Extending the Promise of Chimeric Antigen Receptor T-Cell Therapy Beyond Targeting CD19^{+} Tumors

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INTRODUCTION

Chimeric antigen receptor T cells (CAR-Ts) targeted against CD19 have shown impressive activity in patients with B-cell leukemias and lymphomas. This has revolutionized the treatment landscape for patients with relapsed or refractory (r/r) disease, providing hope for a possible curative approach for those who previously had limited available treatment options.[1] The success of this approach has prompted interest in studying CAR-Ts for other hematologic malignancies and the development of CARs specific for other antigens. In this review, we discuss recent developments in CAR-Ts in multiple myeloma (MM), Hodgkin lymphoma (HL), T-cell malignancies, and acute myelogenous leukemia (AML; Fig 1).

CHALLENGES BEYOND THE CD19 ANTIGEN

CD19 is an excellent target for CAR-Ts given its almost universal expression in B-cell malignancies and tolerable on-target/off-tumor toxicity caused by the depletion of normal B lymphocytes that also express CD19.[2] In contrast, targeting lineage-restricted and nonlineage-restricted antigens in non–B-cell hematologic malignancies raises concerns about the manufacturing of CAR-T products and the clinical consequences associated with selecting antigens shared by normal cells. For example, in T-cell malignancies, targeting with CAR antigens that are lineage restricted and, thus, also expressed by healthy T cells will inevitably cause the reciprocal elimination of T cells, a phenomenon known as fratricide, preventing the generation, expansion, and persistence of CAR-Ts.[3] Furthermore, infusion of CAR-Ts specific for T-cell–associated antigens in patients with T-cell malignancies, or myeloid-associated antigens in patients with AML, may seriously compromise host immunity because of prolonged T-cell depletion and myelosuppression, respectively. On the other hand, nonlineage-specific markers upregulated in malignant cells are inevitably characterized by heterogeneous expression within tumor cells, which can lead to tumor escape as a result of insufficient levels of antigen expression. With knowledge of these critical issues that move beyond CD19 targeting, we describe here some of the strategies implemented to overcome these challenges, such as gene editing techniques and conditional dual targeting.

MM

CAR-Ts that target CD19 (CD19.CAR-Ts) have been tested in a small number of patients with MM following the hypothesis that removing the B-cell precursor of plasma cells (PCs) could promote the eradication of differentiated PCs. While some responses were observed in patients with MM treated with CD19.CAR-Ts,[4,5] direct elimination of tumor PCs through targeting of PC-associated antigens seems a more viable option. To date, numerous antigens have been tested for eliminating malignant PCs. Prioritization was given to those with promising safety profiles (ie, high expression on tumor cells), with minimal expression on normal tissues.

B-cell maturation antigen. B-cell maturation antigen (BCMA) is a type 3 transmembrane protein commonly expressed on MM cells at varying receptor density.[6] BCMA expression is restricted to late memory B cells, plasmablasts, and differentiated PCs[7,8] while being absent on nonhematologic tissues, hematopoietic stem cells (HSCs), naive B cells, and other hematopoietic lineages.[9,10]

CAR-Ts that target BCMA (BCMA.CAR) in MM have had the farthest progress to date. The first trial was piloted by the National Cancer Institute, which accrued 12 heavily pretreated patients with r/rMM.[11] The therapy showed a dose-dependent increase in both toxicity and efficacy: Patients enrolled at the highest dose (9×10^6 CAR-T cells/kg) developed severe, albeit reversible, cytokine release syndrome (CRS) and experienced clinical responses, including one complete response (CR) that lasted 17 weeks and a very good partial response (VGPR) that lasted 66 weeks.[11] This early success jump-started many subsequent and ongoing clinical trials that targeted BCMA (Table 1; Appendix Table A1, online only).

A phase I study led by the University of Pennsylvania using a fully human BCMA.CAR enrolled 25 patients with r/rMM treated in a dose escalation fashion with

CONTEXT

Key Objective
What are the recent advances of chimeric antigen receptor T cells (CAR-Ts) that target antigens other than CD19 in hematologic malignancies?

Knowledge Generated
Challenges of developing CAR-Ts for non–B-cell malignancies include the selection of suitable antigens, limitation of on-target/off-tumor toxicities, and complexity of the tumor microenvironment. We describe developments and future directions of CAR-T cells in multiple myeloma, Hodgkin lymphoma, T-cell malignancies, and acute myeloid leukemia.

Relevance
Novel CAR-Ts for hematologic malignancies are entering the clinic, proving that this strategy holds promise for other targets and supporting future work in solid tumors.

and without lymphodepletion.[12] Twelve (48%) of 25 patient achieved a partial response (PR) or better, including seven (64%) of 11 patients who received lymphodepleting chemotherapy and the higher dose of BCMA.CAR-Ts ($> 10^8$ CAR-Ts/kg). Three of these participants had ongoing remissions that lasted > 11 months, including one stringent CR (sCR) that lasted 2.5 years. An important observation from this trial was the decreased BCMA expression on residual MM cells in patients who responded to BCMA.CAR-Ts.[12] Another phase I trial (bb2121) reported similar results in patients with r/rMM: 85% objective response rate (ORR) with 45% either CR or sCR.[13] Of note, responses occurred within a median time of 1 month after BCMA.CAR-T cell infusion with a trend for slower decreases in serum M-protein levels over up to 9 months.[13]

Other trials with BCMA.CAR-Ts have shown similar efficacy and toxicity profiles (Table 1; Appendix Table A1), including LCAR-B38M,[14] MCARH171,[15] JCARH125,[16] and FCARH143.[17] The latter study documented a patient who relapsed with low BCMA expression at day 79,[17] suggesting that antigen loss may be a mechanism of resistance, which supports a theme noted throughout different CAR-T therapies. Of note, while the great majority of clinical trials used viral vectors to manufacture BCMA.CAR-Ts, one phase I/II trial used a nonviral gene transfer approach, such as the transposon-based DNA modification system PiggyBac, to generate BCMA.CAR-Ts.[18] Twenty-one patients with r/rMM were enrolled, and 9.5% developed CRS grade ≤ 2, with no grade ≥ 3 events reported. The ORR was 100% in the higher dose cohort and 70% in the lower dose cohort.[18] A phase II, open-label, efficacy and safety study is under way (ClinicalTrials.gov identifier: NCT03288493).

Although BCMA.CAR-Ts have generally demonstrated high response rates, the majority of patients ultimately experienced a relapse, stimulating interest in identifying mechanisms of resistance. While low BCMA expression plays a role in tumor escape, BCMA can also be cleaved from the cell membrane by γ-secretase.[19] A clinical trial is currently ongoing that is testing whether combining BCMA.CAR-Ts with a small-molecule γ-secretase inhibitor that blocks cleavage of BCMA can improve the efficacy of this therapy.[20] Alternatively, to limit tumor escape, the targeting of other antigens in combination with BCMA is being explored (Table 2).

CD138. CD138 (syndecan-1) is a member of the type 1 transmembrane heparan sulfate proteoglycans that acts as an extracellular matrix receptor and is highly expressed by terminally differentiated normal PCs and MM. Expression of CD138 on epithelial cells and endothelial cells presents a major concern for on-target/off-tumor toxicity after CD138-targeted therapy.[21,22] Preclinical studies with CD138-redirected CAR-Ts (CD138.CAR-Ts) showed anti-MM activity both in vitro and in vivo, with no evidence of targeting epithelial and endothelial cells.[23] A pilot phase I trial enrolled five patients with r/rMM who received an average dose of 0.756×10^7 CD138.CAR-Ts/kg[24] without lymphodepleting chemotherapy. Four patients achieved stable disease, which lasted between 3 and 7 months. The fifth patient had progressive disease despite the detection of CD138.CAR-Ts in the patient's bone marrow. A phase I dose escalation trial with CD138.CAR-Ts is under way at the University of North Carolina at Chapel Hill (UNC; ClinicalTrials.gov identifier: NCT03672318; Appendix Table A2, online only).

