CAR T-cell therapy in acute myeloid leukemia

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ABSTRACT

سرطان الدم النخاعي الحاد هو ورم خبيث سرطاني شديد يؤثر على الخلايا النقوية (النخاعية) في نخاع العظم. لا يزال مرضى سرطان الدم النخاعي الحاد المنتكسون أو المقاومون يعانون من سوء التشخيص، مما يستلزم تطوير بدائل علاجية جديدة. يعد العلاج باستخدام الخلايا التائية المعبرة لمستقبلات المستضدات الخيمرية (CAR T-cell) احتمالاً مثيرًا للاهتمام في مجال علاج سرطان الدم. يتم الآن اختبار هذا العلاج في التجارب السريرية في المرحلة الأولى والمرحلة الثانية باستخدام محددات بروتينية للخلايا السرطانية شاملة CD33 وCD123 و CLL-1 وغيرها. أظهرت البيانات الأولية نتائج واعدة. ومع ذلك، نظرًا لعدم التجانس الخلوي والجزيئي للخلايا السرطانية والتعبير المشترك مع الخلايا الجذعية المكونة للدم، أظهرت هذه الدراسات السريرية آثار جانبية منها استهداف الخلايا الطبيعية مما يدعو إلى مزيد من البحث. في هذه المراجعة، يتم عرض أحدث الإنجازات المهمة في علاج سرطان الدم النخاعي (النقوي) الحاد باستخدام الخلايا التائية المعبرة لمستقبلات المستضدات الخيمرية. أيضًا، تتم مناقشة التحديات المتعلقة بهذه التقنية الجديدة وبعض الحلول للتغلب على هذه التحديات.

Acute myeloid leukemia (AML) is an aggressive leukemic malignancy that affects myeloid lineage progenitors. Relapsed or refractory AML patients continue to have poor prognoses, necessitating the development of novel therapy alternatives. Adoptive T-cell therapy with chimeric antigen receptors (CARs) is an intriguing possibility in the field of leukemia treatment. Chimeric antigen receptor T-cell therapy is now being tested in clinical trials (mostly in phase I and phase II) using AML targets including CD33, CD123, and CLL-1. Preliminary data showed promising results. However, due to the cellular and molecular heterogeneity of AML and the co-expression of some AML targets on hematopoietic stem cells, these clinical investigations have shown substantial "on-target off-tumor" toxicities, indicating that more research is required. In this review, the latest significant breakthroughs in AML CAR T cell therapy are presented. Furthermore, the limitations of CAR T-cell technology and future directions to overcome these challenges are discussed.

Keywords: acute myeloid leukemia, CAR T-cells, adoptive T-cell therapy, chimeric antigen receptor, leukemia, cancer therapy

Acute myeloid leukemia (AML) is the fast-advancing destructive neoplasm of myeloid precursors that causes partial/complete differentiation block and results in bone marrow (BM) failure.[1] Acute myeloid leukemia is characterized by genomic mutations and chromosomal abnormalities within BM cells, interferes with natural cell processes, and leads to uncontrolled cell expansion and compromised differentiation. It represents approximately 23% of all leukemia cases.[2] Although AML is common in adults, the incidence estimates in the United States are 7.8 per million individuals aged 0-14 and 9.1 per million individuals aged 15-19 years.[3] In 2023, the American Cancer Society estimated 20,380 new cases of AML, with approximately 11,310 cases becoming deadly and an average age of diagnosis of ~68 years.[4]

Acute myeloid leukemia results from 2 or more mutations, one of which promotes proliferation (class I mutations) while the other inhibits differentiation (class II mutations).[5] FLT3-ITD, KRAS, and KIT mutations are classified as class I, while mutations involving core binding factor (CBF) fusions and CEBPA are classified as class II.[5] Approximately half of individuals suffering from AML display a normal karyotype and mutations in certain genes, such as FLT3, IDH1, IDH2, and NPM1, while the remaining exhibit chromosomal abnormalities affecting chromosomes 5, 7, 9, 16, 8, 21, 15, or 17.[6]

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a conventional treatment for AML. Meanwhile, targeted therapies using inhibitors of FLT3, IDH1, IDH2, BCL2, and others and antibody-based therapies that employ anti-CD33 and

anti-CD3 have emerged as novel AML treatments.[7-11] However, treatment resistance and relapse are still major challenges, emphasizing the pressing demand for innovative therapeutic strategies.[12]

An exciting immunotherapeutic strategy that has revolutionized the field of blood cancer treatment involves modifying autologous/allogenic T-cells using chimeric antigen receptors (CARs), enabling the specific targeting of leukemia-associated antigens. This review provides an overview of the various antigens used in CAR T-cell therapy for AML, presents current clinical findings, and discusses the limitations of CAR T-cell technology and future directions to overcome these challenges.

CAR T-cell therapy. Chimeric antigen receptors are recombinant antigenic determinants designed by uniting the attaching domain of immunoglobulins with that of a signal-transducing domain derived from T-cells.[13] By utilizing lentiviral/retroviral vectors, CARs can be genetically expressed on the surface of αβ CD4+ and CD8+ T-cells. This modification directs T-cells toward a specific antigen, initiating a signaling cascade inside CAR-engineered T-cells, resulting in self-renewal and tumor-specific cytotoxic activity similar to that of stimulated T-cells.[14] Figure 1 shows a timeline of the major developments in CAR T-cell therapy.

