REVIEW ARTICLE

blood advances

Dual-targeting CAR T cells for B-cell acute lymphoblastic leukemia and B-cell non-Hodgkin lymphoma

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Relapse after CD19-directed chimeric antigen receptor (CAR) T-cell therapy remains a major challenge in B-cell acute lymphoblastic leukemia (ALL) and B-cell non-Hodgkin lymphoma (B-NHL). One of the main strategies to avoid CD19-negative relapse has been the development of dual CAR T cells targeting CD19 and an additional target, such as CD22 or CD20. Different methods have been used to achieve this, including coadministration of 2 products targeting 1 single antigen, cotransduction of autologous T cells, use of a bicistronic vector, or the development of bivalent CARs. Phase 1 and 2 trials across all manufacturing strategies have shown this to be a safe approach with equivalent remission rates and initial product expansion. CAR T-cell persistence remains a significant issue, with the majority of relapses being antigen-positive after CAR T-cell infusion. Further, despite adding a second antigen, antigen-negative relapses have not yet been eliminated. This review summarizes the state of the art with dual-targeting CAR T cells for B-cell ALL and B-NHL, the challenges encountered, and possible next steps to overcome them.

Introduction

In B-cell acute lymphoblastic leukemia (ALL), the first trials using CD19-directed chimeric antigen receptor (CAR) T cells[1-5] showed response rates of ~80% to 90% in a patient population that was previously unsalvageable with conventional therapies (Table 1). This led to licensing of tisagenlecleucel (tisa-cel) (Kymriah) for patients aged ≤25 years with B-cell ALL in 2018 and brexucabtagene autoleucel (Tecartus or KTE-X19) for patients aged >18 years in 2021. Since then, both trial and real-world data have shown that 40% to 50% of patients who respond to CAR T cells are cured without further therapy.[6,12] Although most patients respond initially, ~50% relapse after CAR T-cell therapy and have a poor prognosis. In B-cell non-Hodgkin lymphoma (B-NHL), the first multicenter trials targeting CD19[13-18] showed complete response rates ranging from 40% to 74%, a practice-changing breakthrough in this highly chemorefractory population (Table 2). For large B-cell lymphoma (LBCL), 30% to 40% of patients have sustained responses with CAR T cells as a stand-alone therapy, and median progression-free survival ranges from 3 to 55 months.[23,24] Pediatric real-world data in B-NHL show best sustained responses in B-cell lymphoblastic lymphoma histology.[25] A detailed overview of the licensed products including axicabtagene ciloleucel (axi-cel), brexucabtagene autoleucel, tisa-cel, and lisocabtagene maraleucel has recently been published.[26]

Relapse after CAR T-cell therapy follows 2 main patterns: CD19-positive ($CD19^+$) relapse, usually due to poor CAR T-cell persistence, and CD19-negative ($CD19^-$) relapse due to antigen escape or lineage switch,[27,28] although other mechanisms have been described.[29] The ELIANA study for B-cell ALL

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Table 1. Main trials in single antigen–targeted CAR T cells for B-cell ALL