CD38. CD38, a glycoprotein with ectoenzymatic functions, represents an attractive target for CAR-T therapy because of its high expression on MM.[25] However, CD38 is also expressed on normal nonmalignant cells of the hematopoietic system that include RBCs, natural killer (NK) cells, and T cells, among others, which poses safety concerns. To overcome this risk,
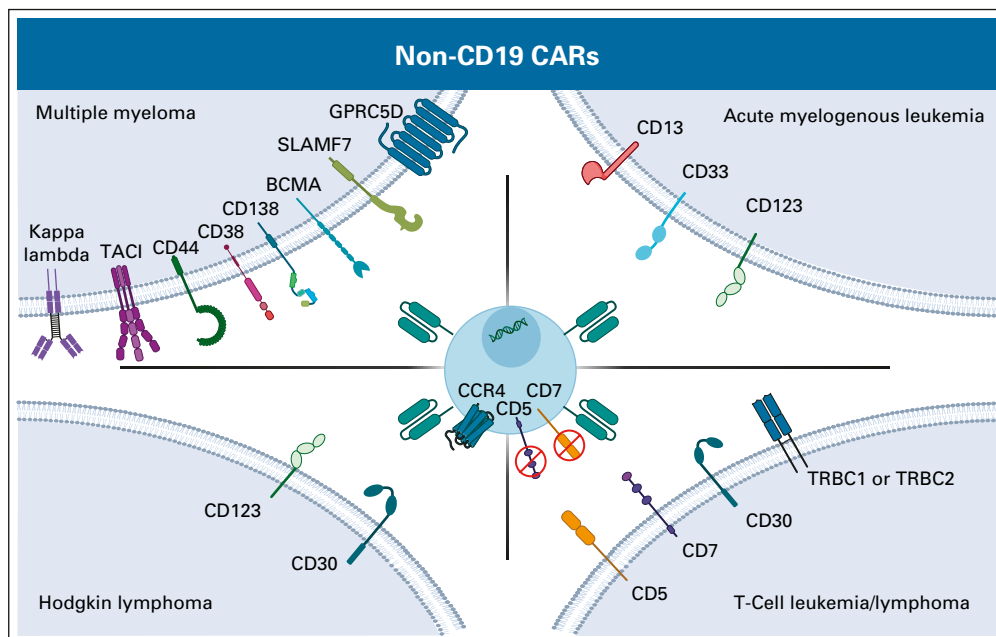
FIG 1. Composite diagram of leading targets for chimeric antigen receptor (CAR)–based therapy across multiple myeloma, acute myelogenous leukemia, Hodgkin lymphoma, and T-cell leukemia/lymphoma. Included in this diagram is the knockdown of CD5 and CD7 on transduced CAR T cells (CAR-Ts) by some active trials in an attempt to avoid CAR-T fratricide. Created with BioRender (BioRender, Toronto, Ontario, Canada). BCMA, B-cell maturation antigen; GPRC5D, G protein-coupled receptor class C group 5 member D; Kappa, κ-light chain; Lambda, λ-light chain; TACI, transmembrane activator and CAML interactor.

preclinical studies explored single-chain variable fragments characterized by low affinity for CD38 aiming to generate CD38.CAR-Ts targeting preferentially MM cells characterized by higher CD38 density while sparing other cells.[26,27] A phase I trial that is evaluating CD38.CAR-T safety and efficacy is under way (ClinicalTrials.gov identifier: NCT03464916; Appendix Table A2).

SLAMF7. SLAMF7 (signaling lymphocyte-activation molecule F7/CD319/CS1CRACC) is a member of the SLAM family of transmembrane receptors expressed by NK cells, B cells, T cells, dendritic cells, NK T cells, and monocytes.[28,29] Relevant to this review, SLAMF7 is overexpressed in MM. A CAR targeting SLAMF7 (SLAMF7.CAR), derived from the humanized anti-SLAMF7 antibody elotuzumab, was generated and showed activity against MM.[30] Although SLAMF7 is also expressed by activated T cells, fratricide cytotoxic activity was largely restricted to those with high expression of SLAMF7, while lymphocytes with low expression were spared. More recently, a single-chain, bispecific CAR that targets both BCMA and SLAMF7 has been developed and has shown activity in vitro and in vivo in murine models.[31] Preclinical evidence also suggests potential synergy between SLAMF7.CAR-Ts and the anti-MM agents lenalidomide and daratumumab.[32] There are at least four SLAMF7.CARs

under clinical investigation (ClinicalTrials.gov identifiers: NCT03710421, NCT03958656, and NCT04142619; EudraCT identifier: 2019-001264-30; Table 2; Appendix Table A2). One of these studies (UCARTCS1) is testing a so-called off-the-shelf CAR. Briefly, allogeneic T cells obtained from healthy donors are engineered to express the SLAMF7.CAR. These cells are also manipulated to have the expression of their inherent T-cell receptor (TCR) and endogenous SLAMF7 disrupted, thereby preventing alloreactivity and fratricide effects, respectively.

Future directions. There has been substantial growth in the development of CAR-T therapies for MM over the years. In addition to known canonical antigens expressed in MM already targeted with monoclonal antibodies, other antigens are currently being investigated in MM, which include CD44 variant 6[33,34] (ClinicalTrials.gov identifier: NCT04097301), G-protein–coupled receptor family class C group 5 member D,[35,36] New York esophageal squamous cell carcinoma 1[37] (ClinicalTrials.gov identifier: NCT03941626), NK group 2 member D,[38-40] CD56[41,42] (ClinicalTrials.gov identifiers: NCT03473496 and NCT03271632), and integrin-β7[43] (ClinicalTrials.gov identifier: NCT03778346; Table 2; Appendix Table A2). In the meantime, future studies should focus on identifying where BCMA.CAR-Ts fit in the current treatment paradigm for MM to maximize depth and

TABLE 1. BCMA CAR-T Clinical Trials for Multiple Myeloma

Trial	Phase	No. of Patients	Median Lines of Prior Therapy	CAR Generation	Costimulatory Domain	Cell Dose	LD Regimen	Best Response	Toxicity (grade ≥ 3 CRS/ neurotoxicity)	Status	Study
Idecabtagene vicleucel (bb2121, KarMMa)	II	128 of 140 enrolled	6	Second	4-1BB	150, 300, 450 × 10^6 CAR-Ts	3 doses of 300 mg/m² Cy and of 30 mg/m² fludarabine	ORR, 73% 42 patients (33%) in CR/sCR	Grade ≥ 3 CRS, 7 (5%; one grade 5 event) Grade ≥ 3 neurotoxicity, 4 (3%)	Ongoing	ClinicalTrials.gov identifier: NCT03361748
LCAR-B38M (LEGEND-2)	I/II	57 (of 100 planned)	3	Second	4-1BB	0.5 × 10^6 cells/kg median CAR-Ts	3 doses of 300 mg/m² Cy	ORR, 88% 39 in CR (68%) 3 in VGPR (5%) 8 in PR (14%) mPFS, 15 months	Grade ≥ 3 CRS, 4 (7%) Grade ≥ 3 neurotoxicity, 0	Ongoing	ClinicalTrials.gov identifier: NCT03090659
LCAR-B38M[a]	I/II	17	4	Second	4-1BB	0.21-1.52 × 10^6 CAR-Ts/kg	Cy and fludarabine	ORR, 88.2% (15 of 17) 13 in sCR 2 in VGPR	Grade ≥ 3 CRS, 6 (1 grade 5 event from a very severe toxic reaction) Grade ≥ 3 neurotoxicity, 0	Ongoing	ChiCTR-ONH-17012285
JNJ-4528 (CARTITUDE-1)	I/II	29 (of planned 118)	5	Second	4-1BB	Target dose: 0.75 × 10^6 viable CAR-Ts/kg Median administered: 0.73 × 10^6 viable CAR-Ts/kg	Cy 300 mg/m² + fludarabine 30 mg/m² over 3 days used for LD	ORR, 100% 22 in sCR (76%) 6 in VGPR (21%) 1 in PR (3%)	Grade ≥ 3 CRS, 2 (7%; one grade 5 event) Grade ≥ 3 neurotoxicity, 1 (3%)	Ongoing	ClinicalTrials.gov identifier: NCT03548207
JCARH125 (orva-cel, first two dose levels of EVOLVE)	I/II	44	7	Second	4-1BB	50, 150 × 10^6 CAR-Ts	3 doses of 300 mg/m² Cy and of 30 mg/m² fludarabine	ORR, 82% across all dose levels 48% in VGPR 27% in PR	Grade ≥ 3 CRS, 9% Grade ≥ 3 neurotoxicity, 7%	Ongoing	ClinicalTrials.gov identifier: NCT03430011
JCARH125 (orva-cel dose escalation of EVOLVE)	I/II	62	6	Second	4-1BB	300, 450, 600 × 10^6 CAR-Ts	3 doses of 300 mg/m² Cy and of 30 mg/m² fludarabine	ORR, 92% 36% in CR/sCR 32% in VGPR 24% in PR	Grade ≥ 3 CRS, 2 (3%) Grade ≥ 3 neurotoxicity, 2 (3%)	Ongoing	ClinicalTrials.gov identifier: NCT03430011
PiggyBac	I/II	21	6	Second	4-1BB	48-430 × 10^6 P-BCMA-101 CAR-Ts	3 doses of 300 mg/m² Cy and of 30 mg/m² fludarabine	ORR (6 treated above cohort 1 dose), 83% 1 in sCR 1 in VGPR 3 in PR	Grade ≥ 3 CRS, 0 Grade ≥ 3 neurotoxicity, 0	Ongoing	ClinicalTrials.gov identifier: NCT03288493
SZ-CART-MM02 (dual CD19/BCMA CAR)	I/II	10 of 15 planned		Third (BCMA CAR)	CD28/OX40	2-6 × 10^7 BCMA⁺ CAR-Ts/kg	Tandem auto-HSCT (busulfan and Cy conditioning)	ORR, 100% (10 of 10) 80% in CR/sCR 20% in VGPR	Grade ≥ 3 CRS, 0 Grade ≥ 3 neurotoxicity, 0	Ongoing	ClinicalTrials.gov identifier: NCT03455972 (dual CD19/BCMA)