In 2017, the Food and Drug Administration (FDA) sanctioned tisagenlecleucel as the pioneer CAR T-cell treatment regime for treating pediatric and young adult acute lymphoblastic leukemia (ALL).[15] This was followed by the approval of the following additional CD19-based CAR T-cell therapies for various B-cell leukemias such as: axicabtagene ciloleucel, brexucabtagene autoleucel, and lisocabtagene maraleucel.[16-18] In subsequent years, the FDA granted approval for 2 B-cell maturation antigen (BCMA)-specific CAR T-cell therapies for treating multiple myeloma (MM) such as: idecabtagene vicleucel and ciltacabtagene autoleucel.[19,20]

CAR T-cell therapy in AML. In 2013, a noteworthy clinical trial marked a pivotal moment in CAR T-cell therapy for AML. This study involved the first reported use of second-generation anti-CD28-ζ CAR T-cells targeting Lewis Y in AML. Although leukemia progression was observed, this investigation established the tolerability profile of administering CAR T-cells to individuals with AML; however, on-target/off-tumor effects were reported.[21]

A key step in the development of CAR T-cell therapy in AML involves selecting the appropriate surface antigen, which should have a robust expression on leukemic cells and negligible or low expression on normal cells to avoid on-target/off-tumor effects. Several cell surface antigens, including CD123, CLL-1, CD33, CD44v6, and FLT3, have been identified as possible targets. Table 1 lists the clinical trials for the treatment of AML employing various targets.

CD33 is a ubiquitous membrane-spanning member of the sialic acid-binding immunoglobulin-like lectin (SIGLEC) group. While it is frequently present in tissue-resident macrophages, normal progenitors, and approximately 90% of blast cells, it is rarely detected on embryonic CD34+ hematopoietic stem cells (HSCs).[22,23]

CD33 remains one of the most researched drug candidates for AML management. Experiments using second-generation anti-CD33 CAR T-cells in NOD SCID gamma (NSG) mouse models with AML have demonstrated a notable decrease in AML burden and an increase in animal survival.[24] Additionally, preclinical outcomes revealed that anti-CD33 CAR T-cells reduced AML blasts and sustained mouse endurance in xenogeneic animal models while causing cytopenia and a decrease in myeloid progenitors.[25] Furthermore, a third-generation CAR T-cell directed at CD33 (3G. CAR33-T) demonstrated enhanced viability, secretion of cytokines, and robust cytolytic action when exposed to normal and leukemic CD33+ cells in comparison to second-generation anti-CAR33 CAR T-cells.[26]

A phase 1/1b study (NCT03927261) investigating PRGN-3006 UltraCAR T-cells, featuring a CD33-specific CAR construct, membrane-bound IL-15 (mbIL15), and a kill switch in patients with relapsed or refractory (R/R) CD33+ AML is ongoing. In a preclinical investigation, 6 different anti-CD33 CAR T-cells incorporating CD3ζ peptide and CD28 or 4-1BB costimulatory domains exhibited notable anticancer action.[27] Encouraged by these promising outcomes, a phase I clinical trial (NCT03971799) is currently ongoing to establish the maximum tolerated dose (MTD) of lentivirally transduced autologous and allogenic anti-CD33 CAR T-cells for individuals with R/R AML and post-HSCT R/R AML.[27] In an early phase I trial (NCT01864902) for R/R AML, a patient administered approximately 109 autologous T-cells (of which 38% were transduced) exhibited cytokine release syndrome (CRS) and pancytopenia. Despite a noticeable decrease in observed myeloid blast cells 2 weeks after treatment, there was subsequent blast cell rebound, resulting in relapse 9 weeks post-therapy.[28] A
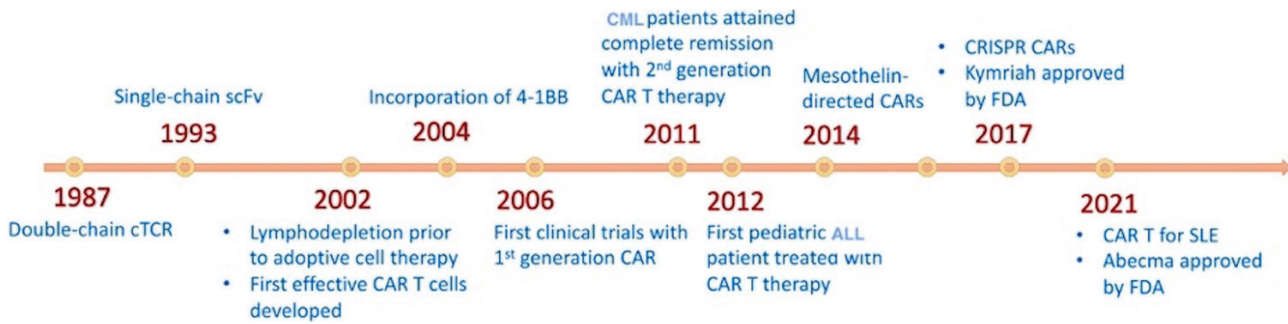
Figure 1 - The timeline of major developments in chimeric antigen receptor T-cell therapy. CML: chronic myeloid leukemia, CAR: chimeric antigen receptor, CRISPR: clustered regularly interspaced short palindromic repeats, FDA: Food and Drug Administration, cTCR: chimeric T-cell receptor, SLE: systemic lupus erythematosus

safety evaluation with varying dosages of autologous CD33-CAR T-cells (NCT03126864) showed adverse events, including CRS and neurotoxicity.[29]

IL-3 receptor subunit (IL-3Rα, CD123) levels are elevated not only in leukemia stem cells (LSCs) and AML blasts but also in early HSPCs, resulting in the risk of long-lasting or even permanent myeloablation.[30] CD123 stimulates cell propagation when exposed to IL-3 and has high expression in AML cells, with a negligible expression on CD34+ BM cells, rendering it a suitable candidate for CAR T-cell therapy in AML.[31,32]

