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Off-the-shelf CAR-T cell therapies for relapsed or refractory B-cell malignancies: latest update from ASH 2023 annual meeting

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Abstract

Currently, many off-the-shelf chimeric antigen receptor (CAR)-T cell products are under investigation for the treatment of relapsed or refractory (R/R) B-cell neoplasms. Compared with autologous CAR-T cell therapy, off-the-shelf universal CAR-T cell therapies have many potential benefits, such as immediate accessibility for patients, stable quality due to industrialized manufacturing and additional infusions of CAR-T cells with different targets. However, critical challenges, including graft-versus-host disease and CAR-T cell elimination by the host immune system, still require extensive research. The most common technological approaches involve modifying healthy donor T cells via gene editing technology and altering different types of T cells. This article summarizes some of the latest data from preclinical and clinical studies of off-the-shelf CAR-T cell therapies in the treatment of R/R B-cell malignancies from the 2023 ASH Annual Meeting (ASH 2023).

To the editor

Off-the-shelf chimeric antigen receptor (CAR)-T cells can improve the availability of clinical treatments for patients with relapsed or refractory (R/R) B-cell malignancies by overcoming the limitations of inconsistent quality, high cost, long manufacturing cycles and occasional production failures. The proliferation, persistence and allogeneity of off-the-shelf CAR-T cells, as well as graft rejection by the host immune system, are the primary determinants of the safety and therapeutic effects of these treatments. The two fundamental methods for universal CAR-T cell production are the use of gene editing technology and the adoption of specific T-cell subtypes endowed with universality. We presented several recent studies on the use of off-the-shelf CAR-T cells for the treatment of R/R B-cell malignancies from the 2023 ASH Annual Meeting (ASH 2023).

Off-the-shelf CAR-T cell therapies based on gene editing

To reduce the risk of graft-versus-host disease (GVHD) and host-versus-graft reactions, knockout (KO) of the genes that encode the T cell receptor alpha (TCR) constant (TRAC) and CD52 by transcription activator-like effector nuclease (TALEN) technology in combination with an anti-CD52 antibody can be used to prevent TCR-mediated recognition of histocompatibility antigens and protect CAR-T cells from lymphodepletion (LD) mediated by an anti-CD52 antibody. [1–3]. Through the application of Cellectis' TALEN® technology, healthy donor T cells are manipulated to generate ALLO-501/ALLO-501A, which is an allogeneic anti-CD19 CAR-T cell line with TRAC and CD52 deletions (Table 1). ALLO-501/ALLO-501A infusions have shown favorable safety and efficacy in patients with R/R large B-cell

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Table 1 Properties of off-the-shelf CAR-T cell therapies updated at ASH 2023