Abbreviations: auto-HSCT, autologous hematopoietic stem-cell transplantation; BCMA, B-cell maturation antigen; CAR, chimeric antigen receptor; CAR-T, chimeric antigen receptor T cell; ChiCTR, Chinese Clinical Trial Register; CR, complete response; CRS, cytokine release syndrome; Cy, cyclophosphamide; LD, lymphodepleting; mPFS, median progression-free survival; orva-cel, orvacabtagene autoleucel; ORR, objective response rate; PR, partial response; sCR, stringent complete response; VGPR, very good partial response.

[a]single-chain variable fragments (scFvs) are directed against two distinct BCMA epitopes (VH1 and VH2).

TABLE 2. Non-BCMA CAR-T Clinical Trials for Multiple Myeloma

Target and Trial	Phase	No. of Patients	Median Lines of Prior Therapy	CAR Generation	Costimulatory Domain	Cell Dose	LD Regimen	Best Response	Toxicity (grade ≥ 3 CRS/ neurotoxicity)	Status	Study
CD38/BCMA											
Dual-specificity CD38 and BCMA	I/II	80	≥ 1	NA	NA	1-5 × 10^6 CAR-Ts/kg	NA	Ongoing	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03767751
CD138											
CART-138	I/II	5 of 10 planned reported	11	Second	4-1BB	Average of 0.756 × 10^7 cytokine-induced killer cells/kg	3 received Cy 1 received PCD 1 received VAD	4 SD 1 PD	Grade ≥ 3 CRS, 4/ 5 (fever) Grade ≥ 3 neurotoxicity, 0	Stopped	ClinicalTrials.gov identifier: NCT01886976
CD138/BCMA/ CD19											
CART-138/BCMA/19	I/II	10	≥ 1	NA	NA	5-100 × 10^6 CAR-Ts/kg	3 doses of 300 mg/ m^2 Cy and of 30 mg/m^2 fludarabine	Ongoing	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03196414
BCMA/TACI											
AUTO2	I/II	12	≥ 3	Third	CD20/OX40	15 × 10^6 to 350 × 10^6 APRIL CAR-Ts	Cy and fludarabine	Terminated after interim analysis showed lack of efficacy	Terminated	Terminated	ClinicalTrials.gov identifier: NCT03287804
SLAMF7/CS1											
CARAMBA-1	I/IIa	38	≥ 2	Second	CD28	NA	NA	Ongoing	Ongoing	Ongoing	EudraCT 2019-001264-30
CD44v6											
MLM-CAR44.1 T-cells	I/II	58	≥ 4	NA	NA	0.5, 1, 2 × 10^6 CAR-Ts/kg	Cy and fludarabine	Ongoing	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT04097301
CD38/BCMA/ NY-ESO-1											
Multitargeted CARs	I/II	73	NA	NA	NA	NA	Cy and fludarabine	Ongoing	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03638206
CD56/CD38/ CD138/BCMA											
Multitargeted CARs	I/II	20	≥ 4	NA	NA	NA	NA	Ongoing	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03271632

Abbreviations: BCMA, B-cell maturation antigen; CAR, chimeric antigen receptor; CAR-T, chimeric antigen receptor T cell; CRS, cytokine release syndrome; Cy, cyclophosphamide; LD, lymphodepleting; PCD, pomalidomide, cyclophosphamide, and dexamethasone; NA, not available in published literature or in trial registry; PD, progressive disease; SD, stable disease; VAD, vincristine, doxorubicin, and dexamethasone.

duration of clinical responses. Furthermore, combinations of BCMA.CAR-Ts with other immunomodulatory agents and the development of bispecific CAR-Ts are warranted to prevent tumor escape. Finally, the tumor microenvironment (TME) of MM likely plays a role in impairing CAR-T function, and countering inhibitory mechanisms should enhance the therapeutic index of CAR-Ts in MM.

HL

CD19. CAR-Ts have been explored in HL with the goal of eradicating the putative CD19-expressing Hodgkin Reed-Sternberg (HRS) clonotypic cells and B cells within the TME.[44,45] Four patients infused with CD19.CAR-Ts obtained through mRNA electroporation did not experience toxicity, but responses were limited and transient at best.[46] Thus, alternative targets must be considered for HL.

CD30, a member of the tumor necrosis factor receptor superfamily,[47] is the most attractive target for HL, being expressed on virtually all HRS cells.[48] CD30 is only found on a small subset of activated T and B cells, so it has a low risk for fratricide and potential toxicity.[49] The feasibility of targeting CD30 in HL has been demonstrated by the success and favorable safety profile of brentuximab vedotin, an antibody drug conjugate directed against CD30,[50] stimulating the development of CD30-specific CAR-Ts (CD30.CAR-Ts; Table 3; Appendix Table A3, online only).

In the first published trial of CD30.CAR-Ts conducted in China, 17 patients with HL were treated, and six achieved PR, but none achieved a CR.[51] Toxicities were minimal, with no CRS observed. Although CAR transgene levels decreased to baseline 4-8 weeks after infusion, there were higher numbers of CD30.CAR-Ts in post-treatment biopsies as well as a decreased number of CD30$^+$ tumor cells, suggesting that even with undetectable levels in peripheral blood, CD30.CAR-Ts can reach and eliminate HL tumor cells.[51]

In a second study conducted at Baylor College of Medicine (BCM), seven patients were treated with CD30.CAR-Ts without previous lymphodepleting chemotherapy.[52] One patient achieved CR, which has been maintained for > 2.5 years, whereas another patient, who was in CR at the time of treatment, continued to be in CR for > 2 years. There were no toxicities attributed to CD30.CAR-Ts and no impairment of T-cell–mediated immunity, defined as incidence of viral infections and loss of virus-specific T cells, despite that activated T cells can upregulate CD30.[52] Phase Ib/II trials of the same CD30.CAR-Ts, but preceded by lymphodepletion, were conducted at BCM and UNC and have shown higher response rates in heavily pretreated patients, with CRs seen in more than half treated with optimal lymphodepletion and low rates of toxicity, including CRS[53] (Table 3). The success of these studies is encouraging for further investigation of CD30.CAR-Ts for patients with HL.