In preclinical studies, anti-CD123 CAR T-cell therapy effectively eliminated AML blast cells while demonstrating acceptable levels of toxicity to HSPCs.[33,34] Furthermore, 4-1BB- and CD28-specific CD123-CAR T-cells proved capable of targeting AML cells while profoundly ablating normal hematopoietic regeneration.[35]

In an initial phase I trial (NCT02623582), autologous T-cells with anti-CD123 linked to TCR/4-1BB regions were evaluated in 5 patients with R/R AML. The patients underwent lymphodepletion therapy and then received CD123 CAR T-cells. However, due to adverse events, including CRS, a lack of anti-leukemic efficiency, and reported on-target/off-tumor toxicity, the trial was terminated.[36] Another ongoing investigation into allogeneic CD123 CAR T-cells was halted due to patient death 9 days after infusion. The trial resumed with conditions including a reduction in the dose of CART123 and chemotherapy, as well as not exceeding the age of 65 years.[37]

To enhance safety, novel anti-CD123 CAR T-cell therapy, which exploits the rapidly adjustable universal CAR T-cell platform (UniCAR) to improve tolerability while retaining full anti-AML effectiveness, was developed. A dose-escalating clinical study (NCT04230265) is currently underway to investigate the therapeutic advantage of the novel UniCAR

system.[38] Moreover, demethylating agent-based drugs have shown promise in enhancing immunological reactions and assisting cancer cell eradication by anti-CD123 CAR T-cells.[39]

Initial findings from the first cohort of an ongoing first-in-human (FIH) investigation using lentiviral-infected CD123-specific CD28-CAR T-cells demonstrated that one of 2 patients who underwent treatment with a dose of 50 million CAR+ T-cells achieved an AML-free status phenotypically for a duration of 2 months (NCT02159495). Shortly after, the individual underwent a second HSCT therapy, which resulted in a blast cell decrease from 77.9% to 0.9%.[40] Furthermore, among the 4 subjects tested, one individual achieved complete remission (CR) with a higher dose of 200 million CAR T-cells, another with CR prior to the start of the therapy continued to have CR, and the remaining 2 subjects experienced a decrease in the number of AML blasts with no relapse. Notably, all toxicities were reversible and tolerable, with no known dose-limiting toxicities or concomitant cytopenia.[41]

The natural killer (NK) group 2D (NKG2D) receptor in AML exhibits selective expression on monoblasts, while myeloblasts and chemoresistant LSCs either lack or show weak expression, which enables them to evade immune defense and prevent NK-mediated apoptosis.[42] Hence, utilizing CAR constructs to activate NKG2D ligands represents a valuable strategy for immunotherapy in AML.[43] As such, a bispecific FLT3-CAR therapy incorporating scFv and NKG2D-CAR T-cells has been developed, demonstrating effective eradication of AML blasts in preclinical studies.[44]

CYAD-01 are recombinant autologous T-cells expressing a CAR engineered by linking the NKG2D target with the CD3ζ signaling component.[45] A preliminary phase I FIH study (NCT02203825) revealed encouraging activity of CYAD-01 in subjects

Table 1 - Registered clinical trials using chimeric antigen receptor T-cell for acute myeloid leukemia in https://clinicaltrials.gov/ as of 8/3/2024.

Trial identifiers	Study names	Condition(s)	Study type	Antigens	Phases	Enrollments[†]	Start dates	Completion dates	Responsible parties	Status
NCT04033302	Multi-CAR T-cell therapy targeting CD7-positive malignancies	AML, T-cell acute lymphoblastic leukemia/lymphoma, NK cell lymphoma	Interventional	CD7	I/II	30	2019	2023	Shenzhen Geno-Immune Medical Institute	Unknown
NCT04762485	Humanized CD7 CAR T-cell therapy for r/r CD7+ acute leukemia	AML, T lymphoblastic leukemia/lymphoma, mixed phenotype acute leukemia	Interventional	CD7	I/II	20	2021	2024	The First Affiliated Hospital of Soochow University	Unknown
NCT05377827	Dose-escalation and dose-expansion study to evaluate the safety and tolerability of anti-CD7 allogeneic CAR T-cells (WU-CART-007) in patients with CD7+ hematologic malignancies	AML, T-Cell non-Hodgkin lymphoma, others	Interventional	CD7	I	54	2023	2026	Washington University School of Medicine	Recruiting
NCT05995028	Universal 4SCAR7U targeting CD7-positive malignancies	AML, T-cell acute lymphoblastic leukemia/lymphoma, NK cell lymphoma	Interventional	CD7	I	30	2023	2026	Shenzhen Geno-Immune Medical Institute	Recruiting
NCT03896854	CART-19 T-cell in CD19 positive relapsed or refractory acute myeloid leukemia (AML)	AML	Interventional	CD19	I/II	15	2017	2025	Shanghai Unicar-Therapy Bio-medicine Technology Co.,Ltd	Recruiting
NCT04796441	Clinical study of universal CAR-γδT-cell injection in the treatment of patients with relapsed AML after transplantation	AML	Interventional	CD19	NA	20	2020	2022	Hebei Senlang Biotechnology Inc., Ltd	Unknown
NCT04257175	CAR-T CD19 for acute myelogenous leukemia with t 8:21 and CD19 expression	AML	Interventional	CD19	II/III	10	2020	2024	Sheba Medical Center	Recruiting
NCT05513612	Novel CAR-T cell therapy in the treatment of hematopoietic and lymphoid malignancies	AML, B-NHL, others	Interventional	CD19	I	0	2020	2026	Shanghai Pudong Hospital	Withdrawn
NCT05388305	Universal CAR-γδT cell injection in the AML patients	AML	Interventional	CD19	NA	30	2022	2023	Hebei Senlang Biotechnology Inc., Ltd	Recruiting
NCT01864902	Treatment of relapsed or chemotherapy refractory CD33 positive acute myeloid leukemia by CART-33 (CART33)	R/R AML	Interventional	CD33	I/II	10	2013	2017	Chinese PLA General Hospital	Unknown
NCT02799680	Allogeneic CART-33 for relapsed/refractory CD33+ AML	R/R AML	Interventional	CD33	-	12	2015	2018	The Affiliated Hospital of the Chinese Academy of Military Medical Sciences	Unknown
NCT03971799	Study of anti-CD33 chimeric antigen receptor-expressing T-cells (CD33CART) in children and young adults with relapsed/refractory acute myeloid leukemia	AML	Interventional	CD33	I/II	52	2020	2039	Center for International Blood and Marrow Transplant Research	Active, not recruiting