	Product	T cell sources	Allogeneic technology	Target antigen	Disease	CAR vector	Current research stage	References
1	ALLO-501/501A	Healthy donor T cells	Cellectis' TALEN® mRNA-mediated TRAC and CD52 KO	CD19	R/R LBCL and FL	Lentiviral transduction	Phase 1 ALPHA (ALLO-501; NCT03939026) and ALPHA2 (ALLO-501A; NCT04416984)	[1]
2	UCART22	Healthy donor T cells	Cellectis' TALEN® mRNA-mediated TRAC and CD52 KO	CD19	R/R CD22+B-ALL	Lentiviral transduction	Phase 1 BALLI-01 (NCT04150497)	[2]
3	UCART20×22	Healthy donor T cells	Cellectis' TALEN® mRNA-mediated TRAC and CD52 KO	CD20 and CD22	R/R NHL	Lentiviral transduction	Phase 1/2a NatHaLi-01 (NCT05607420)	[3]
4	P-BCMA-ALLO1	Healthy donor T cells	Cas-CLOVER™ Site-Specific Gene Editing System-mediated TCRβ and B2M KO	BCMA	R/R MM	PiggyBac® DNA Delivery System	Phase 1 P-BCMA-ALLO1-001 (NCT04960579)	[4]
5	RevCAR T	Healthy donor T cells	CRISPR/Cas9-mediated TRAC, HLA-A and MHC II KO	CD19 and CD20	B-cell malignancies	Lentiviral transduction	Preclinical	[5]
6	SC262	Healthy donor T cells	Overexpression of CD47 CRISPR-Cas12b-mediated B2M, CIITA and TRAC KO	CD22	LBCL	Lentiviral transduction	Preclinical	[6]
7	ThisCART19A	Healthy donor T cells	Intracellular retention of TCRαβ/CD3 by a KDEL-tagged anti-CD3 scFV	CD19	R/R B-ALL	Lentiviral transduction	Phase 1 (NCT05350787)	[7]
8	19-28z CAR EBV-CTLs	Healthy donor T cells	EBV specific cytotoxic T lymphocytes	CD19	R/R B-cell malignancies	Lentiviral transduction	Phase 1 (NCT01430390)	[8]
9	ATA3431	Healthy donor T cells	EBV specific cytotoxic T lymphocytes	CD19 and CD20	B-cell malignancies	Retroviral transduction	Preclinical	[9]
10	ADI-001	Healthy donor γδT cells	Vδ1 γδ T cells	CD20	R/R B-NHL	Retroviral transduction	Phase 1 (NCT04735471)	[10]
11	tFas/CD19. CAR-Vδ2 T cells	Healthy donor γδT cells	Vγ9Vδ2 T cells	CD19	B-cell malignancies	NA	Preclinical	[11]
12	anti-CD38-CAR iPS-rCTLs	IPSCs	iPS-derived cytotoxic T lymphocytes	CD38	MM	Retroviral transduction	Preclinical	[12]
13	RJMty19	Healthy donor T cells	DN T cells	CD19	R/R B-NHL	Lentiviral transduction	Phase 1 RJMty19-B-NHL001 (NCT05453669)	[13]

CAR chimeric antigen receptor, *iPSCs* induced pluripotent stem cells, *TALEN* transcription activator-like effector nuclease, *KO* knock-out, *CRISPR* clustered regularly interspaced short palindromic repeats, *scFv* single-chain variable fragment, *EBV* Epstein–Barr virus, *DN* double negative, *R/R* refractory or relapse, *LBCL* large-B-cell lymphoma, *FL* follicular lymphoma, *B-ALL* B-cell acute lymphoblastic leukemia, *NHL* non-Hodgkin's lymphoma, *MM* multiple myeloma, *B-NHL* B cell-NHL, *NA* not available

lymphoma and follicular lymphoma. Twenty-four percent (20/87) of patients experienced cytokine release syndrome (CRS); grade 3–4 CRS and immune effector cell-associated neurotoxicity syndrome (ICANS) both occurred in 1% of patients (Table 2) [1]. There was no significant increase in the occurrence of adverse events associated with enhanced LD. Allogeneic UCART22 and UCART20X22 CAR-T cells were also developed using TALEN technology to disrupt the TRAC gene and CD52 gene. UCART22 Process 2 was administered to three patients with CD22-positive B-cell acute lymphoblastic leukemia (B-ALL), and two patients achieved minimal residual disease (MRD)-negative complete response (CR) without any severe CRS or ICANS [2]. One hundred percent (3/3) of patients with R/R lymphoma who received UCART20×22 had a response accompanied by grade 1–2 CRS; neither ICANS nor GVHD occurred. Notably, two patients who previously received anti-CD19 CAR-T-cell therapy achieved metabolic CR [3]. P-BCMA-ALLO1 is a piggyBac® transposon-generated allogeneic CAR-T cell-targeting B-cell maturation antigen (BCMA). The TCRβ 1 chain gene and the β-2 microglobulin gene (B2M) were disrupted by the Cas-CLOVER™ site-specific editing system [4]. The median time from enrollment to infusion was reduced to 7 days. Twenty-one percent (7/22) of patients experienced CRS of low severity, and one had grade 1 ICANS. The overall response rate (ORR) in arms P1 and P2 using optimized LD was 82%, and two patients in arm P2 reached stringent CR [4].

