the FLT3 receptor, which prolongs the survival time and accelerates the increase of leukemia cells, thus shortening the overall survival of patients and is one of the predictors of poor prognosis.[25,27,28] The *FLT3* mutation is present in about 30% of all AML patients.[29] Therefore, *FLT3* has become one of the new targets for CAR-T cell immunotherapy. Chen et al.[30] published one of

the first preclinical studies on CAR-T cell immunotherapy targeting FLT3 in 2017. The authors created a second-generation CAR construct with intracellular domains of CD28 and CD3ζ. The study not only demonstrated, through in vivo and in vitro experiments, that anti-FLT3 CAR-T cells effectively eliminated FLT3(+) AML cells and increased interferon-γ (IFN-γ) production, prolonging the survival of AML mice without affecting the ability of hematopoietic stem cells to implant and differentiate. Similar results were obtained in the study by Niswander et al.,[31] who demonstrated that anti-FLT3 CAR-T cells had significant anti-leukemic effects with minor side effects on non-hematopoietic and hematopoietic tissues in the preclinical experiments. However, some of the findings contradicted those of Chen et al. and Niswander et al., the preclinical data by Jetani et al.[32] showed that anti-FLT3 CAR-T cells destroyed normal hematopoietic stem cells and impaired hematopoiesis. To reduce the risk of adverse events in anti-FLT3 CAR-T cell immunotherapy, Sommer et al.[33] reported a preclinical evaluation of the anti-FLT3 CAR-T cells with an off switch for AML treatment. After the anti-FLT3 CAR-T cells eliminated AML cells, rituximab was given to deplete the CAR-T cells, thereby limiting hematologic toxicity. In summary, all of the above data demonstrate the preclinical efficacy of anti-FLT3 CAR-T cells, which may help to support the clinical translation of CAR-T cells in the future.

Lewis Y

Lewis Y (LeY) is a carbohydrate-based antigen that is overexpressed in various malignant tumors but has limited expression in normal tissues. The anti-LeY CAR-T cell immunotherapy developed by Peinert et al.[34] was successfully validated in vitro experiments and mouse models. The T-cells not only effectively cleared LeY(+) tumor cells but also secreted IFN-γ. In a phase I clinical study (CTX 08-0002), Ritchie et al.[35] generated second-generation CAR-T cells by coupling scFv of mAb against the tumor-associated antigen LeY with CD28/CD3ζ signaling domain. The study included five patients with recurrent AML, and four patients who successfully received anti-LeY CAR-T cells therapy achieved different degrees of remission. One patient died of relapse, one achieved a transient cytogenetic remission, one exhibited a transient blast reduction, and the other achieved a cytomorphologic and immunophenotypic remission lasting 23 months. This study once again demonstrates the feasibility of CAR-T cells in the treatment of AML.

C-type lectin-like molecule-1

C-type lectin-like molecule-1 (CLL-1) is a type II transmembrane glycoprotein. Since CLL-1 is highly expressed in AML but not or lowly expressed in normal hematopoietic stem cells,[36] it is an ideal target for CAR-T cells to treat AML with less hematotoxicity.[37] Ataca Atilla et al.[38] optimized the anti-leukemic activity of CLL-1 CAR-T cells by incorporating transgenic IL-15 into CAR-T cell constructs. That study demonstrated that IL-15 enhanced expansion, toxicity, and persistence of CAR-T cells in vitro. However, more severe toxic side effects associated with the production of tumor necrosis factor α (TNFα), IL-15 and IL-15 were seen in AML xenograft mouse models. This could be achieved by using anti-TNFα antibodies and/or the activation of an inducible caspase nine safety switch by administration of dimerizing agent to reduce their toxic effects and maintain antitumor activity. In a clinical trial, Zhang et al.[39] successfully treated a patient with secondary AML with the fourth-generation anti-CLL-1 CAR-T cells (containing CD28-CD27-CD3ζ intracellular domains) and achieved long-term (10 months) CR, effectively proving that CAR-T cells targeting CLL-1 may be a valuable tool for the treatment of AML. In another phase I clinical trial (ChiCTR2000041054), Jin et al.[40] enrolled 10 patients with R/R AML. After receiving an infusion of anti-CLL-1 CAR-T cells, 7/10 patients achieved CR/CRi. The six patients who subsequently received HSCT were still alive at the last follow-up. This indicates that anti-CLL-1 CAR-T cells can offer an opportunity to prolong survival in patients with R/R AML and support the bridge HSCT therapy. Ma et al.[41] treated two AML patients who had relapsed after stem cell transplantation and had failed anti-CD38 CAR-T cell therapy by PD-1 silenced anti-CLL-1 CAR-T cells. Both patients were assessed on day 28 after infusion of anti-CLL-1 CAR-T cells and found to have achieved molecular CRi and maintained continuous remission for 8 and 3 months, respectively. This prompted the authors to conduct a prospective clinical trial (NCT04884984) to

confirm the efficacy, safety, and power of PD-1 knockdown anti-CLL-1 CAR-T cell therapy.

Natural Killer Group 2 member D

Natural killer group 2 member D (NKG2D) ligand, a type II transmembrane receptor, is widely expressed in various hematological malignancies, including AML and solid tumors, but is weakly expressed in healthy tissues, thus providing a useful target for immunotherapy. In an in vitro experiment, Driouk et al.[42] found that the expression of NKG2D was relatively low in AML but could be selectively increased by HDAC inhibitors in AML cells. Also, the anti-leukemic activity of anti-NKG2D CAR-T cells could be enhanced without affecting peripheral blood mononuclear cells. In a phase I trial of autologous CAR-T cells targeting NKG2D ligands in patients with AML, myelodysplastic syndrome (MDS), and multiple myeloma (MM; NCT02203825), seven patients with AML were treated with various doses of anti-NKG2D CAR-T cells. Among them, one AML patient developed "disease stabilization" defined by the new AML guidelines[43] at month 3 after treatment, remained clinically stable for 6 months without further treatment, and was alive at the last follow-up.[44] In another phase I clinical trial (NCT02203825) reported by Sallman et al.,[45] 16 patients (12 AML, 3 MM, 1 MDS) received different doses of autologous NKG2D CAR-T cells (CYAD-01) infusion, but only 12 patients were finally evaluated for efficacy. Among the 12 evaluable patients, 3/12 achieved objective remission, and 4/12 reached disease stabilization. Notably, two AML patients received allogeneic HSCT (allo-HSCT) after CYAD-01 treatment, maintaining remission for 5 and 61 months, respectively. Despite the small efficacy, this illustrates the anti-leukemic activity of NKG2D CAR-T cells.