[†]The 'estimated' enrollment is the target number of participants that the researchers need for the study. CAR: chimeric antigen receptor, AML: acute myeloid leukemia, NK: natural killer, B-NHL: B-cell non-Hodgkin lymphomas, R/R: relapsed/refractory, NA: not applicable

Table 1 - Registered clinical trials using chimeric antigen receptor T-cell for acute myeloid leukemia in https://clinicaltrials.gov/ as of 8/3/2024. Continued

Trial identifiers	Study names	Condition(s)	Study types	Antigens	Phases	Enrollments[†]	Start dates	Completion dates	Responsible parties	Status
NCT05105152	PLAT-08: a study of SC-DARIC33 CAR T-cells in pediatric and young adults with relapsed or refractory CD33+ AML	AML	Interventional	CD33	I	18	2021	2041	Colleen Annesley, Seattle Children's Hospital	Recruiting
NCT04835519	Phase I/II study of enhanced CD33 CAR T-cells in subjects with relapsed or refractory acute myeloid leukemia	R/R AML	Interventional	CD33	I/II	25	2021	2024	Beijing Boren Hospital	Recruiting
NCT05672147	CD33-CAR T-cell therapy for the treatment of recurrent or refractory acute myeloid leukemia	R/R AML	Interventional	CD33	I	27	2023	2026	City of Hope Medical Center	Recruiting
NCT05445765	Anti-CD33 CAR-T cells for the treatment of relapsed/refractory CD33+ acute myeloid leukemia	R/R AML	Interventional	CD33	I	10CD33	2022	2024	iCell Gene Therapeutics	Not yet recruiting
NCT05473221	Evaluate the safety and efficacy of CD33 CAR-T in patients with R/R AML	AML	Interventional	CD33	I	20	2022	2025	Zhejiang University	Not yet recruiting
NCT05945849	CD33KO-HSPC infusion followed by CART-33 infusion(s) for refractory/relapsed AML	R/R AML	Interventional	CD33	I	16	2024	2044	University of Pennsylvania	Not yet recruiting
NCT05984199	Donor-derived anti-CD33 CAR T-cell therapy (VCAR33) in patients with relapsed or refractory AML after allogeneic hematopoietic cell transplant	R/R AML	Interventional	CD33	I/II	24	2023	2026	Vor Biopharma	Recruiting
NCT04351022	CD38-targeted chimeric antigen receptor T-cell (CART) in relapsed or refractory acute myeloid leukemia	R/R AML	Interventional	CD38	I/II	20	2017	2023	The First Affiliated Hospital of Soochow University	Unknown
NCT05239689	Clinical study of CD38 CAR-T cells in the treatment of hematological malignancies	AML	Interventional	CD38	I	36	2022	2024	Zhejiang University	Recruiting
NCT05442580	CART-38 in adult AML and MM patients	AML, MM	Interventional	CD38	I	36	2023	2041	University of Pennsylvania	Recruiting
NCT04662294	CD 70 CAR T for patients with CD70 positive malignant hematologic diseases	AML, NHL, MM	Interventional	CD70	I	108	2021	2027	Zhejiang University	Recruiting
NCT04692948	TAA6 cell injection in the treatment of patients with relapsed/refractory acute myeloid leukemia	AML	Interventional	CD276	NA	5	2019	2023	PersonGen BioTherapeutics (Suzhou) Co., Ltd.	Unknown
NCT05731219	UTAA06 injection in the treatment of relapsed/refractory acute myeloid leukemia	R/R AML	Interventional	B7-H3 (CD276)	I	18	2022	2025	PersonGen BioTherapeutics (Suzhou) Co., Ltd.	Recruiting
NCT05722171	Clinical study of UTAA06 injection in the treatment of relapsed/refractory acute myeloid leukemia	R/R AML	Interventional	B7-H3 (CD276)	I	10	2022	2024	PersonGen BioTherapeutics (Suzhou) Co., Ltd.	Recruiting
NCT03585517	Safety and efficacy evaluation of IM23 CAR-T cells (IM23CAR-T)	AML	Interventional	CD123	I	10	2018	2020	Beijing Immunochina Medical Science & Technology Co., Ltd.	Completed
NCT02159495	Genetically modified T-cell immunotherapy in treating patients with relapsed/refractory acute myeloid leukemia and persistent/recurrent blastic plasmacytoid dendritic cell neoplasm	AML, blastic plasmacytoid dendritic cell neoplasm	Interventional	CD123	-	31	2015	2024	City of Hope Medical Center	Active, not recruiting

[†]The 'estimated' enrollment is the target number of participants that the researchers need for the study. CAR: chimeric antigen receptor, AML: acute myeloid leukemia, NK: natural killer, B-NHL: B-cell non-Hodgkin lymphomas, R/R: relapsed/refractory, NA: not applicable MM: multiple myeloma

Table 1 - Registered clinical trials using chimeric antigen receptor T-cell for acute myeloid leukemia in https://clinicaltrials.gov/ as of 8/3/2024. Continued part one.