The reverse universal CAR platform (RevCAR), which targets CD19 and CD20, includes a universal CAR-T cell with KO of the genes encoding the TCR, human leukocyte antigen (HLA)-A and class II major histocompatibility complex transactivator gene (CIITA) and a soluble adaptor called the targeting module (R-TM19/20) [5]. This approaches induces better tumor clearance in B-ALL and lymphoma xenograft models even after CD19-negative relapse [5]. CRISPR-Cas12b is an RNA-guided nuclease platform for genome editing that contains a single RuvC nuclease domain without an HNH domain. It has been used to disrupt B2M, CIIA and TRAC to produce CD22-targeting hypoimmune CAR-T cells (SC262) due to its high efficiency and decreased off-target toxicity in human genome editing [6]. CD47-overexpresing SC262 cells were generated, and these cells can evade killing by NK cells and macrophages by triggering the CD47-SIRPα "don't eat me" signaling pathway and ultimately elicit robust tumor control in vitro and in vivo [6].

In addition, a novel non-gene-editing strategy to generate ThisCART19A (TCRαβ/CD3 and/or HLA-I intracellular sequestered) was used to transduce a construct encoding a CD19 CAR and a KDEL-tagged anti-CD3 single-chain variable fragment (scFv) into T cells. The secretion of the TCRαβ/CD3 complex from the endoplasmic reticulum was prevented, resulting in the loss of TCR expression. A total of 25% (2/8) of patients with R/R B-ALL experienced grade 3–4 CRS, and 37.5% (3/8) of patients had ICANS, while encouraging efficacy profiles revealed that 100% (7/7) of patients had MRD-negative CR/CR with an incomplete hematologic recovery rate, and 57% (4/7) of patients remained in response with a median follow-up of more than 4.9 months [7].

Off-the-shelf CAR-T cell therapies originating from specific T-cell types

Epstein–Barr virus-specific T cells (EBVSTs) can effectively eliminate EB virus-related tumor cells and are considered to be a potential off-the-shelf source due to their restricted TCR repertoire [8, 9]. The 19-28z CAR EBV-CTLs were EBVSTs that were modified with an anti-CD19 CD28-containing CAR and were administered to patients who relapsed or required consolidation therapy after allogeneic or hematopoietic stem cell transplantation [8]. Six percent (1/16) of these patients developed grade 1 CRS, and 18% (3/16) had skin GVHD, including 1 patient with grade 3 GVHD. The 3-year overall survival rate was 74% following multiple infusions. These T cells had lower expression of exhaustion markers, which may contribute to the promising long-term outcomes [8]. ATA3431, which carries a novel CD3ζ signaling domain, 1XX, to extend effector function, is a bispecific CD19- and CD20-targeting allogeneic CAR-T cell based on the EBVST platform that can reliably inhibit B-cell tumor growth according to preclinical evaluations [9].

CAR + γδT cells recognize and kill tumor cells via CAR-dependent and CAR-independent mechanisms, reducing tumor escape without the potential for GVHD. The CD20-targeted universal CAR + Vδ1 γδT cell line ADI-001 exhibited robust dose-dependent expansion and persistence in a phase I trial. Moreover, clinical responses were related to ADI-001 cellular kinetics, regardless of HLA mismatching [10]. Leong et al. altered Vγ9Vδ2 T cells with a reverse fate receptor so that they could avoid activation-induced cell death and augment cytokine signaling via the extracellular domain of Fas and linked intracellular MyD88 [11]. The armed Fas88/CD19.CAR-Vδ2 T cells mediated tumor elimination via massive amplification and persistence in NALM6-bearing mice, suggesting their potential for clinical translation [11]. Induced pluripotent stem (iPS) cells can self-renew, and iPS-derived cytotoxic T lymphocytes (iPS-rCTLs) are expected to survive long term and sustain their cytotoxicity in vivo. IPS-rCTLs transduced with an αCD38 CAR could suppress tumor growth and carry no risk of CD38-mediated fratricide [12]. Double-negative T cells (DNTs) are CD3-positive memory-like T cells that do not have HLA