Reference	Trial phase	CAR construct	n* (age range)	In vivo expansion	Rate of CR or CRi	Toxicity	Persistence	Relapse incidence and phenotype	EFS/OS
B-cell ALL – CD19									
Maude et al, 2018[4] Updated by Laetsch et al, 2021[6] ELIANA study	2	Tisa-cel FMC63 scFv – 4-1BB – Cd3z	n = 79 (3-21 y)	AUC 0-28: 318 000 mean copies per µg C_{max} 34 700 copies per µg in responders[7]	CR: 45/79 (60%) CRi: 16/79 (21%) 65/79 (82%) MRD^- at 3 mo	CRS G3/4: 46% NTx G3/4: 13%	Median time to B-cell recovery in responders 35.3 mo BCA 12 mo: 71% BCA 24 mo: 59%	51% (33/65) $CD19^+$: 2/33 (6%) $CD19^-$: 16/33 (48%) $CD19^{+/-}$: 3/33 (9%) Unknown: 12/33 (36%)	Median EFS 23.7 mo EFS 44% at 3 y OS 63% at 3 y
Gardner et al, 2017[3]	1-2	FMC63-4-1BB-CD3z Defined 1:1 ratio of $CD4^+/CD8^+$ CAR T cells	n = 45 (1-27 y)	Peak 10 d. No correlation of peak expansion with cell dose. Higher expansion with >15% CD19 disease in marrow.	40/45 (89%) MRD^- CR by day 21	CRS G3/4: 10/43 (23%) NTx G3/4: 9/43 (21%)	BCA ≈ 30% at 6 mo	18/40 (45%) $CD19^+$: 11/18 (61%) $CD19^-$: 7/18 (39%)	Median EFS ~13 mo EFS 50.8% at 12 mo OS 70% at 12 mo
Ghorashian et al, 2019[5] CARPALL study	1-2	CAT19 scFv – 4-1BB – CD3z	n = 14 (<25 y)	AUC 0-28: 1 721 355 mean copies per µg C_{max} 128 012 mean copies per µg	86% (12/14) CR MRD^- at 3 mo	No G3/4 CRS NTx G3/4: 1/14 (7%)	B-cell aplasia 21% at 12 mo CAR detectable qPCR 79% (11/14) at last follow-up Median duration 215 d (14-728 d)	50% (6/12) $CD19^+$: 1/6 (16%) $CD19^-$: 5/6 (83%)	Median EFS 9 mo EFS 46% at 12 mo OS 63% at 12 mo
Park et al , 2018[8]	1	FMC63 scFv – CD28 – CD3z	N = 53 (23-74 y)	Higher expansion in patients with preinfusion MRD^- CR	44/53 CR at day 21 32/48 MRD^-	CRS G3/4: 26% (14/53) NTx G3/4/5: 22/53†	Short-persisting CAR T cells. Median duration of CAR T-cell detection: 14 d Most CAR T cells lost before day 40	25/53 $CD19^+$: 21/25 (84%) $CD19^-$: 4/25 (16%)	Median EFS 6.1 mo EFS ~18% at 24 mo Median OS 12.9 mo
Shah et al, 2021[9] ZUMA 3	2	Brexucabtagene autoleucel (KTE-X19) FMC63 scFv – CD28 – CD3z	N = 55 (28-52 y)	Median peak: 40.47 cells per µL (IQR, 6.04-76.70)	39/55 (71%) at median of 1 mo	CRS G3/4: 13/55 (24%) NTx G3/4/5: 14/55 (25%)†	B-cell recovery in 10/12 ongoing responders at month 12	Relapse incidence: 12/55 (22%) $CD19^+$: 6/9 (67%) $CD19^-$: 3/9 (33%) (only 9 patients with available data)	Median EFS 11.6 mo OS 71% at 12 mo 9/55 proceeded to HSCT
B-cell ALL – CD22									
Fry et al, 2018[10] Updated and expanded by Shah et al, 2020[11]	1	Anti-CD22 m971 scFv – 4-1BB – CD3z → Shah et al incorporated CD4/CD8 selection into manufacturing	58 (4-30 y) 36/58 (62 %) previous aCD19 CAR-T 39/58 (67%) previous HSCT	Median peak: 480.5 CAR T cells per µL (range, 39.7-11 346)	40/57 (70%) at 1 mo	CRS G3/4: 12/58 (24%) NTx G3/4/5: 1/58 (2%) → 19/58 (33%) developed HLH (HLH incidence increased after incorporating CD4/CD8 selection at target dose)	NR	30/58 (75%) Downregulation of cD22 expression in most patients.	Median EFS 6 mo Median OS 13.4 mo 14 patients proceeded to HSCT

AUC, area under the curve; BCA, B-cell aplasia; C_{max}, peak serum concentration; CR, complete remission; CRi, complete remission with incomplete recovery; CRS, cytokine release syndrome; HLH, hemophagocytic lymphohistiocytosis; HSCT, hematopoietic stem cell transplantation; IQR, interquartile range; MRD^-, negative minimal residual disease; NR, not reported; NTx, neurotoxicity; OS, overall survival; qPCR, quantitative polymerase chain reaction.

*Showing the final number of patients who received infusions.

†Used American Society for Transplantation and Cellular Therapy consensus criteria for CRS grading and Common Terminology Criteria for Adverse Events grading for neurotoxicity.

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Table 2. Main trials in single antigen–targeted CAR T cells for B-NHL