Another target investigated in HL is CD123, which is expressed on HRS cells in 50%-60% of patients with HL, in addition to immune cells in the HL TME, most importantly tumor-associated macrophages (TAMs).[54] Preclinical studies of CD123-specific CAR-Ts (CD123.CAR-Ts) have confirmed their antilymphoma activity as well as their ability to eliminate TAMs.[55,56] However, because CD123 is expressed by myeloid precursors, myelosuppression may represent a serious adverse effect,[57] and this treatment would likely require a rescue with HSC transplantation.[56]

Future directions. Despite the encouraging high rate of response in patients with HL receiving CD30.CAR-T cells, relapses still occur. It is encouraging that HL that relapses after CD30. CAR-T therapy seems to retain CD30 expression.[53] Thus, tumor recurrence may more likely be related to the inhibitory effects of the TME, suboptimal persistence, or issues with trafficking of CAR-Ts to the tumor site. The unique biology of the TME of HL makes this tumor an excellent model to test some of the issues that CAR-T therapies face and to study the next generation of CAR-T modifications. For example, HRS cells produce thymus and activation-regulated chemokine/CC chemokine ligand 17 and macrophage-derived chemokine to attract suppressive cells that express their cognate receptor CC chemokine receptor 4 (CCR4).[58-60] In preclinical studies, CD30.CAR-Ts that co-expressed CCR4 showed improved trafficking and antilymphoma activity compared with CD30.CAR-Ts.[61] There is currently a phase I clinical trial at UNC that is testing the hypothesis that CD30.CAR-Ts co-expressing CCR4 may have improved tumor trafficking in patients with r/rHL (Appendix Table A3).

Given the rich inhibitory TME seen in HL, other venues for improvement include combination therapies. Because PD-1 is expressed by infused CD30.CAR-Ts,[52] these cells remain susceptible to the inhibitory effect from PD-L1–expressing HRS cells.[62] Checkpoint inhibitors, already approved for HL, should interrupt this pathway, improve the expansion and persistence of CAR-Ts, and potentially overcome the inhibitory TME.

T-Cell Malignancies

The prognosis of patients with relapsed acute T-cell acute lymphoblastic leukemia (T-ALL) and T-cell lymphoma (TCL) is poor with limited treatment options,[63,64] so there has been interest in developing CAR-T therapies for these malignancies. However, unique challenges when targeting T-cell–associated antigens in T-cell malignancies raise significant concerns in developing CAR-T–based approaches.

The first problem is that targeting T-cell lineage–associated antigens shared by malignant and normal T cells can directly impair the manufacturing of CAR-Ts because of fratricide.[3] This necessitates the identification of antigens expressed on T cells but rapidly downregulated upon binding.[65] CD5 is believed to be one of these antigens. In

TABLE 3. CD30 CAR-T Clinical Trials for HL and CD30^{+} NHL

Trial Site	Phase	Disease	No. of Patients	Median Lines of Prior Therapy	CAR Generation	Costimulatory Domain	Cell Dose	LD Regimen	Best Response	Toxicity (grade ≥ 3 CRS/ neurotoxicity)	Study
Tessa Therapeutics	II	HL	90	≥ 3	Second	4-1BB	NA	Fludarabine and bendamustine	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT04268706
Chinese PLA General Hospital	I/II	HL/NHL	30	≥ 2[a]	Second	4-1BB	Mean of 1.56×10^7 CAR-Ts/kg	25 mg/m^2 fludarabine + 30 mg/kg Cy	Ongoing, 7 of 18 in PR 6 of 18 with SD	Ongoing Of initial 18 patients, no grade 3/4 events reported to date	ClinicalTrials.gov identifier: NCT02259556
UNC/BCM	I/II	HL	42	7	Second	CD28	$0.2\text{-}2 \times 10^8$ cells/m^2	Fludarabine and bendamustine or fludarabine and Cy	ORR, 72%, CR, 59%	Grade ≥ 3 CRS, 24% No grade ≥ 3 neurotoxicity	ClinicalTrials.gov identifiers: NCT02690545 and NCT02917083
Southwest Hospital, China	I/II	CD30^{+} leukemia/ lymphoma	45	r/r	NA	NA	NA	NA	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT02958410
UNC	II	CD30^{+} T-cell lymphoma	20	r/r, ≥ 2	Second	CD28	2×10^8 CAR-Ts/ m^2	Fludarabine and bendamustine then Cy and fludarabine	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT04083495

Abbreviations: BCM, Baylor College of Medicine; CAR, chimeric antigen receptor; CAR-T, chimeric antigen receptor T cell; CR, complete response; CRS, cytokine release syndrome; Cy, cyclophosphamide; HL, Hodgkin lymphoma; LD, lymphodepleting; NA, not available in published literature or in trial registry; NHL, non-Hodgkin lymphoma; ORR, objective response rate; PLA, People's Liberation Army; PR, partial response, r/r, relapsed or refractory; SD, stable disease; UNC, University of North Carolina at Chapel Hill.

[a]Also enrolling newly diagnosed patients not fit for other therapy.

preclinical models, T cells transduced with a CAR targeting CD5 (CD5.CAR-Ts) experienced only transient fratricide because these T cells subsequently reduced their expression of CD5 while increasing expression of protective molecules that inhibit T-cell–mediated cytotoxicity and apoptosis.[65] CD5.CAR-Ts eradicated TCL lines and T-ALL blasts in vitro and controlled progression of T-ALL in mouse models.[65] A clinical trial of CD5.CAR-Ts is currently ongoing[66] (ClinicalTrials.gov identifier: NCT03081910; Appendix Table A4, online only). Preliminary results suggest safety of this approach, with responses seen in four of nine reported patients, although two patients who did not proceed to HSC transplantation experienced a relapse.

Another method to prevent fratricide effects during CAR-T manufacturing is testing the elimination of the target antigen in CAR-Ts themselves through gene editing. This strategy is being explored for targeting CD7, which is highly expressed by normal T and NK cells without downregulation during CAR-T expansion.[67] CRISPR/Cas9 editing to disrupt the CD7 gene before transduction with a CD7-directed CAR allowed CAR-T expansion ex vivo without fratricide effects.[68] Several trials of CD7.CAR-Ts for patients with CD7+ T-cell malignancies are currently recruiting (Table 4; Appendix Table A4).

The second and most concerning challenge of developing CAR-Ts for T-cell malignancies is the potential deleterious effect of specific CAR-Ts on the normal compartments of T cells and NK cells upon infusion in patients. Edited CD7.CAR-Ts seemed to retain response to viral peptides through their native TCR, which offers hope that infused cells will preserve some pathogen specificity.[68] However, if T-cell aplasia occurs in case of robust persistence in vivo of CAR-Ts targeting pan-T antigens, patients will be at high risk for life-threatening opportunistic infections.[67] In consideration of this risk, CAR-Ts that target pan-T antigens may only be indicated as a bridge to allogeneic HSC transplantation. Alternatively, antigens with limited expression on normal T cells compared with malignant T cells should be considered to develop CAR-Ts as a long-term therapy for patients with T-cell malignancies. An example is the CAR specific for the TCR β-chain constant region domain 1 (TRBC1).[69] Physiologically, TRBC1 and TRBC2 are expressed on normal T cells, in a mutually exclusive manner, and in a majority of T-cell lymphomas as well as in one third of T-ALL.[69] Although normal and antigen-specific T-cell populations contain both TRBC1+ and TRBC2+ cells, malignant clones express only either TRBC1 or TRBC2. In preclinical studies, CAR-Ts that target TRBC1 eliminated TRBC1+ normal and malignant T cells but preserved TRBC2+ normal T cells in vitro and in T-ALL mouse models.[69] A clinical trial of TRBC1.CAR-T cells for T-cell lymphoma is ongoing (ClinicalTrials.gov identifier: NCT03590574; Table 4).