Table 2 Outcomes of clinical studies of off-the-shelf CAR-T cell therapies in relapsed or refractory B-cell malignancies

	Product	Clinical trial	Disease	PTS enrolled	Cohorts	LD regimens	Median time to LD	Median time to infusion	ORR/CR	Long-term outcomes	CRS/NT	GvHD	References
1	ALLO-501 and ALLO-501A	NCT03939026; NCT04416984	R/R LBCL and FL	87	NA	3 to 5-day of F (30 mg/m^2/d), C (300–500 mg/m^2/d) and A (39, 60, or 90 mg/d)	3 days (FCA90)	NA	FCA90: ORR 67% (8/12), CR 58% (7/12)	NA	Grade 1/2 CRS: 23% (19/87), Grade 3 CRS: 1% (1/87)	No	[1]
2	UCART22	NCT04150497	R/R CD22 + B-ALL	3	P1: manufacture by external CDMO; P2: manufacture by Cellectis Biologics	FC: 4-day of F (30 mg/m^2/d), 3-day of C (1 g/m^2/d) FCA: 3-day of F (30 mg/m^2/d), C (0.5 g/m^2/d) and A (20 mg/d)	NA	NA	ORR 67% (2/3), MRD-CR 67% (2/3)	NA	Grade 1/2 CRS: 67% (2/3)	No	[2]
3	UCART20×22	NCT05607420	R/R B-NHL	3	NA	3-day of F (30 mg/m^2/d), C (500 mg/m^2/d), A (60 mg total)	NA	NA	ORR 100% (3/3), CR 67% (2/3), PR 33% (1/3)	NA	Grade 1/2 CRS: 100% (3/3)	No	[3]

Table 2 (continued)

	Product	Clinical trial	Disease	PTS enrolled	Cohorts	LD regimens	Median time to LD	Median time to infusion	ORR/CR	Long-term outcomes	CRS/NT	GvHD	References
4	P-BCMA-ALLO1	NCT04960579	R/R MM	24	arm S (C 300 mg/m^2 + F 30 mg/m^2 X 3 days); arm P1 (C 500 mg/m^2 + F 30 mg/m^2 X 3 days); arm P2 (C 1000 mg/m^2 + F 30 mg/m^2 X 3 days) arm C (multiple P-BCMA-ALLO1 doses following C 300 mg/m^2 + F 30 mg/m^2 X 3 days)	3-day of F (30 mg/m^2/d) and C (300, 500 or 1000 mg/m^2/d)	2 days	7 days	Arm S: ORR 0 (0/8) Arm P1: ORR 80% (4/5), VGPR 40% (2/5), PR 40% (2/5) Arm P2: ORR 83% (5/6), sCR 33% (2/6), VGPR 50% (3/6)	NA	Grade 1 CRS: 21% (7/22) Grade 1 ICANS: 4% (1/22)	No	[4]
8	ThisCART19A	NCT05350787	R/R B-ALL	10	NA	5-day of F (30 mg/m^2/d) and C (300 mg/m^2/d) and etoposide (100 mg/d)	NA	NA	ORR 100% (7/7), MRD-neg CR/CRi 100% (7/7)	NA	Grade 1/2 CRS: 100% (8/8), Grade ≥ 3 CRS: 25% (2/8) ICANS: 37.5% (3/8)	No	[7]

Table 2 (continued)