Trial identifiers	Study names	Condition(s)	Study types	Antigens	Phases	Enrollments[†]	Start dates	Completion dates	Responsible parties	Status
NCT03114670	Donor-derived anti-CD123-CART cells for recurred AML after Allo-HSCT	Adult AML	Interventional	CD123	-	20	2017	2021	Affiliated Hospital to Academy of Military Medical Sciences	Unknown
NCT03190278	Study evaluating safety and efficacy of UCART123 in patients with relapsed/refractory acute myeloid leukemia (AMELI-01)	R/R AML	Interventional	CD123	-	65	2017	2024	Cellectis S.A.	Recruiting
NCT03556982	CART-123 for relapsed/refractory scute myelocytic leukemia (AML)	R/R AML	Interventional	CD123	I/II	10	2018	2020	The Affiliated Hospital of the Chinese Academy of Military Medical Sciences	Unknown
NCT04265963	CD123-targeted CAR-T cell therapy for relapsed/refractory acute myeloid leukemia	AML	Interventional	CD123	I/II	45	2019	2024	Chongqing Precision Biotech Co., Ltd	Recruiting
NCT04272125	Safety and efficacy of CD123-targeted CAR-T therapy for relapsed/refractory acute myeloid leukemia	AML	Interventional	CD123	I/II	40	2019	2024	Chongqing Precision Biotech Co., Ltd	Recruiting
NCT05949125	Dose-escalating trial with Allo-RevCAR01-T cells in combination with CD123 target module (R-TM123) for participants with selected hematologic malignancies positive for CD123	AML	Interventional	CD123	I	37	2024	2025	AvenCell Europe GmbH	Recruiting
NCT04318678	CD123-directed autologous T-cell therapy for acute myelogenous leukemia (CATCHAML)	AML, MDS, B-ALL, T-ALL, BPDCN	Interventional	CD123	I	32	2020	2025	St. Jude Children's Research Hospital	Active, not Recruiting
NCT04678336	CD123 redirected T-cells for AML in pediatric subjects	R/R AML, pediatric AML	Interventional	CD123	I	12	2021	2036	University of Pennsylvania	Active, not Recruiting
NCT04884984	Anti-CLL1 CAR T-cell therapy in CLL1 positive relapsed/refractory acute myeloid leukemia (AML)	AML	Interventional	CLL1	I/II	20	2017	2024	The First Affiliated Hospital of Soochow University	Recruiting
NCT05467202	Evaluate the safety and efficacy of CLL1 CAR-T in patients with R/R AML	AML	Interventional	CLL1	I	20	2022	2025	Zhejiang University	Not yet recruiting
NCT04219163	Chimeric antigen receptor T-cells for the treatment of AML expressing CLL-1 antigen	AML	Interventional	CLL1	I/II	18	2020	2038	Baylor College of Medicine	Recruiting
NCT04923919	Clinical study of chimeric antigen receptor T lymphocytes (CAR-T) in the treatment of myeloid leukemia	AML	Interventional	CLL1	I	100	2021	2024	920th Hospital of Joint Logistics Support Force of People's Liberation Army of China	Recruiting
NCT05252572	Clinical study of CLL1 CAR-T cells in the treatment of hematological malignancies	AML	Interventional	CLL1	I	36	2022	2024	Zhejiang University	Recruiting

[†]The 'estimated' enrollment is the target number of participants that the researchers need for the study. CAR: chimeric antigen receptor, AML: acute myeloid leukemia, NK: natural killer, B-NHL: B-cell non-Hodgkin lymphomas, R/R: relapsed/refractory, NA: not applicable MM: multiple myeloma

Table 1 - Registered clinical trials using chimeric antigen receptor T-cell for acute myeloid leukemia in https://clinicaltrials.gov/ as of 8/3/2024. Continued part 2.