Another alternative target is CD1a, expressed in cortical T-cell leukemia, that accounts for approximately 40% of cases of T-ALL, but not on mature T cells.[70] In preclinical studies, CAR-Ts that target CD1a expanded ex vivo without fratricide effects and showed activity against cortical T-cell blasts, while preserving normal T cells.[71] Although this approach cannot be applied to all T-ALL, it holds promise for cortical T-ALL.

CD30 is another potential target expressed by anaplastic large-cell lymphoma (ALCL) and other peripheral TCLs.[72] In a phase I clinical trial of CD30.CAR-Ts, one patient with cutaneous ALCL was treated without lymphodepleting chemotherapy with a PR. In a different phase I clinical trial of CD30.CAR-Ts without lymphodepletion, two patients with ALCL were treated, and one patient achieved CR that lasted 9 months.[52] There are several clinical trials of CD30.CAR-Ts that are enrolling patients with CD30+ TCL (Table 3; Appendix Table A3).

A third obstacle in developing CAR-Ts for T-ALL and TCL revolves around the risk of contamination of the CAR-T product with malignant T cells that can be accidentally transduced with a viral vector.[3] This phenomenon was reported in a patient with B-cell ALL who received CD19.CAR-Ts, which included leukemic blasts that inadvertently expressed the CAR.[73] The presence of the CD19.CAR in cells expressing the same antigen led to the masking of the CD19 epitope, creating a resistant clone able to expand and lead to relapse. While this is a rare occurrence in patients with B-cell malignancies, it seems a more real possibility in patients with T-cell malignancies because T cells are the intended target of transduction and are highly susceptible to vector-mediated gene transfer compared with B cells. As more clinical trials of autologous CAR-Ts for T-cell malignancies are developing, it will be important to consider whether CAR insertion in malignant T cells, in addition to masking antigen recognition, can also cause insertion mutagenesis in tumor cells, exacerbating their malignant phenotype.

Future directions. Patients with T-cell malignancies often have been heavily pretreated and may have low T-cell counts as well as functionally defective T cells. In addition, these patients may have aggressive diseases with a restricted window for receiving treatment. All these aspects make T-cell malignancies an appealing clinical setting to test the use of off-the-shelf CAR-Ts. One example of off-the-shelf CAR-Ts being explored is the UCART7 study in which the deletion of CD7 and TCR through CRISPR/Cas9 gene editing in allogeneic T cells is coupled with CD7.CAR expression.[74] The UCART7 T-cell product was found to be active against T-ALL without causing fratricide effects or xenogeneic graft-versus-host disease.[74] These universal CAR-Ts are generally expected to have shorter persistence as a result of rejection by host cells.[3,74] However, this phenomenon may have some advantages in this specific setting given the concerns for immunosuppression when targeting T and NK cells. Universal CAR-Ts for T-cell malignancies are currently being investigated in clinical trials (Appendix Table A4).

TABLE 4. CAR-T Clinical Trials for T-Cell Malignancies

Target and Trial	Phase	Disease	No. of Patients	Median Lines of Prior Therapy	CAR Generation	Costimulatory Domain	Cell Dose	LD Regimen	Best Response	Toxicity (grade ≥ 3 CRS/ neurotoxicity)	Study
CD7											
Shenzhen Geno-Immune Medical Institute	I/II	CD7+ NK or T-cell leukemia/ lymphoma	30	r/r	NA	NA	NA	NA	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT04033302
TRBC1											
Autolus Limited	I/II	TRBC1+ T-cell Leukemia/ lymphoma	55	r/r	NA	NA	$25\text{-}225 \times 10^6$ RQR8/ aTRBC1 CAR-Ts	Fludarabine and Cy	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03590574

Abbreviations: CAR, chimeric antigen receptor; CAR-T, chimeric antigen receptor T cell; LD, lymphodepleting; NA, not available in published literature or in trial registry; NK, natural killer; r/r, relapsed or refractory.

Preliminary data were recently reported for TruUCART GC027 (Gracell Biotechnologies, Shanghai, China), another universal CD7.CAR-T platform.[75] Four of five treated patients achieved minimal residual disease (MRD)–negative CR without subsequent allogeneic HSC transplantation and modest toxicity, including CRS that resolved with treatment and supportive care. Longer follow-up is needed to assess the durability of these responses as well as long-term effects on T-cell immunity.

There has been much progress in the preclinical realm in overcoming the unique obstacles associated with targeting T-cell malignancies with CAR-Ts. In the coming years, we expect to see results of the ongoing clinical trials. Future challenges remain in identifying targets and approaches that can lead to more-prolonged persistence while avoiding intolerable toxicities.

AML

Developing CAR-Ts for AML has been particularly puzzling because of the difficulty of selecting a suitable antigen not shared by the leukemic blasts and healthy HSCs.[1] There is a particular concern for significant on-target/off-tumor toxicity leading to life-threatening infections, long-term transfusion requirements, or bleeding complications.[76] The majority of the work in AML to date has been preclinical, although there have been a few clinical trials with limited data reported (Table 5; Appendix Table A5, online only). The first published clinical trial tested CAR-Ts that targeted the Lewis-Y antigen in AML. This phase I study showed transient clinical responses, and CAR-Ts persisted for up to 10 months without causing severe toxicities.[77] Two other antigens of particular interest have been CD33 and CD123 because of their almost universal expression on AML blasts.[78]

In a case report, one patient treated with CD33.CAR-Ts had a very transient reduction of leukemic blasts followed by progression within 9 weeks.[79] Dual CD33 and CLL-1 (C-type lectin molecule-1).CAR-Ts have been developed in an attempt to improve efficacy and overcome tumor escape. In a preliminary report, two patients with AML achieved MRD-negative CR and subsequently underwent allogeneic HSC transplantation.[80]

There have been two reported clinical trials with CD123.CAR-Ts in AML. In the first trial at the University of Pennsylvania, mRNA electroporated CD123.CAR-Ts were used to assess the safety of targeting CD123.[81] No significant toxicities were reported, but despite multiple infusions of CD123.CAR-Ts, all five patients progressed before day 28, and the trial was discontinued because of futility. However, the lack of toxicity supported the development of the subsequent study targeting CD123 using CD123.CAR-Ts in which the CAR is stably expressed by T cells through lentiviral transduction. Another trial of CD123.CAR-Ts obtained through lentiviral transduction is currently ongoing at City of Hope Medical Center. In the preliminary report, two

of six treated patients with AML achieved CR and successfully proceeded to allogenic HSC transplantation.[82] Still, more data are needed to draw conclusions about long-term toxicities in responding patients not eligible for transplantation.

Future directions. Despite the promise of some of the aforementioned approaches, there is still concern about the profound and prolonged myeloablation after CAR-Ts targeting myeloid-associated antigens, and alternative methods are urgently needed to minimize on-target/off-tumor toxicity. Novel approaches include using gene editing to remove the AML-specific antigen from normal cells.[76] In preclinical models, CRISPR/Cas9 gene editing was successful in creating CD33-deficient HSCs that are resistant to CD33.CAR-Ts but still have normal hematologic and immunologic function.[83] However, the clinical feasibility of this complex procedure remains a major concern. Alternative approaches consider the targeting of multiple antigens that must be simultaneously expressed by leukemic blasts and not by normal HSCs to fully activate CAR-Ts.[84,85]

DISCUSSION

There have been promising developments in CAR-Ts for non–CD19-expressing hematologic malignancies. BCMA.CAR-Ts for MM are farthest along, with upcoming US Food and Drug Administration approval. Approaches for other hematologic diseases such as HL have also recently made significant progress. Toxicities of BCMA.CAR-Ts in MM, such as CRS and neurotoxicity, seem similar to those observed with CD19.CAR-Ts in B-cell malignancies. In contrast, targets such as CD30 in HL showed limited incidence of CRS and no neurotoxicity. Additional studies are needed to determine the etiology of these differences, which are likely multifactorial and related to antigen specificity, CAR construct design, and disease type. As these novel CAR-Ts move to the clinic, challenges remain, including mitigating on-target/off-tumor toxicities either through antigen selection or logic-gating strategies,[86] understanding resistance to treatment, addressing manufacturing limitations in heavily pretreated patients, and reducing the high costs. We anticipate continued development of novel approaches to meet these obstacles and enhance the effectiveness of CAR-Ts.