CD7

CD7 is a transmembrane glycoprotein expressed in about 90% of lymphomas and lymphoblastic leukemias and about 30% of AML cases, but not in myeloid and erythroid cells.[46,47] Since CD7 is also expressed on the surface of T-cells, the production of anti-CD7 CAR-T cells requires the KO of CD7 from T-cells to prevent fratricide.[48] Such gene-edited anti-CD7 CAR-T cells not only resisted self-incineration but also effectively eliminated CD7-expressing AML cells and colony-forming cells, while preserving the normal function of bone marrow and erythroid progenitors.[49] To enhance the antitumor activity of anti-CD7 CAR-T cells, Hu et al.[50] constructed RD13-01 cells that contain anti-CD7 scFv, CD8 hinge, 4-1BB, CD3ζ, cytokine receptor γ chain (γc), and NK cell inhibitory receptor (NKi). According to the authors, the introduction of γc promoted IL-2 production in CAR-T cells and enhanced in vitro cytotoxicity, as reflected by enhanced cytolytic activity and higher levels of perforin/granzyme A. The NKi incorporation into CAR constructs reduced NK cell-mediated CAR-T cell lysis. To assess the safety and efficacy of RD13-01 cells, a phase I clinical trial (NCT04538599) was conducted by Hu et al.[50] A total of 12 patients were enrolled in the trial, including one patient with R/R AML. The patient achieved CRi on day 28 after treatment with RD13-01 cells. Subsequently, the patient underwent allo-HSCT, maintained long-term CR, and was still alive at follow-up 10 months later. This further demonstrates the feasibility of anti-CD7 CAR-T cell therapy for AML and also provides a guarantee for bridging allo-HSCT. The conventional allo-HSCT requires a pretreatment regimen (myeloablation and GVHD prophylaxis) before infusing hematopoietic stem cells, which not only has toxic effects but also clears residual CAR-T cells and reduces the efficacy of the treatment. Therefore, Hu et al.[51] presented a novel "all-in-one" strategy combining sequential CD7 CAR T-cell therapy with haploidentical HSCT without pharmacologic myeloablation or GVHD prophylaxis. A total of seven AML patients were recruited for the study, all of whom exhibited CRi after infusion of CAR-T cells, followed by haploidentical HSCT infusion at an interval of 19 days (median time). Four AML patients had full donor chimerism at 1 month after HSCT. The overall and disease-free survival rates at 1 year were 4/7 and 3/7, respectively. This approach directly realizes the "seamless interface" between CAR-T cell therapy and allo-HSCT, avoids the use of pharmacological myeloablation and GVHD prophylaxis agents before HSCT, reduces the toxicity of the agents on patients, and opens a new strategic pathway for allogeneic HSCT.

CD70

CD70, the ligand of CD27, belongs to the TNF family. It is highly expressed in AML cells and LSCs but not expressed or low expressed

in normal hematopoietic stem cells.[52,53] The second-generation anti-CD70 CAR-T cells containing the 4-1BB/CD3ζ intracellular signaling domain was constructed by Wu et al.[53] They proved strong cytotoxic effects of the anti-CD70 CAR-T cells on AML cells by in vitro experiments and validated in the AML mouse models, which showed potent anti-leukemic activity and prolonged survival. Similar results were obtained by Sauer et al.[54] The anti-CD70 CAR-T cells (based on the CD70 ligand CD27 as the CAR construct) created by the authors exhibited superior antitumor efficacy in both in vitro and in vivo experiments, while preserving normal hematopoietic stem cells, thus avoiding potential off-tumor toxicity, compared with standard CAR T cells based on anti-CD70 scFv.

CD38

CD38 is a type II transmembrane glycoprotein expressed on AML cells. Therefore, CD38 is one of the potential targets for the treatment of AML. Glisovic-Aplenc et al.[55] recently published a report that anti-CD38 CAR-T cells had potent anti-AML effects and prolonged the survival of AML mice. Due to the fact that CD38 expression in AML cells is lower than that in B lymphoma and myeloma cells, Yoshida et al.[56] found that the expression of CD38 on the surface of AML cells could be enhanced by all-trans retinoic acid (ATRA), which greatly improved the efficacy of anti-CD38 CAR-T cells in the treatment of AML. A clinical trial (NCT04351022) evaluated the clinical efficacy and safety of anti-CD38 CAR-T cell therapy in six patients with recurrent AML after allogeneic HSCT. In this clinical study, after 4 weeks of CAR-T cell therapy, three of the six patients achieved CR, one achieved CRi, five patients developed mild CRS (grades 1–2), and no patient developed ICANS, which preliminarily demonstrated the feasibility of anti-CD38 CAR-T cell therapy in AML patients.[57] To prevent antigen escape in AML, Atar et al.[58] developed an adapter CAR (AdCAR) platform that couples multiple adapter molecules (AMs) in the presence of specific linkers. The authors generated a group of AMs targeting the AML-associated antigens CD33, CD38, CD123, CD135, and CD371, and AdCAR-T cells were activated against target cells only in the presence of AMs. The authors proved via in vitro cell experiments and in vivo mouse models that AdCAR-T cells, in combination with multiple AMs, could successfully address intratumoral heterogeneity and antigen escape due to heterogeneous antigen expression. This multiplex targeting by AdCAR-T cells technology paves the way for precision immunotherapy.

CD44v6

CD44v6, a splice variant of the adhesion molecule CD44, is highly expressed in hematologic tumors such as AML, diffuse large B-cell lymphoma, and MM, but is lowly expressed in normal cells such as T-cells and monocytes.[59,60] Since the frequency of *FLT3* and DNA methyltransferase 3A (*DNMT3A*) mutations is about 37% and 23% in AML, respectively, AML patients with these mutations have higher CD44v6 expression, which often implies a poor prognosis. Thus, to show the advantage of anti-CD44v6 CAR-T cells in AML with *FLT3* or *DNMT3A* mutations, Tang et al.[60] constructed a CAR construct containing the CD44v6-scFv, CD8 hinge, 4-1BB co-stimulatory domain, and CD3ζ signaling domain. The authors found by in vitro and in vivo experiments that anti-CD44v6 CAR-T cells had strong anti-leukemic effects on CD44v6(+) AML cells, especially in AML cells with *FLT3* or *DNMT3A* mutations. This indicates that anti-CD44v6 CAR-T therapy may be an effective option for the treatment of AML, especially for AML with *FLT3* or *DNMT3A* mutations, conversely having negligible effects on normal tissue cells instead. Additionally, the authors' other preclinical study[61] also revealed that hypomethylating agents (HMAs) decitabine or azacitidine could enhance the antitumor effect of anti-CD44v6 CAR-T cells by upregulating CD44v6 expression in AML cells. Notably, decitabine exhibited superior effects. Hence, combining HMAs with anti-CD44v6 CAR-T cells is a more prospective combination therapy.

Folate receptor β

Folate receptor β (FRβ) is a myeloid antigen expressed in approximately 70% of AML cells.[62] Lynn et al.[63] described, first in 2015, the ability of humanized FRβ CAR-T cells to mediate AML cell regression without affecting CD34 hematopoietic stem cells. The expression of FRβ is also enhanced by ATRA. Similar results were obtained in a preclinical study, which additionally found that high-affinity anti-FRβ CAR-T cells had an

even more superior anti-leukemic effect.[64] However, so far, no clinical trials have applied anti-FRβ CAR-T cells to treat AML patients.

CD117

CD117 (C-KIT) is a homologous receptor of stem cell factor, expressed in about 80%–90% of AML cells, related to drug tolerance and poor prognosis.[65,66] Myburgh et al.[65] constructed anti-CD117 CAR-T cells from healthy donors and AML patients, and verified through in vitro and in vivo experiments that anti-CD117 CAR-T cells could effectively target CD117(+) cells. Finally, rituximab combined with antihuman thymocyte globulin was utilized to deplete the remaining CD117 CAR-T cells. Currently, CD117 targets are also in the clinical trial phase, such as trial NCT03473457, although this trial was terminated early due to less than expected efficacy.

B7-H3

B7-H3 (CD276) is a co-receptor of the B7 family of immune checkpoint molecules. It is highly expressed in some AML patients (mainly differentiated from monocytes) and various solid tumors.[67] Anti-B7-H3 CAR-T cells demonstrated significant antitumor activity without unacceptable toxicity to normal myeloid progenitor cells in vitro and in xenograft models, proving that B7-H3 is a potential target for CAR-T cell-targeted therapy of AML.[68] Similar results were confirmed in studies by others.[69]

Leukocyte immunoglobulin-like receptor B4

LILRB4 (ILT3) is the leukocyte immunoglobulin-like receptor-B family, expressed in all patients with monocyte-differentiated AML (FAB M4/M5). LILRB4 is a highly sensitive and specific marker that distinguishes monocyte-differentiated AML from other types of AML.[70] In a preclinical study, the novel anti-LILRB4 CAR-T cells generated by John et al.[71] showed potent anti-leukemic function and minimized the risk of off-tumor toxicity in both in vitro and in vivo experiments.