Trial identifiers	Study names	Condition(s)	Study types	Antigens	Phases	Enrollments†	Start dates	Completion dates	Responsible parties	Status
NCT06128044	CRISPR-edited allogeneic anti-CLL-1 CAR-T cell therapy in patients with relapsed/refractory acute myeloid leukemia (AMpLify)	R/R AML	Interventional	CLL1	I	70	2023	2028	Caribou Biosciences, Inc.	Recruiting
NCT06118788	Phase I clinical study: BG1805 injection in the treatment of relapsed or refractory acute myeloid leukemia	R/R AML	Interventional	CLL1	I	24	2023	2025	Guangzhou Bio-gene Technology Co., Ltd	Not yet recruiting
NCT05023707	Anti-FLT3 CAR T-cell therapy in FLT3 positive relapsed/refractory acute myeloid leukemia	R/R AML	Interventional	FLT3	I/II	5	2021	2025	The First Affiliated Hospital of Soochow University	Recruiting
NCT05432401	TAA05 injection in the treatment of adult patients with FLT3-positive relapsed/refractory acute myeloid leukemia	FLT3-positive R/R AML	Interventional	FLT3	I	18	2022	2025	PersonGen BioTherapeutics (Suzhou) Co., Ltd.	Recruiting
NCT05445011	Anti-FLT3 CAR-T cell (TAA05 cell injection) in the treatment of relapsed/refractory acute myeloid leukemia	AML	Interventional	FLT3	I	12	2022	2027	Wuhan Union Hospital, China	Recruiting
NCT03018405	A dose escalation Phase I study to assess the safety and clinical activity of multiple cancer indications (THINK)	MDS/AML/MM	Interventional	NKG2D	-/II	146	2016	2021	Celyad Oncology SA	Unknown
NCT04658004	NKG2D CAR-T cell therapy for patients with relapsed or refractory acute myeloid leukemia	AML	Interventional	NKG2D	I	36	2021	2027	Zhejiang University	Not yet recruiting
NCT04599543	IL3 CAR-T cell therapy for patients with CD123 positive relapsed or refractory acute myeloid leukemia	AML	Interventional	IL3	I	36	2020	2026	Zhejiang University	Not yet recruiting
NCT05266950	Safety and efficacy study of CI-135 CAR-T cells in subjects with relapsed or refractory acute myeloid leukemia	AML	Interventional	CI-135	I	7	2021	2025	Beijing Boren Hospital	Recruiting
NCT04803929	Clinical study of anti-ILT3 CAR-T therapy for R/R AML (M4/M5)	R/R AML (M4/M5)	Interventional	ILT3	I	25	2021	2026	Carbiogene Therapeutics Co. Ltd.	Recruiting
NCT05463640	Evaluate the safety and efficacy of ADGRE2 CAR-T in patients with R/R AML	AML	Interventional	ADGRE2	I	20	2022	2025	Zhejiang University	Not yet recruiting
NCT05488132	Administration of anti-siglec-6 CAR-T cell therapy in relapsed and refractory acute myeloid leukemia (rr/AML)	R/R AML	Interventional	Siglec-6	I/II	20	2022	2025	Xuzhou Medical University	Recruiting
NCT06197672	Chimeric antigen receptor T-cell redirected to target CD4 positive relapsed refractory acute myeloid leukemia (AML) as a bridge to allogeneic stem cell transplant	R/R AML	Interventional	CD4	I	30	2024	2042	Indiana University	Not yet recruiting

†The 'estimated' enrollment is the target number of participants that the researchers need for the study. CAR: chimeric antigen receptor, AML: acute myeloid leukemia, NK: natural killer, B-NHL: B-cell non-Hodgkin lymphomas, R/R: relapsed/refractory, NA: not applicable MM: multiple myeloma

Table 1 - Registered clinical trials using chimeric antigen receptor T-cell for acute myeloid leukemia in https://clinicaltrials.gov/ as of 8/3/2024. Continued part 3.

Trial identifiers	Study names	Condition(s)	Study types	Antigens	Phases	Enrollments[†]	Start dates	Completion dates	Responsible parties	Status
NCT06281847	An adaptive open-label multicentre Phase 1/2 trial, to determine the recommended Phase 2 dose of CCTx-001, and to assess safety, tolerability, and clinical activity in patients with relapsed/refractory acute myeloid leukaemia (RESOLVE AML001)	R/R AML	Interventional	IL-1RAP	I/II	143	2024	2041	Advesya SAS	Not yet recruiting
NCT06017258	A study of CD371-YSNVZIL-18 CAR T-cells in people with acute myeloid leukemia	AML	Interventional	CD371	I	6	2023	2026	Memorial Sloan Kettering Cancer Center	Recruiting
NCT05016063	Dual CD33-CLL1-CAR-T cells in the treatment of relapsed/refractory acute myeloid leukemia	AML	Interventional	CD33/CLL1	I	32	2021	2023	Xinqiao Hospital of Chongqing	Unknown
NCT05248685	Optimized dual CD33/CLL1 CAR T-cells in subjects with refractory or relapsed acute myeloid leukemia	AML	Interventional	CD33/CLL1	I	20	2022	2024	Beijing Boren Hospital	Unknown
NCT03795779	CLL1-CD33 cCAR in patients with relapsed or refractory, high risk hematologic malignancies	AML, MDS, MPN, CML	Interventional	CLL1/CD33	I	20	2018	2022	iCell Gene Therapeutics	Unknown
NCT05467254	Evaluate the safety and efficacy of CLL1+CD33 CAR-T in patients with R/R AML	AML	Interventional	CLL1/CD33	I	20	2022	2025	Zhejiang University	Not yet recruiting
NCT06110208	Study to evaluate the safety and preliminary efficacy of CLL1 and CD38 dual CAR-T in r/r AML	R/R AML	Interventional	CLL1/CD38	I	18	2023	2026	920th Hospital of Joint Logistics Support Force of People's Liberation Army of China	Recruiting
NCT04010877	Multiple CAR-T cell therapy targeting AML	AML	Interventional	CLL1, CD33, CD123	I/II	10	2019	2023	Shenzhen Geno-Immune Medical Institute	Unknown
NCT03222674	Multi-CAR T-cell therapy for acute myeloid leukemia	AML	Interventional	Muc1/CLL1/CD33/CD38/CD56/CD123	I/II	10	2017	2020	Shenzhen Geno-Immune Medical Institute	Unknown
NCT03291444	CAR-T cells combined with peptide specific dendritic cell in relapsed/refractory leukemia/MDS	AML	Interventional	CD33, CD38, CD56, CD117, CD123, CD34, Muc1	I	30	2017	2025	Zhujiang Hospital	Recruiting
NCT05995041	Universal CAR-T cells targeting AML	R/R AML	Interventional	CLL-1, CD33, CD38, CD123	I	30	2023	2026	Shenzhen Geno-Immune Medical Institute	Recruiting
NCT04766840	Donor-derived CAR-T cells in the treatment of AML patients	AML	Interventional	not shown	I	9	2021	2023	Beijing Immunochina Medical Science & Technology Co., Ltd.	Unknown

[†]The 'estimated' enrollment is the target number of participants that the researchers need for the study. CAR: chimeric antigen receptor, AML: acute myeloid leukemia, NK: natural killer, B-NHL: B-cell non-Hodgkin lymphomas, R/R: relapsed/refractory, NA: not applicable MM: multiple myeloma

Table 1 - Registered clinical trials using chimeric antigen receptor T-cell for acute myeloid leukemia in https://clinicaltrials.gov/ as of 8/3/2024. Continued part 4.