In conclusion, while targeting CD19 has driven the field of CAR-Ts, recent advances support the notion that CAR-T technology can be applied to other targets and to other hematologic diseases. It is, however, also becoming evident that by moving away from B-cell malignancies, we also face more challenges related to both CAR-T manufacturing and the complexity of the TME. Ongoing work will continue to inspire researchers to further advance CAR-T cells and hopefully move into the arena of solid tumors.

TABLE 5. CAR-T Clinical Trials for AML

Target and Trial	Phase	Disease	No. of Patients	Median Lines of Prior Therapy	CAR Generation	Costimulatory Domain	Cell Dose	LD Regimen	Best Response	Toxicity (grade ≥ 3 CRS/ neurotoxicity)	Study
CD33											
CIBMTR	I/II	CD33+ AML	34	r/r, ≥ 1	NA	NA	Starting dose: 3 × 10^5 transduced CAR-Ts/kg	3 days fludarabine 25 mg/m²/dose, 1 day Cy 900 mg/m²/dose	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03971799
Southwest Hospital, China	I/II	CD33+ myeloid malignancy	45	r/r	NA	NA	NA	NA	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT02958397
CD123[a]											
Chongqing Precision Biotech	I/II	CD123+ AML	40	r/r	NA	NA	NA	NA	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT04272125
Chongqing Precision Biotech	I/II	CD123+ AML	45	r/r, ≥ 2	NA	NA	NA	NA	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT04265963
Southwest Hospital, China	I/II	CD123+ myeloid malignancy	45	r/r	NA	NA	NA	NA	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT02937103
Mustang Bio	I/II	CD123+ MDS/ AML/BPDCN	126	r/r BPDCN/ AML or high-risk MDS	Second	CD28	Maximum: 600 × 10^6 CAR-Ts	5 days 20 mg/m² decitabine, fludarabine 30 mg/m²/ d, 3 days of Cy 300-500 mg/m²/d	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT04109482
CLL1/CD123											
Fujian Medical University	II/III	AML	20	r/r	NA	NA	NA	NA	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03631576
CLL1/CD33/ CD123											
Shenzhen Geno-Immune Medical Institute	I/II	CLL1+ and/or CD33+ and/or CD123+ AML	10	r/r	NA	NA	NA	NA	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT04010877

(continued on following page)

TABLE 5. CAR-T Clinical Trials for AML (continued)

Target and Trial	Phase	Disease	No. of Patients	Median Lines of Prior Therapy	CAR Generation	Costimulatory Domain	Cell Dose	LD Regimen	Best Response	Toxicity (grade ≥ 3 CRS/ neurotoxicity)	Study
CD38											
First Affiliated Hospital of Soochow University	I/II	CD38+ AML	20	r/r	NA	NA	5-20 × 10⁶ CAR-T-38 cells/kg	NA	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT04351022
CD44ᵇ											
Molecular Medicine	I/II	CD44v6+ AML/MM	58	r/r	NA	NA	0.5, 1, or 2 × 10⁶/kg	Fludarabine and Cy	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT04097301

Abbreviations: AML, acute myelogenous leukemia; BPDCN, blastic plasmacytoid dendritic cell neoplasma; CAR, chimeric antigen receptor; CAR-T, chimeric antigen receptor T cell; CIBMTR, Center for International Blood and Marrow Transplant Research; Cy, cyclophosphamide; LD, lymphodepleting; MDS, myelodysplastic syndrome; MM, multiple myeloma; NA, not available in published literature or in trial registry; r/r, relapsed or refractory.

aBiodegradable T electroporated with anti-CD123 CAR mRNA.

bHSV-TK Mut2 gene (suicide gene).

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Patents, Royalties, Other Intellectual Property: Patents in the field of T/NKT-cell therapies and royalties derived from licensed patents.

Barbara Savoldo
Consulting or Advisory Role: Tessa Therapeutics
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APPENDIX

TABLE A1. BCMA CAR-T Clinical Trials for Multiple Myeloma

Trial	Phase	No. of Patients	Median Lines of Prior Therapy	CAR Generation	Costimulatory Domain	Cell Dose	LD Regimen	Best Response	Toxicity (grade ≥ 3 CRS/ neurotoxicity)	Status	Study
NCI	I	24	9.5	Second	CD28	0.3, 1, 3, or 9 × 10^6 CAR-Ts/kg	3 doses of 300 mg/m^2 Cy and of 30 mg/m^2 fludarabine	ORR, 81% 63% in VGPR or CR mEFS, 31 weeks	Grade ≥ 3 CRS, 6 of 24 Grade ≥ 3 neurotoxicity, 1 of 24	Completed	ClinicalTrials.gov identifier: NCT02215967
UPenn/Novartis/NIH (CART-BCMA)	I	25 (in three cohorts)	7	Second	4-1BB	C1: 1-5 × 10^8 CAR$^+$ cells alone C2: 1-5 × 10^7 CAR-Ts + LD C3: 1-5 × 10^8 CAR-Ts + LD	1 dose of 1.5 g/m^2 Cy	ORR, 48% 12 PR or better 3 remissions > 11 months, including 1 sCR > 2.5 years	Grade ≥ 3 CRS, 8 (32%) Grade ≥ 3 neurotoxicity, 3 (12%)	Completed	ClinicalTrials.gov identifier: NCT02546167
Idecabtagene vicleucel (bb2121, KarMMa)	I	33	7	Second	4-1BB	50, 150, 450, or 800 × 10^6 CAR$^+$ cells	3 doses of 300 mg/m^2 Cy and of 30 mg/m^2 fludarabine	ORR, 85% 15 in CR (45%) mPFS, 11.8 months	Grade ≥ 3 CRS, 2 (6%) Grade ≥ 3 neurotoxicity, 1 (3%)	Completed	ClinicalTrials.gov identifier: NCT02658929
bb21217	I	8	9	Second	4-1BB	150, 450, 800, or 1,200 × 10^6 CAR$^+$ cells	3 doses of 300 mg/m^2 Cy and of 30 mg/m^2 fludarabine	ORR, 86% 6 achieving PR or better, including 1 sCR	Grade ≥ 3 CRS, 1 (12.5%) Grade ≥ 3 neurotoxicity, 1 (12.5%)	Ongoing	ClinicalTrials.gov identifier: NCT03274219
MCARH171	I	11	6	Second	4-1BB	C1: 1-5 × 10^8 CAR$^+$ cells alone C2: 1-5 × 10^7 CAR-Ts + LD C3: 1-5 × 10^8 CAR-Ts + LD C4: 1-5 × 10^8 CAR-Ts + LD 72-818 × 10^6 CAR$^+$ cells	1 dose of 3 g/m^2 Cy or 3 doses of 300 mg/m^2 Cy and of 30 mg/m^2 fludarabine	ORR, 64% mDOR, 106 days	Grade ≥ 3 CRS, 2 (20%) Grade ≥ 3 neurotoxicity, 0	Ongoing	ClinicalTrials.gov identifier: NCT03070327
FCAR143	I	7	11	Second	4-1BB	5 × 10^7 CAR-Ts	3 doses of Cy and fludarabine	ORR (at 28 days), 100% 1 relapse at day 60 with BCMA-negative disease	Grade ≥ 3 CRS, 0 Grade ≥ 3 neurotoxicity, 0	Ongoing	ClinicalTrials.gov identifier: NCT03338972
CT053	I	16	4	Second	4-1BB	0.5, 1.5, or 1.8 × 10^8 cells	3 doses of 300-500 mg/m^2 Cy and 20-25 mg/m^2 fludarabine	ORR, 100% (13 of 13) 3 in CR 6 in VGPR 4 in PR	Grade ≥ 3 CRS, 1 Grade ≥ 3 neurotoxicity, 0	Ongoing	ClinicalTrials.gov identifier: NCT03915184
CT103A	I	16	NA (≥ 3 prior lines)	Second	4-1BB	1, 3, 6, or 8 × 10^6 CAR-Ts/kg	Cy and fludarabine	ORR, 100%, with 75% in CR/sCR, and 25% in VGPR.	CRS/CRES, 5 grade 3, 1 grade 4, 1 DLT (highest dose)	Ongoing	ChiCTR-1800018137
BRD015	I	28	7	Second	CD28	5.4-25.0 × 10^6 CAR-Ts/kg	Cy and fludarabine	ORR, 87% (BCMA strong expression group) and 100% (BCMA weak expression group)	Grade ≥ 3 CRS, 4 (14%) Grade ≥ 3 neurotoxicity, 0	Ongoing	ChiCTR-OPC-16009113
ALLO-715[a]	I	90	≥ 3	Second	4-1BB	40, 120, or 320 × 10^6 CAR-Ts	3 doses of 300 mg/m^2 Cy and of 30 mg/m^2 fludarabine	Ongoing	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT04093596
BCMA nano-antibody CAR-T	I	7 of 15 planned	10	Second	4-1BB	5, 10, or 15 × 10^6 CAR-Ts/kg	Cy and fludarabine	ORR, 100% (7 of 7) 42.8% in CR/sCR 14.3% in VGPR 42.8% in PR	Grade ≥ 3 CRS, 2 of 7 Grade ≥ 3 neurotoxicity, 0	Ongoing	ClinicalTrials.gov identifier: NCT03661554