Granulocyte-macrophage colony-stimulating factor receptor

GMR is the granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor expressed in approximately 100% of juvenile myelomonocytic leukemia (JMML) and 63%–83% of AML.[72] NaKazawa[72] and Hasegawa et al.[73] created novel ligand-based CAR-T cells targeting GMR using a piggyBac-based gene transfer system. It was found that T-cells with a modified spacer (G4S) or a mutant ligand of GM-CSF (E21K) in CAR constructs not only showed potent antitumor effects but also exhibited negligible toxicity to most of the normal blood cells in vitro and AML xenograft mouse models, which is a new idea for CAR-T cells in the field of AML treatment. Subsequently, the authors performed a phase I/II clinical trial (jRCT20333210029) utilizing CAR-T cells targeting GMR to treat patients with AML and JMML.

Siglec-6

Siglec-6 belongs to the salivary acid-binding immunoglobulins expressed on approximately 60% of AML cells. The preclinical study by Jetani et al.[74] demonstrated that targeting Siglec-6 could lead to effective treatment of AML patients without inducing myeloablation, making it a new target for treating AML. CAR-T cell therapies targeting Siglec-6 may especially help patients who are medically unsuitable for HSCT or who have relapsed after transplantation.

T-cell immunoglobulin and mucin-3

TIM3 is a T-cell immunoglobulin and mucin-3 that plays an important role in regulating inflammatory responses.[75] Since TIM3 is highly expressed in most AML but not in normal hematopoietic stem cells, it is a promising target for clear AML cells.[76,77] Studies[78] demonstrated that anti-TIM3 CAR-T cells exhibited strong anti-leukemic activity against both AML cells and mouse models. Interestingly, CAR-T cells targeting TIM3 could also effectively kill primary LSCs isolated from patients, providing an opportunity to eradicate LSCs present in minimal residual disease (MRD).

Dual-target CAR-T cells

Factors such as high heterogeneity of AML cells, antigen escape, lack of specific target antigens, and the generation of immunosuppressive microenvironment contribute to the poor prognosis of patients, and these can be ameliorated by using dual-target CAR-T cells. Wang et al.[79]

constructed tandem bispecific CD123/CLL1 CAR-T cells (containing CD28 and OX40 co-stimulatory domains) that exhibited significant killing effects on both CLL1(+)/CD123(+) leukemia cells and primary AML tumor cells, compared to the ability of single-target CAR-T cells by in vitro experiments. This helps to overcome the challenges caused by tumor heterogeneity and antigen escape. Interestingly, Xie et al.[80] constructed the bicistronic CAR-T cells, with the suicide gene RQR8 introduced into the CAR construct, which not only could target AML cells with CD123 and/or CLL-1 but also showed significant anti-AML activity in animal transplantation models. Simultaneously, it could also eliminate excessive CAR-T cells in emergencies through the safety switch RQR8, providing a valuable and safe approach for treating AML. In 2024, Teppert et al.[81] constructed CAR'TCR-T cells co-expressing CD33-CAR and dNPM1-TCR to open a new therapeutic pathway for the treatment of AML. CAR'TCR-T cells under dual stimulation with CD33-CAR and dNPM1-TCR not only showed increased and prolonged antitumor activity in vitro but also eliminated AML cells in the xenograft mouse models, showing superior cytotoxic capabilities compared to individual CAR-T and TCR-T cells. In a phase I clinical study evaluating the safety and efficacy of dual-targeted CD33/CLL-1 CAR-T (cCAR-T) cells in AML, Liu et al.[82] found that a patient diagnosed with complex karyotype AML achieved MRD(−) CR after treatment with cCAR-T cells, and no leukemic blasts were detected by flow cytometry. Currently, other clinical trials on dual-targeted CAR-T cells are actively underway, such as a phase I trial (NCT06110208) evaluating the safety and efficacy of targeting CLL-1 and CD38 CAR-T cells for the treatment of R/R AML and a phase I/II trial targeting TIM3 with CD123 (NCT06125652).

Another specific type of dual-target CAR-T cell is the logically gated CAR-T cell. It is linked by signaling ("AND" gated or "NOT" gated) through two synthetic receptors that recognize different antigens. The new RevCAR-T cells constructed by Feldmann et al.,[83] which followed the "AND" gating logic for targeting combinations, increasing the antitumor effect of CAR-T cells and reducing the side effects of off-target effects. Richards et al.[84] developed a "NOT-Gated" CAR-T cell with an inhibitory CAR (iCAR) construct that efficiently targets AML cells. When the iCAR recognized targets expressed in normal tissues, it activated intracellular inhibitory signals to inhibit T-cell activation and killing, avoiding nontumor toxicity.

Novel CAR-T cells

The sharing of multiple antigens between AML and normal hematopoietic cells or other nontarget tissues makes conventional CAR-T cell immunotherapy susceptible to off-target effects, limiting the therapeutic efficacy of CAR-T cells; therefore, there is an urgent need to develop a switchable novel CAR construct to improve safety.

UniCAR, a novel modular technology with "ON" and "OFF" switching functions developed by Cartellieri et al.[19] in 2016, not only effectively eliminates AML cells but also turns off the antitumor effect of CAR-T cells by stopping TM infusion. It provides a solution to the safety problem of CAR-T cell applications. Subsequently, Sommer et al.[33] inserted a "suicide gene" into CAR-T cells targeting FLT3, causing them to express CD20, and then induced apoptosis of CAR-T cells using agents such as rituximab. Peschke et al.[85] also described an UniCAR system with a reversible on/off function for the treatment of AML, which was validated by in vitro and in vivo experiments. A phase I clinical trial of UniCAR-T cells targeting CD123 for treating AML (NCT04230265), although still ongoing, has shown encouraging signs of efficacy with some of its results.

"Biodegradable" CAR-T cells involve transient expression of T-cell CARs through transient transfection of mRNAs encoding CARs. In 2017, Smith et al.[86] developed a biodegradable nanoparticle carrying specific CAR genes that could program T-cells in vivo to recognize and attack cancer cells. Cummins et al.[87] initiated a clinical trial (NCT02623582) to explore the therapeutic efficacy of constructing "biodegradable" CAR-T cells by using electroporation to deliver mRNA from a CAR targeting CD123 into T-cells. Seven patients with R/R AML were enrolled in the study, and the five patients who received the infusion did not experience any clinically significant vascular, neurologic, or hematologic adverse

effects, although the effects were transient. Overall, this is a promising and worthwhile approach to explore.

Conditional rather than constitutive CAR expression is another way to avoid or mitigate toxicity. The activity of conditioned CAR-T cells can be modulated by adding small molecules. This CAR-T cell can not only exhibit cytotoxicity comparable to that of conventional CAR-T cells, but its toxicity can be reversibly attenuated by small molecules. The CAR-T cell constructed by Park et al.[88] could be directly controlled by the small molecule agent methotrexate. Appelbaum et al.[89] combined pharmacology with CAR to design a unique dimerizing agent-regulated immunoreceptor complex (DARIC33), which reversibly controls the effector function of CAR-T cells on AML via rapamycin. The authors obtained the first clinical data on the unique CAR-T cell platforms. The inducible CAR systems must be activated in the presence of inducing factors, such as doxycycline. Inducible CAR systems could also serve this purpose, in the presence of inducible factors, such as doxycycline,[90] to activate CAR expression and CAR-T cell function. This provides a new idea of the safety issues associated with conventional CAR-T cells.