Trial identifiers	Study names	Condition(s)	Study types	Antigens	Phases	Enrollments[†]	Start dates	Completion dates	Responsible parties	Status
NCT03473457	CAR-T cells therapy in relapsed/refractory acute myeloid leukemia (AML)	R/R AML	Interventional	Not shown	NA	2	2018	2020	Zhujiang Hospital	Terminated[*]
NCT04097301	Study of CAR T-cell therapy in acute myeloid leukemia and multiple myeloma	R/R AML, MM	Interventional	CD44v6	I/II	8	2019	2021	AGC Biologics S.p.A	Terminated[Δ]

[†]The 'estimated' enrollment is the target number of participants that the researchers need for the study. [*]The therapeutic effect was not as expected. [Δ]Inability to close the study in a clinically relevant time frame. CAR: chimeric antigen receptor, AML: acute myeloid leukemia, NK: natural killer, B-NHL: B-cell non-Hodgkin lymphomas, R/R: relapsed/refractory, NA: not applicable MM: multiple myeloma

with AML.[46] Another trial (NCT03018405) is being carried out to assess the safety and tolerability of large doses and repeated injections of NKG2D-CAR T-cells.[45,47] Although it showed antileukemic activity, patients experienced adverse events and CRS associated with the 3 dose levels, warranting further investigations into safety and efficiency.[45]

CLL-1 is highly expressed in approximately 92% of AML blasts and granulocytes, while it is expressed in approximately 2.5% of HSCs, making it a suitable target for CAR T-cell therapy.[48,49] Anti-CLL1 CAR T-cell therapy in a child with secondary AML resulted in favorable outcomes, including molecular CR for more than 10 months.[50] A phase I/II anti-CLL1 CAR T-cell therapy trial involving 4 children with R/R AML resulted in 3 cases of CR with minimal residual disease (MRD) negativity.[51] In a phase 1 anti-CLL1 CAR T-cell therapy trial involving adult R/R AML, most patients experienced CRS, although it was tolerable when patients were followed up for 6 months.[52] Treatment with dual-targeting CD33- and CLL-1-specific CAR T-cell therapy in a phase I trial involving a child with complex karyotype AML achieved CR.[53] As CLL-1 is highly expressed in granulocytes, it is recommended that anti-CLL1 CAR T-cell therapy be bridged with HSCT to avoid granulocytopenia.

FLT3-ITD and FLT3-TKD aberration is present in ~20% of all AML cases and FLT3-TKD aberration is present in 7% of all AML cases, with only a subset of HSPCs expressing FLT3.[54] Nevertheless, tailored CAR technologies are not aimed at mutant FLT3 but rather at FLT3 as a whole.[55,56] Second-generation FLT3-specific CAR with a 4-1BB costimulatory domain displayed noteworthy cytolytic effects on FLT3+ AML as well as on primary cell lines, with negligible off-tumor damage to healthy HSCs.[57] Some studies have reported blood cell-related toxicity in animal models.[56]

CD44v6, splicing variant 6 of the hyaluronic acid receptor (CD44, a class I transmembrane glycoprotein), is elevated in AML but not in HSCs and has insignificant expression in normal cells.[58,59] A second-lineage CAR redirected toward CD44v6 was developed and showed cytotoxic activity against AML blasts but not HSCs.[60] Clinical trials of CD44v6-based CAR T-cell therapy for the management of AML patients (NCT04097301) are currently underway.[61]

CD7 expression is reported in approximately 30% of AML cases and its absence in HSCs makes it a good candidate for CAR T-cell therapy.[62,63] Utilizing CD7-specific CAR T-cell therapy in CD7 knockout T-lymphocytes has been proven to target CD7+ AML cells with no toxic effects on myeloid and erythroid cells.[64]

CD38 is highly expressed in AML samples and hematopoietic progenitors but is absent in long-term HSCs.[65,66] Preclinical studies using CD38-CAR T-cells have shown antileukemic activities in AML cell lines and primary AML associated with improved survival while targeting normal hematopoietic progenitors.[67]

Limitations of CAR T-cell therapy. Chimeric antigen receptor T-cell therapy faces a formidable challenge when tumor cells downregulate the target CAR antigen. This results in a partial or complete loss of antigen expression and contributes to disease relapse following CAR T-cell therapy.[68] This has been observed in ALL and MM patients in which CD19 and BCMA expression, were downregulated or lost after treatment with CAR T-cells.[69,70] This phenomenon of antigen escape has also been reported in solid tumors. The expression of IL13Ra2 was downregulated in recurring glioblastoma cases after CAR T-cell therapy.[71] To overcome this issue, increase the efficiency of CAR T-cell therapy, and reduce tumor recurrence/relapse rates, strategies involving targeting multiple antigens using dual CAR constructs or tandem CARs have been developed. Data from clinical trials using CD19/CD22 and CD19/BCMA dual-targeted CAR T-cells for ALL and MM, have shown promising results and prolonged remission rates.