	Product	Clinical trial	Disease	PTS enrolled	Cohorts	LD regimens	Median time to LD	Median time to infusion	ORR/CR	Long-term outcomes	CRS/NT	GvHD	References
5	19-28z CAR EBV-CTLs	NCT01430390	R/R B-cell malignancies	16	cohort 1: disease recurrence after allo-HCT or auto-HCT cohort 2: consolidative therapy after auto-HCT cohort 3: consolidative therapy after allo-HCT	NA	NA	NA	NA	mFU 53.3 m, OS at 12 months 81%, OS at 24 months 74%	Grade 1 CRS: 6% (1/16)	Skin GvHD of any grade 12% (2/16), Grade 3 skin GvHD 6%(1/16)	[8]
6	ADI-001	NCT04735471	R/R B-NHL	24	NA	sLD:3-day of F (30 mg/m^2) and C (500 mg/m^2) eLD:4-day of F (30 mg/m^2) and 3-day of C (1000 mg/m^2)	NA	NA	ORR 7/9 (78%), CR 7/9 (78%)	mFU 1.2–8.8 m, 57.1% pts still CR	Grade 1/2 CRS: 22.2%, Grade 1 ICANS: 11.1%	No	[10]
7	RJMty19	NCT05453669	R/R B-NHL	15	NA	sLD:3-day of F (25 mg/m^2) and C (500 mg/m^2) rLD:3-day of F (20 mg/m^2) and C (300 mg/m^2)	NA	NA	DL ≥ 3, DCR 100% (5/5), ORR 40% (2/5)	NA	Grade 2 CRS: 9% (1/11)	No	[13]

R/R refractory or relapsed, *LBCL* large-B-cell lymphoma, *FL* follicular lymphoma, *B-ALL* B-cell acute lymphoblastic leukemia, *NHL* non-Hodgkin's lymphoma, *MM* multiple myeloma, *B-NHL* B cell-NHL, *PTS* patients, *NA* not available, *HCT* hematopoietic stem cell transplantation, *LD* lymphodepletion, *F* fludarabine, *C* cyclophosphamide, *A* anti-CD52 antibody, *sLD* standard LD, *eLD* enhanced LD, *rLD* reduced LD, *ORR* overall response rate, *CR* complete response, *MRD* minimal residual disease, *PR* partial response, *VGPR* very good partial response, *sCR* stringent complete response, *CRi* complete response with incomplete marrow recovery, *DL* dose level, *DCR* disease control rate, *mFU* median follow-up, *OS* overall survival, *CRS* cytokine release syndrome, *NT* neurotoxicity, *ICANS* immune effector cell-associated neurotoxicity syndrome, *GVHD* graft-versus-host disease

restriction owing to the absence of CD4 and CD8 expression. The first-in-class humanized anti-CD19 allogeneic DNT RJMty19 was well tolerated in 12 patients with non-Hodgkin's lymphoma, and grade 2 CRS was observed in 1 patient without any GVHD or ICANS. The ORR and disease control rate were 40% and 100%, respectively, at the highest dose [13].

Conclusion and further perspective

Overall, preclinical and clinical research on the use of universal, off-the-shelf CAR-T cells to treat R/R B-cell malignancies has been progressing rapidly, and these cells have demonstrated reliable safety profiles. Most allogeneic CAR-T cell therapies are barely superior to the approved autologous CAR-T cell therapies in terms of efficacy. Gene-editing strategies can transform allogeneic T cells into universal T cell sources through the disruption of critical genes related to allogeneity, such as TRAC, CIITA, and B2M. However, the impact of TCR disruption on the capacity of CAR-T cells to proliferate and kill tumors is still inconclusive. In addition, manufactured CAR-T cells derived from EBVSTs, iPS-rCTLs, γδT cells and DNTs usually equip off-the-shelf CAR-T cells with some inherent properties, including minimal alloreactivity and tumor cytotoxicity. With the increased accessibility and potency of CAR-T cell therapy, establishing platforms for clinical high-yield off-the-shelf CAR-T cell therapy is expected to provide patients with more standardized, dependable, and effective therapeutic approaches.

Author contributions

HM designed the study. YK and CGL drafted the manuscript. YK prepared the tables. All the authors participated in the process of drafting and revising the manuscript. All the authors have read and approved the final manuscript.

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Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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