Abbreviations: BCMA, B-cell maturation antigen; CAR, chimeric antigen receptor; CAR-T, chimeric antigen receptor T cell; C, cycle; ChiCTR, Chinese Clinical Trials Repository; CR, complete response; CRES, chimeric antigen receptor–related encephalopathy syndrome; CRS, cytokine release syndrome; Cy, cyclophosphamide; DLT, dose-limiting toxicity; LD, lymphodepleting; mDOR, median duration of response; mEFS, median event-free survival; mPFS, median progression-free survival; NA, not available in published literature or in trial registry; NCI, National Cancer Institute; NIH, National Institutes of Health; ORR, objective response rate; PR, partial response; sCR, stringent complete response; UPenn, University of Pennsylvania; VGPR; very good partial response.

[a]Allogeneic off-the-shelf CAR product.

TABLE A2. Non-BCMA CAR-T Clinical Trials for Multiple Myeloma

Target and Trial	Phase	No. of Patients	Median Lines of Prior Therapy	CAR Generation	Costimulatory Domain	Cell Dose	LD Regimen	Best Response	Toxicity (grade ≥ 3 CRS/ neurotoxicity)	Status	Study
CD38											
CAR2 Anti-CD38 A2	I	72	≥ 2	NA	NA	NA	NA	Ongoing	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03464916
CD138											
ATLCAR.CD138	I	33	≥ 2	Second	CD28	5, 10, 25, 50, 100, or 200 × 10^6 CAR-Ts/kg	3 doses of 300 mg/m² Cy and of 30 mg/m² fludarabine	Ongoing	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03672318
CD38/CD138/BCMA/ SLAMF7/integrin β7											
Multitargeted CARs	I	30	≥ 2	Fourth	Unknown costimulatory domain (simultaneously expressing IL-7 and CCL19)	10^6-10^7 CAR-Ts/kg	NA	Ongoing	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03778346
SLAMF7/CS1											
CS1-CAR T	I	30	≥ 3	Second	4-1BB	Dose escalation	Cy and fludarabine	Ongoing	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03710421
Anti-SLAMF7	I	42	≥ 3	NA	NA	0.3 × 10^6 to 12.0 × 10^6 CAR-Ts/kg	3 doses of 300 mg/m² Cy and of 30 mg/m² fludarabine	Ongoing	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03958656
MELANI-01 (UCARTCS1A)[a]	I	18	≥ 1	NA	NA	NA	NA	Ongoing	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT04142619
NKG2D											
CM-CS1	I	12	1	First	(NKG2D contains an endogenously expressed costimulatory domain, Dap10)	1 × 10^6 to 3 × 10^7 total viable T cells	None	Objective clinical responses to NKG2D-CAR-T therapy alone not seen	Grade ≥ 3 CRS, 0 Grade ≥ 3 neurotoxicity, 0	Completed	ClinicalTrials.gov identifier: NCT02203825
CD56/CD38/ CD138/BCMA											
Multitargeted CARs	I	50	NA	NA	NA	10^6-10^7 CAR-T/kg	NA	Ongoing	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03473496
κ-Light chain											
CHARKALL	I	7	≥ 1	Second	CD28	2, 10, of 20 × 10^7 κ.CAR-T/m²	3 doses of 500 mg/m² Cy and of 30 mg/m² fludarabine	4 of 7 with SD for 2-17 months	Grade ≥ 3 CRS, 0 Grade ≥ 3 neurotoxicity, 0	Ongoing	ClinicalTrials.gov identifier: NCT00881920
ATLCAR.κ.28	I	20	≥ 2	Second	CD28	5, 10, or 20 × 10^5 cells/kg	3 doses of 70 mg/m² bendamustine and of 30 mg/m² fludarabine	Ongoing	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT04223765

Abbreviations: BCMA, B-cell maturation antigen; CAR, chimeric antigen receptor; CAR-T, chimeric antigen receptor T cells; CRS, cytokine release syndrome; Cy, cyclophosphamide; IL-7, interleukin 7; LD, lymphodepleting; NA, not available in published literature or in trial registry; SD, stable disease.

[a]Allogeneic off-the-shelf CAR-T product with endogenous T-cell receptor and SLAMF7 disrupted.

TABLE A3. CD30 CAR-T Clinical Trials for HL and CD30$^+$ NHL

Trial Site	Phase	Disease	No. of Patients	Median Lines of Prior Therapy	CAR Generation	Costimulatory Domain	Cell Dose	LD Regimen	Best Response	Toxicity (grade ≥ 3 CRS/ neurotoxicity)	Study
RELY30	I	HL/NHL	66	r/r	Second	CD28	2, 10, 20 × 10^7 cells/ m^2	Cy and fludarabine	Ongoing 6 in CR lasting up to > 9 months 3 with PD	Ongoing, no grade 3/ 4 events reported to date	ClinicalTrials.gov identifier: NCT02917083
Immune Cell/ Weifang People's Hospital	I	HL/ALCL	20	r/r	NA	NA	NA (dose escalation)	NA	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03383965
NCI	I	HL/NHL/ ALCL	79	≥ 2	NA	NA	0.3-18 × 10^6 cells/kg	Cy and fludarabine	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03049449
UNC[a]	I	HL/cutaneous T-cell lymphoma	59	≥ 2	Second	CD28	2, 5, 10 × 10^7 cells/ m^2	Fludarabine and bendamustine	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03602157
Wuhan Union Hospital	I	HL/NHL/ ALCL	50	≥ 1[b]	Third	NA	NA	NA	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT04008394
UNC[c]	I	HL/NHL	18	Recurrent disease	Second	CD28	2, 10, 20 × 10^7 cells/ m^2 (maximum dose, 50 × 10^7 cells)	None	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT02663297
BCM[d]	I	HL/NHL	18	r/r[b]	Second	NA	2, 5, 10 × 10^7 cells/ m^2	None	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT01192464
BCM	I	HL/NHL	9	r/r, ≥ 3	Second	CD28	0.2-2 × 10^8 CD30.CAR-Ts/m^2	None	2 in CR 3 with SD	Grade ≥ 3 CRS, 0 Grade ≥ 3 neurotoxicity, 0	ClinicalTrials.gov identifier: NCT01316146

Abbreviations: ALCL, anaplastic large-cell lymphoma; BCM, Baylor College of Medicine; CAR, chimeric antigen receptor; CAR-T, chimeric antigen receptor T cell; CR, complete response; CRS, cytokine release syndrome; Cy, cyclophosphamide; HL, Hodgkin lymphoma; LD, lymphodepleting; NA, not available in published literature or in trial registry; NCI, National Cancer Institute; NHL, non-Hodgkin lymphoma; r/r, relapsed/refractory; PD, progressive disease; SD, stable disease; UNC, University of North Carolina at Chapel Hill.

[a]Co-administration with CAR-T co-expressing CCR4.