The "IF-better" strategy is another innovative approach. This strategy modulates CAR-T cell activity based on the expression levels of antigens in leukemia and healthy cells, meaning that when target antigen 1 is overexpressed, the target cells can be recognized and cleared by CAR-T cells, but if target antigen 1 is low, the target cells can only be recognized and cleared by CAR-T cells in the presence of target antigen 2.[91,92] Haubner et al.[93] developed a novel combinatorial CAR construct (ADCLEC.syn1) consisting of an ADGRE2-targeting CAR and a chimeric co-stimulatory receptor (CCR) targeting CLEC12A, and conducted a preclinical study. The authors found that CLEC12A-CCR could enhance ADGRE2-CAR sensitivity, effectively eliminate AML cells with low or high ADGRE2 expression, and avoid antigen escape and off-target toxicity in AML. This brings a new idea for the safe treatment of AML with CAR-T cells at present.

In the emerging topic of genetic determinants of drug tolerance to CAR T-cell therapy in hematologic malignancies, dysregulation of the mevalonate, and Wnt pathways contributes to drug tolerance in TP53-mutant AML. Mueller et al.[94] argued that targeting these pathways is expected to improve CAR-T cell therapy for TP53-mutant AML, and experimentally demonstrated that inhibition of the mevalonate pathway or enhancement of the Wnt pathway could increase the sensitivity of AML cells to CAR-T cell-mediated killing, thus improving the killing efficacy of CAR-T cells.

Clinical trials

The results of preclinical studies have largely shown the effectiveness of CAR-T cells in fighting AML cells, leading to various clinical trials. Table 1 shows published clinical trials using CAR-T cells in AML. Table 2 presents the ongoing clinical trials. All data are from "clinicaltrials.gov."

Conclusion

Following the success of CAR T-cell therapy in treating B acute lymphoblastic leukemia and other BCLs, CAR T-cell therapy has also shown tremendous potential for treating AML, offering new hope for patients with limited treatment options. As, Pérez-Amill et al.[91] listed clinical trials that demonstrated favorable efficacy in the treatment of AML with CAR-T cells. Currently, multiple CAR constructs targeting different target antigens (such as CD33, CD123, FLT3, LeY, CLL-1, NKG2D, and CD7) have been developed and have shown varying degrees of success in preclinical trials. Some have even been used in clinical trials and have shown significant efficacy in inducing CR and prolonging survival in AML patients.

However, challenges remain in treating AML with CAR-T cells. First, one of the main reasons preventing the widespread use of CAR-T cells in AML patients is the lack of specific target antigens, which causes CAR-T cells to misidentify and attack normal cells, especially HSPCs in the bone marrow, when identifying and attacking tumor cells. This can suppress bone marrow function, resulting in persistent pancytopenia and other hematologic toxicities, which may even lead to severe infections.[95] Secondly, low expression or loss of target antigens (antigen escape) is one of the reasons for relapse in AML patients after treatment. Tumor cells can downregulate the

Table 1. The published clinical trials using CAR-T cells in AML.

Target	ID	Total of patients treated	CAR-T product	Dose	CRS	ICANS	Other adverse events	Domain	Efficacy after CAR-T infusion	LC	DOI
CD33	NCT01864902	1 AML	Autologous	1.12×10^9 cells	1/1	No	Pancytopenia, transient hyperbilirubinemia, cytotoxic activity escape of renascent CD33 blasts	4-1BB	Disease progression	No	10.1038/mt.2014.164
	NCT03126864	3 AML	Autologous	0.3×10^6/kg	2/3	1/3	TLS, acute kidney injury, mucositis, leukopenia, intermittent orthostatic hypotension, increased bilirubin, increased ALT and AST	4-1BB	Disease progression	Flu + Cy	10.1038/s41375-021-01232-2
	NCT03927261	24 (20 AML, 1 CMML, 3 MDS)	Autologous	$1.8–83 \times 10^6$ cells	17/24	No	NA	4-1BB	1 SD, 1 CRi, 1 CRh, 1 PR, 4 decreased bone marrow blasts, 16 NA	No or 14 Flu + Cy	10.1182/blood-2022-169142
	NCT03971799	19 AML	Autologous	$0.3–10 \times 10^6$/kg	13/19	1/19	Myeloid aplasia	NA	2 MRD-/CR, 17 NA	Flu + Cy	10.1182/blood-2023-179667
	NCT04835519	4 AML	Autologous, donor	0.5×10^6/kg	4/4	2/4	Neutropenia, monocytopenia, thrombocytopenia	4-1BB	2 MRD−/CRi1 MRD+/CRi, 1 NR	Flu + Cy	10.1038/s41467-024-50485-9
CD33	NCT05105152	3 AML	Autologous	$1–10 \times 10^6$/kg	1/3	No	Increased sites of hemorrhagic necrosis	4-1BB	NA	Yes	10.1172/JCI162593
CLL-1	NCT03222674	4 AML	Autologous	$1–1.98 \times 10^6$/kg	3/4	1/4	Neutropenia, anemia	CD28/CD27	3 CRm/MRD−, 1 poor response	Flu + Cy	10.1158/1078-0432.ccr-20-4543
	NCT03222674	8 AML	Autologous	$0.35–1 \times 10^6$/kg	8/8	No	Pancytopenia	4-1BB	4 CRm/MRD−, 2 CRm/MRD+, 1 PR, 1 SD	Flu + Cy	10.1038/s41375-022-01703-0
	NCT04884984	2 AML	Autologous, donor	$5–10 \times 10^6$/kg	2/2	No	Neutropenia, thrombocytopenia	CD28/OX40	1 MRD−/CR, 1 MRD−/CRi	Cy	PMID: 35261791
	NCT03222674	7 AML	NA	$9.4–19.8 \times 10^5$/kg	7/7	1/7	Impaired liver function, pneumonia, neutropenia, thrombocytopenia, monocytopenia, anemia	CD28/CD27 or 4-1BB	2 MRD−/CR, 3 MRD+/CR, 2 PR	Flu + Cy	10.1002/cam4.5916

(Continued)

Table 1. (Continued)