However, increased on-target off-leukemia toxicity has been associated with dual-target CAR constructs versus single constructs.[72,73] In AML, compound constructs have been developed to enhance anti-AML activity. Chimeric antigen receptor T-cell dual-target CD123/NKG2DLs and CAR T-cell dual-target CD33/TIM3 have been shown to be efficacious against AML cell lines and primary AML.[74,75] Although the dual-target strategy has proven to be effective against antigen escape, further improvements are needed to overcome other hurdles, such as on-target off-tumor effects.

Targeting AML antigens is challenging due to their frequent expression in normal hematopoietic cells, necessitating careful antigen selection in CAR design to achieve high therapeutic efficiency and avoid on-target off-tumor toxicity.[76] As this is a common issue between hematological and solid tumors, an emerging strategy to address this challenge involves directing CARs toward tumor-associated posttranslational modifications (PTMs), such as overexpressed truncated O-glycans in solid tumors.[77] A total of 4 primary PTMs directed toward CAR T-cell glycated antigens have been explored.[76]

Optimizing the affinity of scFvs can help mitigate the on-target off-tumor effect of antigens that are highly expressed in tumor cells but have low expression in normal cells. CD38-CAR T-cells engineered to have a low affinity have been shown to eliminate CD38+ MM cells, while CD38+ hematopoietic cells remain unaffected.[78] This strategy may be effective in designing CAR T-cells for AML due to the abundance of common antigens and the variable expression between AML and normal blood cells.

The toxicity associated with CAR T-cell therapy represents an obstacle to achieving the maximum benefit of CAR T-cell therapy as a standard of care therapy. Such toxicities include CRS, hemophagocytic lymphohistiocytosis/macrophage activation syndrome (HLH/MAS), and immune effector cell-associated neurotoxicity syndrome (ICANS).[79] Severe and life-threatening events have been reported, even for FDA-approved CD19-targeted CARs.[80,81] In CAR T-cell treatment for ALL, nearly all patients experience some toxicity, with 23-46% developing CRS and extensive T-cell proliferation in vivo.[82,83] CRS and ICANS become more severe following the infusion of a high number of CAR T-cells and upon the secretion of the cytokines IL-1 and IL-6.[84] Further research is required to identify new strategies to overcome the toxicity associated with CAR T-cells.

The magnitude and dynamics of CAR T-cell stimulation are intricately affected by multiple factors, including the extent of cancer antigen expression, the affinity of the antigen-binding domain to its target epitope, and the integration of costimulatory components within the CAR.[85] Thus, optimizing therapeutic efficacy and minimizing toxicity require careful consideration of multiple components within the CAR's modular structure.

Making modifications to the hinge and transmembrane domains of CARs is one strategy to reduce toxicity.[86] In a phase 1 trial involving patients with B-cell lymphoma, CD19-targeted CAR T-cells with genetic alterations resulting in a longer CD8α hinge and intracellular domain sequences led to lower cytokine production, reduced CAR T-cell propagation, absence of grade 1 CRS or neurotoxicity in all patients, and CR in 54.5% of patients when compared to shorter CD8α hinge and intracellular domain sequences.[87] Additionally, the host's immune response to CAR constructs may pose a challenge. Thus, using human-derived fragments instead of murine-derived fragments is an optimal solution to avoid immunological-related toxicity.[88]

Another recent approach to preventing CAR T-cell cytokine toxicity involves the inhibition of granulocyte-macrophage colony-stimulating factor (GM-CSF) production either by modifying CAR-transduced T-cells or by combined treatment. Preclinical studies indicate that mutational inactivation of GM-CSF by generating GM-CSF-deficient CD19-CAR T-cells through the CRISPR/Cas9 disruption of GM-CSF or the inhibition of GM-CSF with lenzilumab decreases neurotoxicity and CRS while enhancing CAR T-cell efficacy.[89]

Employing "off-switches" or suicidal genes is another strategy to reduce CAR T-cell toxicity. These approaches enable the selective reduction of customized CAR T-cells during adverse reactions by treating them with an additional stimulating agent.[90] Various approaches have been developed, including inducible Cas9 switches with immediate effects on eliminating CAR T-cells in approximately 30 minutes, protease-based small molecule-assisted shutoff CARs (SMASh-CARs), and the administration of dasatinib (a tyrosine kinase inhibitor) immediately after CAR T-cell injection to reversibly inhibit CAR T-cell activation, thereby protecting model animals from CRS-induced mortality.[91-93] Such strategies would be highly beneficial if they are engineered to be temporally controlled to allow for the inhibition and activation of CAR T-cells when needed.

Conclusion and future directions. Despite advancements in understanding AML etiology and pathophysiology, disease relapse continues to be the primary cause of mortality, even following HSCT.

Chimeric antigen receptor-based immunotherapeutic intervention is a promising approach with the potential for long-term protection in individuals with AML relapse. However, the success of CAR T-cell therapy in AML depends on various factors. Overcoming immune escape and identifying AML-specific target antigens remain critical hurdles in successfully instigating adoptive CAR T-cell therapies in the future. Other ongoing considerations include optimizing CAR signaling, managing CAR T-cell persistence post-treatment to avert extended myeloablation, host conditioning complications, and mitigating T-cell-mediated toxic reactions. With numerous clinical trials in progress, assessing the ongoing safety of CAR T-cell therapy and its ability to target relapse-inducing LSCs will be intriguing. There is optimism that therapeutic alternatives will continue to advance, offering improved efficacy with reduced toxicity for better patient outcomes.

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