[b]Also enrolling newly diagnosed patients not fit for other therapy.

[c]Administered as consolidation after autologous stem-cell transplantation.

[d]Epstein-Barr virus–specific T cells.

TABLE A4. CAR-T Clinical Trials for T-Cell Malignancies

Target and Trial	Phase	Disease	No. of Patients	Median Lines of Prior Therapy	CAR Generation	Costimulatory Domain	Cell Dose	LD Regimen	Best Response	Toxicity (grade ≥ 3 CRS/ neurotoxicity)	Study
CD4											
First Affiliated Hospital with Nanjing Medical	I	CD4+ T-cell lymphoma	34	r/r, ≥ 1	NA	NA	NA	NA	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT04219319
Stony Brook University	I	CD4+ T-cell leukemia/ lymphoma	20	r/r	Third	CD28, 4-1BB	Up to 4×10^6 T cells/kg	None	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03829540
CD5											
BCM	I	CD5+ T-cell leukemia/ lymphoma	21	r/r, > 5	Second	CD28	1, 5, 10×10^7 cells/ m^2	3 doses of fludarabine 30 mg/m² and 3 doses of Cy 500 mg/ m²/d	Ongoing, 4 of 9 evaluable patients obtained an objective response, with 3 achieving a CR	Ongoing No grade 3/4 CRS or neurotoxicity reported to date	ClinicalTrials.gov identifier: NCT03081910
CD7											
PersonGen BioTherapeutics (Suzhou)	I	CD7+ NK- or T-cell leukemia/ lymphoma	10	r/r	NA	NA	$0.5\text{-}5 \times 10^6$ cells/kg	Cy or fludarabine and Cy	Ongoing 2 of 3 patients with MRD-negative CR	Ongoing Grade ≥ 3 CRS, 1 of 3 patients Grade ≥ 3 neurotoxicity, 0	ClinicalTrials.gov identifier: NCT04004637
BCM	I	CD7+ NK- or T-cell leukemia/ lymphoma	21	r/r	Second	CD28	1, 3, 10×10^7 cells/ m^2	3 doses of fludarabine 30 mg/m² and 3 doses of Cy 500 mg/ m²/d	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03690011
Xinqiao Hospital of Chongqing	I	CD7+ NK- or T-cell leukemia/ lymphoma	30	r/r	NA	NA	1, 3, 5×10^7 cells/ m^2	6 doses of fludarabine 30 mg/m² and 2-6 doses of Cy 300 mg/ m²/d and 50-70 mg/ m² melphalan (optional on the basis of tumor burden)	Ongoing 4 of 5 patients with MRD-negative CR, 1 of 5 patients with MRD-positive CR with quick relapse	Ongoing Grade ≥ 3 CRS, 5 of 5 patients Grade ≥ 3 neurotoxicity, 0	ClinicalTrials.gov identifier: NCT04264078

Abbreviations: BCM, Baylor College of Medicine; CAR, chimeric antigen receptor; CAR-T, chimeric antigen receptor T cell; CR, complete response; CRS, cytokine release syndrome; Cy, cyclophosphamide; LD, lymphodepleting; MRD, minimal residual disease; NA, not available in published literature or in trial registry; NK, natural killer; ORR, objective response rate; r/r, relapsed or refractory.

TABLE A5. CAR-T Clinical Trials for AML

Target and Trial	Phase	Disease	No. of Patients	Median Lines of Prior Therapy	CAR Generation	Costimulatory Domain	Cell Dose	LD Regimen	Best Response	Toxicity (grade ≥ 3 CRS/ neurotoxicity)	Study
CD123											
Hebei Senlang Biotechnology	I	CD123$^+$ AML	15	r/r	NA	NA	NA	NA	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03796390
Wuhan Union Hospital, China	I	CD123$^+$ AML	50	r/r, ≥ 2	Third	CD28 and 4-1BB	NA	NA	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT04014881
Cellectis	I	AML	59	r/r	NA	NA	NA	NA	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03190278
City of Hope Medical Center	I	CD123$^+$ AML/ ALL/ BPDCN	42	r/r	Second	CD28	50-200 × 10^6 CAR-Ts	Fludarabine and Cy	Ongoing 2 of 6 in CR	Ongoing No grade 3/4 CRS/ neurotoxicity reported to date	ClinicalTrials.gov identifier: NCT02159495
St Jude Children's Research Hospital	I	CD123$^+$ AML	32	r/r, ≥ 2	NA	NA	3, 10, 30, 100 × 10^5 CAR-Ts/kg Maximum: 2.5 × 10^8 CAR-Ts	Fludarabine and Cy	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT04318678
UPenn	I	AML	12	r/r	Second	4-1BB	1, 2, 5 × 10^6 CAR-Ts/kg	Fludarabine and Cy	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03766126
Beijing Immunochina Medical Science and Technology	I	CD123$^+$ AML	15	r/r	NA	NA	NA	Fludarabine and Cy	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03585517
Affiliated Hospital to Academy of Military Medical Sciences[a]	I	CD123$^+$ AML	20	r/r, post-allogenic HSCT	Second	4-1BB	NA	NA	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03114670
UPenn[b]	I	AML	7	4 (2-6)	Second	4-1BB	Planned 3-6 doses of 4 × 10^6 CAR-Ts/kg	Cy 1 g/m^2 (optional)	0/5 responses All patients experienced progression	Grade ≥ 3 CRS, 58% Grade ≥ 3 neurotoxicity, 0	ClinicalTrials.gov identifier: NCT02623582
Cellectis (UCART123, ABC123)[a]	I	BPDCN	1	r/r	Second	4-1BB	6.25-62.5 × 10^5 CAR-Ts/kg	Fludarabine and Cy	Terminated	Terminated after grade 5 CRS in first patient	ClinicalTrials.gov identifier: NCT03203369
Cellectis (UCART123, AMELI-01)[a]	I	AML	59	r/r	Second	4-1BB	Dose reduced to 6.25 × 10^4 CAR-Ts/kg	Fludarabine and Cy	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03190278

(continued on following page)

TABLE A5. CAR-T Clinical Trials for AML (continued)

Target and Trial	Phase	Disease	No. of Patients	Median Lines of Prior Therapy	CAR Generation	Costimulatory Domain	Cell Dose	LD Regimen	Best Response	Toxicity (grade ≥ 3 CRS/ neurotoxicity)	Study
CLL/CD33											
iCell Gene Therapeutics	I	AML/MDS/ MPN	20	r/r	NA	NA	NA	Fludarabine and Cy	Ongoing 2 in CR	Ongoing	ClinicalTrials.gov identifier: NCT03795779
CLL1											
BCM	I	CLL1$^+$ AML	18	r/r	Second	4-1BB	1, 3, 10 × 10^7 CAR-Ts/m^2	NA	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT04219163
FLT3											
Amgen	I	FLT3$^+$ AML	40	r/r	NA	NA	NA	Fludarabine and Cy	Ongoing	Ongoing	ClinicalTrials.gov identifier: NCT03904069
Lewis-Y											
Peter MacCallum Cancer Center	I	Lewis-Y$^+$ AML	5	r/r, ≥ 1	Second	CD28	5 × 10^8 to 1.3 × 10^9 CAR-T	Fludarabine 30 mg/ m^2 days 1-5 and cytarabine 2 g/ m^2 days 1-5	Transient response in 3 of 4 patients, including 1 cytogenetic CR	Grade ≥ 3 CRS, 0 Grade ≥ 3 neurotoxicity, 0	ClinicalTrials.gov identifier: NCT01716364

Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; BPDCN, blastic plasmacytoid dendritic cell neoplasma; CAR, chimeric antigen receptor; CAR-T, chimeric antigen receptor T cell; CR, complete response; CRS, cytokine release syndrome; Cy, cyclophosphamide; HSCT, hematopoietic stem-cell transplantation; LD, lymphodepleting; MDS, myelodysplastic syndrome; NA, not available in published literature or in trial registry; r/r, relapsed or refractory.

[a]Allogeneic off-the-shelf CAR-T product.

[b]Biodegradable T electroporated with anti-CD123 CAR mRNA.