Target	ID	Total of patients treated	CAR-T product	Dose	CRS	ICANS	Other adverse events	Domain	Efficacy after CAR-T infusion	LC	DOI
CD38	NCT04351022	6 AML	Autologous, donor	$6.1–10\times10^6$/kg	6/6	No	Neutropenia, thrombocytopenia	CD28/4-1BB	3 CR, 1 CRi, 2 NR	Flu + Cy	10.1186/s13045-021-01092-4
CD7	NCT04762485	1 AML	Autologous	$1–4\times10^6$/kg	1/1	No	Hemocytopenia, impaired liver function	4-1BB	MLFS	Flu + Cy	10.1186/s40164-022-00318-6
	NCT04938115	10 AML	Autologous, donor	$0.5–1\times10^6$/kg	10/10	No	Lung infection	4-1BB	6 MRD−/CR, 1 MRD+/CR, 3 NR	Flu + Cy	10.1182/blood-2023-179086
	NCT04538599	12 (7 T-ALL, 4 T-LBL, 1 AML)	Donor	$1–3\times10^7$/kg	10/12 ⩽ grade 2	No	Neutropenia, sepsis	4-1BB	4 CRi, 3 CR, 2 PR, 2 NR, 1 NA	Flu + Cy	10.1038/s41422-022-00721-y
	NCT04599556/ NCT04538599	10 (7 AML, 2 T-ALL, 1 T-LBL)	Donor, Universal	$2–5\times10^6$/kg	9/10	No	Pancytopenia, sepsis, infection	4-1BB	9 MRD−/CRi, 1 MRD+/CRi	Flu + Cy	10.1056/NEJMoa2313812
CD123	NCT04318678	5 AML	Autologous	$0.3–1\times10^6$/kg	1/1	No	Transient hemocytopenia	CD28	1 PR, 1 CR, 3 NR	Flu + Cy	10.1182/blood-2022-170201
	NCT02159495	7 AML	Autologous, donor	50–200M	No ⩾ grade 3	No ⩾ grade 3	Transient hemocytopenia, rash	CD28	1 MLFS, 1 CRi, 1 CR, 3 SD, 1 NA	Flu + Cy	10.1158/2326-6074.TUMIMM18-PR14
	NCT04106076	16 AML	Donor	$2.5–30.3\times10^5$/kg	15/16	1/16	Hemocytopenia	NA	2 SD, 1 MRD-/CR, 1 MLFS, 12 NA	Flu + Cy or Flu + Cy + Ale	10.1182/blood-2022-169928
	NCT04230265	3 AML	Autologous	$10–25\times10^7$ cells	2/3	No	Neutropenia, thrombocytopenia	CD28	2 CRi, 1 PR	Flu + Cy	10.1182/blood.2020009759
NKG2D	NCT02203825	12 (7 AML, 5 MM)	Autologous	$1–30\times10^6$ cells	No	No	Neutropenia, thrombocytopenia, rash, anemia, infection	DAP10	All treatment failures	No	10.1158/2326-6066.CIR-18-0307
	NCT03018405	16 (12 AML, 3 MM, 1 MDS)	Autologous	$0.3–3\times10^9$ cells	15/16	No	Lymphopenia, neutropenia, thrombocytopenia, anemia, etc.	DAP10	3 CRi or marrow CR, 4 SD, 5 disease progression, 4 nonevaluable	No	10.1016/S2352-3026(22):00378-7
CLL1/ CD33	NCT03795779	1 AML	NA	1×10^6/kg	NA	NA	NA	NA	MRD−/CR	Flu + Cy	10.1182/blood-2018-99-110579

Ale, alemtuzumab;ALT, alanine aminotransferase; AML, acute myeloid leukemia; AST, aspartate aminotransferase; CMML, chronic myelomonocytic leukemia; CR, complete remission; CRh, CR with partial hematologic recovery; CRi, CR with incomplete hematologic recovery; CRm, morphologic CR; CRS, cytokine release syndrome; Cy, cyclophosphamide; Flu, fludarabine; ICANS, immune effector cell-associated neurotoxicity syndrome; LC, lymphodepletion chemotherapy; MDS, myelodysplastic syndrome; MLFS, morphologic leukemic-free state; MM, multiple myeloma; MRD, minimal residual disease; NA, not available; NR, no remission or nonresponse; PR, partial remission; SD, stable disease; T-ALL, T acute lymphoblastic leukemia; T-LBL, T-cell lymphoblastic lymphoma; TLS, tumor lysis syndrome.

Table 2. The ongoing clinical trials using CAR-T cells in AML.

Target	ID	LC	Dose	CAR-T product	Status	Phase
CD33	NCT05473221	Yes	$3–9 \times 10^6$/kg	Autologous	No	I
	NCT06326021	NA	$0.5–1 \times 10^6$/kg	Autologous/donor	Recruiting	I
	NCT05672147	Yes	NA	Autologous/donor	Recruiting	I
	NCT05984199	NA	NA	Donor	Recruiting	I/II
	NCT05445765	Yes	NA	NA	No	I
	NCT02799680	NA	NA	Donor	Unknown	I
	NCT05945849	NA	NA	Donor	Recruiting	I
CLL1	NCT05252572	NA	$2–8 \times 10^6$/kg	Donor	Recruiting	I
	NCT05467202	Yes	$3–9 \times 10^6$/kg	Autologous	No	I
	NCT04923919	NA	NA	Autologous	Recruiting	I
	NCT04219163	Yes	$0.1–1 \times 10^8/m^2$	NA	Recruiting	I
	NCT06128044	Yes	NA	Donor	Recruiting	I
CD38	NCT05239689	NA	$2–8 \times 10^6$/kg	Donor	Recruiting	I
ILT3	NCT04803929	NA	NA	Autologous	Recruiting	I
CD123	NCT03796390	NA	NA	Autologous	Unknown	I
	NCT03556982	Yes	NA	Autologous/donor	Unknown	I/II
	NCT04265963	NA	NA	NA	Recruiting	I/II
	NCT04272125	NA	NA	NA	Recruiting	I/II
	NCT04014881	NA	NA	NA	Unknown	I
	NCT03585517	Yes	NA	NA	Completed	I
	NCT03672851	Yes	$0.5–2 \times 10^6$/kg	Autologous	Terminated	I
	NCT03114670	NA	NA	Donor	Unknown	I
	NCT05949125	Yes	NA	Donor	Recruiting	I
	NCT03190278	Yes	NA	Donor	Recruiting	I
CD19	NCT04257175	Yes	$0.5–1.5 \times 10^6$/kg	Autologous	Recruiting	II/III
	NCT03896854	NA	$1–20 \times 10^6$/kg	NA	Recruiting	I/II
Siglec-6	NCT05488132	NA	NA	Autologous	Recruiting	I/II
CD7	NCT04033302	NA	NA	Autologous/donor	Unknown	I/II
CD7	NCT05907603	NA	2×10^8/once	NA	Recruiting	I
	NCT05377827	Yes	NA	Donor	Recruiting	I

(Continued)

Table 2. (Continued)

Target	ID	LC	Dose	CAR-T product	Status	Phase
FLT3	NCT05023707	NA	NA	NA	Recruiting	I/II
	NCT05445011	Yes	$1–4 \times 10^8$	NA	Recruiting	I
	NCT05432401	Yes	$1–4 \times 10^8$	NA	Recruiting	I
	NCT05017883	Yes	NA	Autologous	Recruiting	NA
CD70	NCT04662294	NA	NA	NA	Recruiting	I
	NCT06326463	Yes	NA	Autologous	Recruiting	I
NKG2D	NCT04658004	NA	NA	NA	No	I
Lewis Y	NCT01716364	NA	NA	Autologous	Unknown	I
B7-H3	NCT04692948	NA	NA	Autologous	Unknown	N
ADGRE2	NCT05463640	Yes	$3–9 \times 10^6$/kg	Autologous	No	I
ADGRE2/ CLEC12A	NCT05748197	Yes	$1–45 \times 10^7$	NA	Recruiting	I
TIM3/CD123	NCT06125652	NA	NA	Autologous	Recruiting	I/II
CLL1/CD33	NCT05467254	Yes	$3–9 \times 10^6$/kg	Autologous	No	I
CD33/CD123	NCT06420063	Yes	NA	NA	Recruiting	I/II
	NCT04156256	NA	NA	NA	Unknown	I
CLL1/CD38	NCT06110208	NA	NA	NA	Recruiting	I
CD123/CLL1	NCT03631576	NA	NA	NA	Unknown	II/III
CD33/CLL1	NCT05248685	Yes	$0.5–5 \times 10^6$/kg	Autologous	Unknown	I
	NCT05016063	Yes	0.5×10^6/kg	NA	Unknown	I

Trials that were terminated early due to efficacy not meeting expectations or funding issues are not included.
AML, acute myeloid leukemia; CAR, chimeric antigen receptor; CLL-1, C-type lectin-like molecule-1; LC, lymphodepletion chemotherapy; NA, not available; No, not yet recruiting.

expression of target antigens by gene regulation, making it difficult for CAR-T cells to effectively recognize and kill cancer cells, which ultimately leads to a decrease in therapeutic effect. Third, immune-related side effects are also a major challenge limiting the widespread application of CAR-T cell therapy for AML, such as CRS and ICANS. CRS is a common complication associated with CAR-T cell therapy for AML. As CAR-T cells are activated, large amounts of cytokines are released into the blood, leading to a systemic inflammatory response and potential organ damage. Meanwhile, as T-cells and peripherally activated monocytes enter the central nervous system, ICANS can also occur. In addition, the heterogeneity of AML and the immunosuppressive tumor microenvironment generation also pose challenges. Despite these challenges, new strategies and potential solutions continue to evolve, such as creating multitargeted CAR-T cells, CAR-T cells with "suicide genes" and "safety switches."

With the in-depth research and continuous advancements in CAR-T cell technology, CAR-T cells in combination with other treatments such as radiotherapy, chemotherapy, immune checkpoint inhibitors, targeted therapies, or allo-HSCT are also a viable option worth pursuing, which can enhance therapeutic efficacy and reduce side effects. For instance, CAR-T cell therapy combined with radiotherapy or chemotherapy can exert synergistic antitumor effects; combined with PD-1/PD-L1 inhibitors can prevent the immune escape of tumor cells by blocking the PD-1/PD-L1 signaling pathway; and combined with allo-HSCT can rebuild the patient's immune system and support the sustained antitumor effects of CAR-T cells.[96] These combination therapies comprehensively target tumors through multiple mechanisms to improve patient survival and also provide new insights and strategies to enhance the clinical application of CAR-T cell therapy.

In summary, CAR-T therapy has changed the treatment landscape for AML, offering new hope for patients who have failed to use traditional therapies. As more research is ongoing, CAR-T cells are expected to become an option for the treatment of AML.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Author contributions
Huan Deng: Conceptualization; Investigation; Writing – original draft.

Qi Wang: Data curation.

Xiaodong Tong: Conceptualization.

Zhiwei Cui: Resources.

Yang Yang: Supervision.

Ying Xiang: Writing – review & editing.

Acknowledgements
None.

Funding
The authors received no financial support for the research, authorship, and/or publication of this article.

Competing interests
The authors declare that there is no conflict of interest.

Availability of data and materials
Data sharing is not applicable to this article as no data sets were generated or analyzed during this study.

ORCID iD
Huan Deng https://orcid.org/0009-0008-2209-3654

References

1. Zarychta J, Kowalczyk A, Krawczyk M, et al. CAR-T cells immunotherapies for the treatment of acute myeloid leukemia-recent advances. *Cancers (Basel)* 2023; 15: 2944.
2. Peroni E, Randi ML, Rosato A, et al. Acute myeloid leukemia: from NGS, through scRNA-seq, to CAR-T. Dissect cancer heterogeneity and tailor the treatment. *J Exp Clin Cancer Res* 2023; 42: 259.
3. Shah BD, Bishop MR, Oluwole OO, 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.
4. Daei Sorkhabi A, Mohamed Khosroshahi L, Sarkesh A, et al. The current landscape of CAR T-cell therapy for solid tumors: mechanisms, research progress, challenges, and counterstrategies. *Front Immunol* 2023; 14: 1113882.
5. Sterner RC and Sterner RM. CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer J* 2021; 11: 69.
6. Hudecek M, Sommermeyer D, Kosasih PL, et al. The nonsignaling extracellular spacer domain of chimeric antigen receptors is decisive for in vivo antitumor activity. *Cancer Immunol Res* 2015; 3: 125–135.
7. Huang R, Li X, He Y, et al. Recent advances in CAR-T cell engineering. *J Hematol Oncol* 2020; 13: 86.

8. Zhang ZZ, Wang T, Wang XF, et al. Improving the ability of CAR-T cells to hit solid tumors: challenges and strategies. *Pharmacol Res* 2022; 175: 106036.

9. Chmielewski M and Abken H. TRUCKS, the fourth-generation CAR T cells: current developments and clinical translation. *Adv Cell Gene Ther* 2020; 3: e84.

10. Abbasi S, Totmaj MA, Abbasi M, et al. Chimeric antigen receptor T (CAR-T) cells: novel cell therapy for hematological malignancies. *Cancer Med* 2023; 12: 7844–7858.

11. Benmebarek MR, Karches CH, Cadilha BL, et al. Killing mechanisms of chimeric antigen receptor (CAR) T cells. *Int J Mol Sci* 2019; 20: 1283.

12. Larson RC and Maus MV. Recent advances and discoveries in the mechanisms and functions of CAR T cells. *Nat Rev Cancer* 2021; 21: 145–161.

13. Wu W, Zhou Q, Masubuchi T, et al. Multiple signaling roles of CD3epsilon and its application in CAR-T cell therapy. *Cell* 2020; 182: 855–871 e823.

14. Alnefaie A, Albogami S, Asiri Y, et al. Chimeric antigen receptor T-cells: an overview of concepts, applications, limitations, and proposed solutions. *Front Bioeng Biotechnol* 2022; 10: 797440.

15. Liu Y, Wang S, Schubert ML, 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.

16. Wang QS, Wang Y, Lv HY, et al. Treatment of CD33-directed chimeric antigen receptor-modified T cells in one patient with relapsed and refractory acute myeloid leukemia. *Mol Ther* 2015; 23: 184–191.

17. Tambaro FP, Singh H, Jones E, et al. Autologous CD33-CAR-T cells for treatment of relapsed/refractory acute myelogenous leukemia. *Leukemia* 2021; 35: 3282–3286.

18. Bachmann M. The UniCAR system: a modular CAR T cell approach to improve the safety of CAR T cells. *Immunol Lett* 2019; 211: 13–22.

19. Cartellieri M, Feldmann A, Koristka S, et al. Switching CAR T cells on and off: a novel modular platform for retargeting of T cells to AML blasts. *Blood Cancer J* 2016; 6: e458.

20. El Achi H, Dupont E, Paul S, et al. CD123 as a biomarker in hematolymphoid malignancies: principles of detection and targeted therapies. *Cancers (Basel)* 2020; 12: 3087.

21. Testa U, Pelosi E and Castelli G. CD123 as a therapeutic target in the treatment of hematological malignancies. *Cancers (Basel)* 2019; 11: 1358.

22. Gill S, Tasian SK, Ruella M, et al. Preclinical targeting of human acute myeloid leukemia and myeloablation using chimeric antigen receptor-modified T cells. *Blood* 2014; 123: 2343–2354.

23. Tasian SK, Kenderian SS, Shen F, et al. Optimized depletion of chimeric antigen receptor T cells in murine xenograft models of human acute myeloid leukemia. *Blood* 2017; 129: 2395–2407.

24. Wermke M, Kraus S, Ehninger A, et al. Proof of concept for a rapidly switchable universal CAR-T platform with UniCAR-T-CD123 in relapsed/refractory AML. *Blood* 2021; 137: 3145–3148.

25. Pedersen MG, Moller BK and Bak RO. Recent Advances in the development of anti-FLT3 CAR T-cell therapies for treatment of AML. *Biomedicines* 2022; 10: 2441.

26. Nitika Wei J and Hui AM. Role of Biomarkers in FLT3 AML. *Cancers (Basel)* 2022; 14: 1164.

27. Warren M, Luthra R, Yin CC, et al. Clinical impact of change of FLT3 mutation status in acute myeloid leukemia patients. *Mod Pathol* 2012; 25: 1405–1412.

28. Daver N, Venugopal S and Ravandi F. FLT3 mutated acute myeloid leukemia: 2021 treatment algorithm. *Blood Cancer J* 2021; 11: 104.

29. Fedorov K, Maiti A and Konopleva M. Targeting FLT3 mutation in acute myeloid leukemia: current strategies and future directions. *Cancers (Basel)* 2023; 15: 2312.

30. Chen L, Mao H, Zhang J, et al. Targeting FLT3 by chimeric antigen receptor T cells for the treatment of acute myeloid leukemia. *Leukemia* 2017; 31: 1830–1834.

31. Niswander LM, Graff ZT, Chien CD, et al. Potent preclinical activity of FLT3-directed chimeric antigen receptor T-cell immunotherapy against FLT3- mutant acute myeloid leukemia and KMT2A-rearranged acute lymphoblastic leukemia. *Haematologica* 2023; 108: 457–471.

32. Jetani H, Garcia-Cadenas I, Nerreter T, et al. CAR T-cells targeting FLT3 have potent activity against FLT3(−)ITD(+) AML and

act synergistically with the FLT3-inhibitor crenolanib. *Leukemia* 2018; 32: 1168–1179.

33. Sommer C, Cheng HY, Nguyen D, et al. Allogeneic FLT3 CAR T cells with an off-switch exhibit potent activity against AML and can be depleted to expedite bone marrow recovery. *Mol Ther* 2020; 28: 2237–2251.
34. Peinert S, Prince HM, Guru PM, et al. Gene-modified T cells as immunotherapy for multiple myeloma and acute myeloid leukemia expressing the Lewis Y antigen. *Gene Therapy* 2010; 17: 678–686.
35. Ritchie DS, Neeson PJ, Khot A, et al. Persistence and efficacy of second generation CAR-T cell against the LeY antigen in acute myeloid leukemia. *Mol Ther* 2013; 21: 2122–2129.
36. Ma H, Padmanabhan IS, Parmar S, et al. Targeting CLL-1 for acute myeloid leukemia therapy. *J Hematol Oncol* 2019; 12: 41.
37. Zhang H, Bu C, Peng Z, et al. Characteristics of anti-CLL1 based CAR-T therapy for children with relapsed or refractory acute myeloid leukemia: the multi-center efficacy and safety interim analysis. *Leukemia* 2022; 36: 2596–2604.
38. Ataca Atilla P, McKenna MK, Tashiro H, et al. Modulating TNFα activity allows transgenic IL15-expressing CLL-1 CAR T cells to safely eliminate acute myeloid leukemia. *J Immunother Cancer* 2020; 8: e001229.
39. Zhang H, Gan WT, Hao WG, et al. Successful anti-CLL1 CAR T-cell therapy in secondary acute myeloid leukemia. *Front Oncol* 2020; 10: 685.
40. Jin X, Zhang M, Sun R, et al. First-in-human phase I study of CLL-1 CAR-T cells in adults with relapsed/refractory acute myeloid leukemia. *J Hematol Oncol* 2022; 15: 88.
41. Ma YJ, Dai HP, Cui QY, et al. Successful application of PD-1 knockdown CLL-1 CAR-T therapy in two AML patients with post-transplant relapse and failure of anti-CD38 CAR-T cell treatment. *Am J Cancer Res* 2022; 12: 615–621.
42. Driouk L, Gicobi JK, Kamihara Y, et al. Chimeric antigen receptor T cells targeting NKG2D-ligands show robust efficacy against acute myeloid leukemia and T-cell acute lymphoblastic leukemia. *Front Immunol* 2020; 11: 580328.
43. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood* 2017; 129: 424–447.
44. Baumeister SH, Murad J, Werner L, et al. Phase I trial of autologous CAR T cells targeting NKG2D ligands in patients with AML/MDS and multiple myeloma. *Cancer Immunol Res* 2019; 7: 100–112.
45. Sallman DA, Kerre T, Havelange V, et al. CYAD-01, an autologous NKG2D-based CAR T-cell therapy, in relapsed or refractory acute myeloid leukaemia and myelodysplastic syndromes or multiple myeloma (THINK): haematological cohorts of the dose escalation segment of a phase 1 trial. *Lancet Haematol* 2023; 10: e191–e202.
46. Tiftik N, Bolaman Z, Batun S, et al. The importance of CD7 and CD56 antigens in acute leukaemias. *Int J Clin Pract* 2004; 58: 149–152.
47. Marofi F, Rahman HS, Al-Obaidi ZMJ, et al. Novel CAR-T therapy is a ray of hope in the treatment of seriously ill AML patients. *Stem Cell Res Ther* 2021; 12: 465.
48. Gomes-Silva D, Srinivasan M, Sharma S, et al. CD7-edited T cells expressing a CD7-specific CAR for the therapy of T-cell malignancies. *Blood* 2017; 130: 285–296.
49. Gomes-Silva D, Atilla E, Atilla PA, et al. CD7 CAR T cells for the therapy of acute myeloid leukemia. *Mol Ther* 2019; 27: 272–280.
50. Hu Y, Zhou Y, Zhang M, et al. Genetically modified CD7-targeting allogeneic CAR-T cell therapy with enhanced efficacy for relapsed/refractory CD7-positive hematological malignancies: a phase I clinical study. *Cell Res* 2022; 32: 995–1007.
51. Hu Y, Zhang M, Yang T, et al. Sequential CD7 CAR T-cell therapy and allogeneic HSCT without GVHD prophylaxis. *N Engl J Med* 2024; 390: 1467–1480.
52. Riether C, Schurch CM, Buhrer ED, et al. CD70/CD27 signaling promotes blast stemness and is a viable therapeutic target in acute myeloid leukemia. *J Exp Med* 2017; 214: 359–380.
53. Wu G, Guo S, Luo Q, et al. Preclinical evaluation of CD70-specific CAR-T cells targeting acute myeloid leukemia. *Front Immunol* 2023; 14: 1093750.
54. Sauer T, Parikh K, Sharma S, et al. CD70-specific CAR T cells have potent activity against acute myeloid leukemia without HSC toxicity. *Blood* 2021; 138: 318–330.

55. Glisovic-Aplenc T, Diorio C, Chukinas JA, et al. CD38 as a pan-hematologic target for chimeric antigen receptor T cells. *Blood Adv* 2023; 7: 4418–4430.

56. Yoshida T, Mihara K, Takei Y, et al. All-trans retinoic acid enhances cytotoxic effect of T cells with an anti-CD38 chimeric antigen receptor in acute myeloid leukemia. *Clin Transl Immunology* 2016; 5: e116.

57. Cui Q, Qian C, Xu N, et al. CD38-directed CAR-T cell therapy: a novel immunotherapy strategy for relapsed acute myeloid leukemia after allogeneic hematopoietic stem cell transplantation. *J Hematol Oncol* 2021; 14: 82.

58. Atar D, Ruoff L, Mast AS, et al. Rational combinatorial targeting by adapter CAR-T-cells (AdCAR-T) prevents antigen escape in acute myeloid leukemia. *Leukemia* 2024; 38: 2183–2195.

59. Gutjahr JC, Szenes E, Tschech L, et al. Microenvironment-induced CD44v6 promotes early disease progression in chronic lymphocytic leukemia. *Blood* 2018; 131: 1337–1349.

60. Tang L, Huang H, Tang Y, et al. CD44v6 chimeric antigen receptor T cell specificity towards AML with FLT3 or DNMT3A mutations. *Clin Transl Med* 2022; 12: e1043.

61. Tang L, Kong Y, Wang H, et al. Demethylating therapy increases cytotoxicity of CD44v6 CAR-T cells against acute myeloid leukemia. *Front Immunol* 2023; 14: 1145441.

62. Ross JF, Wang H, Behm FG, et al. Folate receptor type beta is a neutrophilic lineage marker and is differentially expressed in myeloid leukemia. *Cancer* 1999; 85: 348–357.

63. Lynn RC, Poussin M, Kalota A, et al. Targeting of folate receptor beta on acute myeloid leukemia blasts with chimeric antigen receptor-expressing T cells. *Blood* 2015; 125: 3466–3476.

64. Lynn RC, Feng Y, Schutsky K, et al. High-affinity FRβ-specific CAR T cells eradicate AML and normal myeloid lineage without HSC toxicity. *Leukemia* 2016; 30: 1355–1364.

65. Myburgh R, Kiefer JD, Russkamp NF, et al. Anti-human CD117 CAR T-cells efficiently eliminate healthy and malignant CD117-expressing hematopoietic cells. *Leukemia* 2020; 34: 2688–2703.

66. Branella GM, Lee JY, Okalova J, et al. Ligand-based targeting of c-kit using engineered γδ T cells as a strategy for treating acute myeloid leukemia. *Front Immunol* 2023; 14: 1294555.

67. Guery T, Roumier C, Berthon C, et al. B7-H3 protein expression in acute myeloid leukemia. *Cancer Med* 2015; 4: 1879–1883.

68. Lichtman EI, Du H, Shou P, et al. Preclinical evaluation of B7-H3-specific chimeric antigen receptor T cells for the treatment of acute myeloid leukemia. *Clin Cancer Res* 2021; 27: 3141–3153.

69. Fan S, Wang T, You F, et al. B7-H3 chimeric antigen receptor-modified T cell shows potential for targeted treatment of acute myeloid leukaemia. *Eur J Med Res* 2023; 28: 129.

70. Dobrowolska H, Gill KZ, Serban G, et al. Expression of immune inhibitory receptor ILT3 in acute myeloid leukemia with monocytic differentiation. *Cytometry B Clin Cytom* 2013; 84: 21–29.

71. John S, Chen H, Deng M, et al. A novel anti-LILRB4 CAR-T cell for the treatment of monocytic AML. *Mol Ther* 2018; 26: 2487–2495.

72. Nakazawa Y. [A first-in-human clinical trial of piggyBac transposon-mediated GMR CAR-T cells against CD116-positive acute myeloid leukemia and juvenile myelomonocytic leukemia]. *Rinsho Ketsueki* 2022; 63: 776–781.

73. Hasegawa A, Saito S, Narimatsu S, et al. Mutated GM-CSF-based CAR-T cells targeting CD116/CD131 complexes exhibit enhanced anti-tumor effects against acute myeloid leukaemia. *Clin Transl Immunology* 2021; 10: e1282.

74. Jetani H, Navarro-Bailón A, Maucher M, et al. Siglec-6 is a novel target for CAR T-cell therapy in acute myeloid leukemia. *Blood* 2021; 138: 1830–1842.

75. Tabata R, Chi S, Yuda J, et al. Emerging immunotherapy for acute myeloid leukemia. *Int J Mol Sci* 2021; 22: 1944.

76. Klaihmon P, Luanpitpong S, Kang X, et al. Anti-TIM3 chimeric antigen receptor-natural killer cells from engineered induced pluripotent stem cells effectively target acute myeloid leukemia cells. *Cancer Cell Int* 2023; 23: 297.

77. Kikushige Y, Shima T, Takayanagi S, et al. TIM-3 is a promising target to selectively kill acute myeloid leukemia stem cells. *Cell Stem Cell* 2010; 7: 708–717.

78. Lee WS, Ye Z, Cheung AMS, et al. Effective killing of acute myeloid leukemia by TIM-3 targeted chimeric antigen receptor T cells. *Mol Cancer Ther* 2021; 20: 1702–1712.

79. Wang XY, Bian MR, Lin GQ, et al. Tandem bispecific CD123/CLL-1 CAR-T cells exhibit

specific cytolytic effector functions against human acute myeloid leukaemia. *Eur J Haematol* 2024; 112: 83–93.

80. Xie D, Jin X, Sun R, et al. Bicistronic CAR-T cells targeting CD123 and CLL1 for AML to reduce the risk of antigen escape. *Transl Oncol* 2023; 34: 101695.
81. Teppert K, Yonezawa Ogusuku IE, Brandes C, et al. CAR'TCR-T cells co-expressing CD33-CAR and dNPM1-TCR as superior dual-targeting approach for AML treatment. *Mol Ther Oncol* 2024; 32: 200797.
82. Liu F, Cao Y, Pinz K, et al. First-in-human CLL1-CD33 compound CAR T cell therapy induces complete remission in patients with refractory acute myeloid leukemia: update on phase 1 clinical trial. *Blood* 2018; 132: 901.
83. Feldmann A, Hoffmann A, Bergmann R, et al. Versatile chimeric antigen receptor platform for controllable and combinatorial T cell therapy. *Oncoimmunology* 2020; 9: 1785608.
84. Richards RM, Zhao F, Freitas KA, et al. NOT-gated CD93 CAR T cells effectively target AML with minimized endothelial cross-reactivity. *Blood Cancer Discov* 2021; 2: 648–665.
85. Peschke JC, Bergmann R, Mehnert M, et al. FLT3-directed UniCAR T-cell therapy of acute myeloid leukaemia. *Br J Haematol* 2023; 202: 1137–1150.
86. Smith TT, Stephan SB, Moffett HF, et al. In situ programming of leukaemia-specific T cells using synthetic DNA nanocarriers. *Nat Nanotechnol* 2017; 12: 813–820.
87. Cummins KD, Frey N, Nelson A, et al. Treating relapsed/refractory (RR) AML with biodegradable anti-CD123 CAR modified T cells. *Blood* 2017; 130: 1359.
88. Park S, Pascua E, Lindquist KC, et al. Direct control of CAR T cells through small molecule-regulated antibodies. *Nat Commun* 2021; 12: 710.
89. Appelbaum J, Price AE, Oda K, et al. Drug-regulated CD33-targeted CAR T cells control AML using clinically optimized rapamycin dosing. *J Clin Invest* 2024; 134: e162593.
90. Sakemura R, Terakura S, Watanabe K, et al. A tet-on inducible system for controlling CD19-chimeric antigen receptor expression upon drug administration. *Cancer Immunol Res* 2016; 4: 658–668.
91. Pérez-Amill L, Bataller A, Delgado J, et al. Advancing CART therapy for acute myeloid leukemia: recent breakthroughs and strategies for future development. *Front Immunol* 2023; 14: 1260470.
92. Haubner S, Mansilla-Soto J, Nataraj S, et al. "IF-better" gating: combinatorial targeting and synergistic signaling for enhanced CAR T cell efficacy. *Blood* 2021; 138: 2774–2774.
93. Haubner S, Mansilla-Soto J, Nataraj S, et al. Cooperative CAR targeting to selectively eliminate AML and minimize escape. *Cancer Cell* 2023; 41: 1871–1891.e1876.
94. Mueller J, Schimmer RR, Koch C, et al. Targeting the mevalonate or Wnt pathways to overcome CAR T-cell resistance in TP53-mutant AML cells. *EMBO Mol Med* 2024; 16: 445–474.
95. Fiorenza S and Turtle CJ. CAR-T cell therapy for acute myeloid leukemia: preclinical rationale, current clinical progress, and barriers to success. *BioDrugs* 2021; 35: 281–302.
96. Zhou D, Zhu X and Xiao Y. CAR-T cell combination therapies in hematologic malignancies. *Exp Hematol Oncol* 2024; 13: 69.