Reference	Trial phase	CAR construct	n* (age range) and diagnoses	In vivo expansion	Best ORR and CR	Toxicity	Persistence	Relapse incidence and phenotype	EFS/OS
B-NHL CD19									
Neelapu et al, 2017[13] (ZUMA-1)	2	Axicabtagene ciloleucel CD19 scFv – CD28 – CD3z	101 (25-76 y) - DLBCL: 77 - PMBCL: 8 - tFL: 16	Peak at 14 d (peak 10-100 copies per µL)	ORR: 82/101 (82%) CR: 54/101 (54%)	CRS G3/4: 13/101 (13%) NTx G3/4/5: 28/101 (28%)†	Most patients with detectable CAR T cells at 180 d	58/101 (58%) 11 patients available CD19-status: 7/11 $CD19^+$ disease 3/11 had $CD19^-$ disease	Median PFS 5.8 mo 41% PFS at 15 mo OS 52% at 18 mo
Abramson et al, 2020[18] (TRANSCEND)	2	Lisocabtagene maraleucel CD19 scFv – 4-1BB – CD3z (sequential $CD8^+$ then $CD4^+$ components at equal doses)	268 (18-86 y) - DLBCL NOS: 131 - HGBCL: 33 - tFL: 54 - t iNHL: 18 - PMBCL: 14	Peak at 12 d (C_{max} 23 928 copies per µL)	ORR: 186/256 (73%) CR: 136/256 (53%)	CRS G3/4: 6/268 (2%) NTx G3/4/5: 27/268 (10%)†	CAR T cells detectable at 1 y in 35/67 patients (52%) B-cell aplasia at 1 y in 51/70 (73%)	NR	Median PFS 6.8 mo 44% PFS at 12 mo Median OS 21.1 mo
Schuster et al, 2019[14] (JULIET)	2	Tisa-cel CD19 scFv – 4-1BB – CD3z	93 (22-76 y) - DLBCL NOS: 88 - tFL: 21 - Other: 2	Peak at 9 d (C_{max} 5530 copies per µg)	ORR: 48/93 (52%) CR: 37/93 (40%)	CRS G3/4: 24/93 (22%) NTx G3/4/5: 13/93 (14%)†	Not quantified. Long-term persistence up to 2 y observed.	NR	PFS 65% at 12 mo
B-NHL – CD20									
Till et al, 2012[19]	1	CD20 scFv – CD28-4-1BB-CD3z Third-generation CAR	4 Indolent lymphomas	1 patient no expansion	2 patients no evaluable disease 1 partial response	No grade 3/4 toxicities	9-12 mo detectable CAR T cells	1 progression after partial response	NR
Wang et al, 2014[20]	1	CD20 scFv – 4-1BB – CD3z	7 (37-85 y) DLBCL	–	1/7 CR 4/7 PR	CRS G3/4: 1 No NTx Reported delayed-onset CRS and toxicities in tumor involvement sites	NR	NR	NR
Zhang et al, 2016[21]	2	CD20 scFv – 4-1BB – CD3z	11	Peak levels at 4 wk (range, 800-255 044 copies per µg DNA)	Objective response rate: 9/11 (82%) CR: 6/11 (55%) PR: 3/11 (27%)	No CRS or NTx Excluded patients with intrapulmonary involvement, GI involvement, or refractory to debulking therapy	NR	Relapse incidence: 6/11 All with loss of persistence and recovery of $CD20^+$ B-cells	Median PFS 6 mo
B-NHL – CD22									
Baird et al, 2021[22]	1	CD22 scFv (m971) – 4-1BB – CD3z	3	Peak levels at 14 d	CR 3/3 at 6 mo	CRS G3/4: 0/3 NTx G3/4: 0/3	3/3 detectable at last assessment at 6 mo	No relapses at 6 mo	NR

C_{max}, peak serum concentration; CR, complete remission; CRS, cytokine release syndrome; DLBCL NOS, diffuse large B-cell lymphoma not otherwise specified; GI, gastrointestinal; HGBCL, high-grade B-cell lymphoma; NR, not reported; NTx, neurotoxicity; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; PMBCL, primary mediastinal B-cell lymphoma; PR, partial response; tFL, transformed follicular lymphoma; t iNHL, DLBCL transformed from indolent non-Hodgkin lymphoma other than follicular lymphoma.

*Showing the final number of patients who received infusions.

†Used American Society for Transplantation and Cellular Therapy consensus criteria for CRS grading and Common Terminology Criteria for Adverse Events grading for neurotoxicity.

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reports predominant CD19$^-$ relapses (48%), with very few CD19$^+$ relapses (6%).[4,6] In contrast, real-world studies have shown higher rates of CD19$^+$ relapses vs CD19$^-$ relapses (ie, 60% vs 30% in a UK national study[12] or 58% vs 42% in data from the Pediatric Real-World CAR Consortium[30]). Prerelapse and postrelapse sample analysis on the ZUMA-1 study in B-NHL also showed a higher proportion of CD19$^+$ relapses (~64%).[13]

To infer persistence, B-cell aplasia in peripheral blood is most commonly used as a surrogate marker.[3,4,9] Data from studies with tisa-cel suggest that recovery of B cells before 6 months from infusion is associated with a higher risk of relapse and warrants therapeutic intervention.[6,28] Early loss of CAR T-cell persistence may reflect either intrinsic factors making CAR T cells less "fit" (including CAR design, the memory phenotype of the starting material, and production methodology),[31,32] CAR T-cell exhaustion in vivo,[33,34] or immune-mediated rejection.[5,32,35] Currently, it is not known which of these is the dominant cause of early loss of CAR T cells in patients with ALL.

Resistance to CD19-targeted CAR T cells may also result from loss or downregulation of CD19 surface antigen expression due to acquired mutations or splice site alterations.[27,36] Incorporating an additional target represents a logical strategy to overcome this challenge given that a single leukemic stem cell is unlikely to lose or downregulate 2 antigens simultaneously.

In this article, we focus on the different strategies used to deliver dual-targeting CAR T cells to patients and review the published data on construct design, toxicity, expansion, response rates, relapse incidence, and outcomes following dual-targeting CAR T cells for B-cell ALL and B-NHL.

Dual antigen–targeting CAR T cells

Potential targets

CD19 is almost universally expressed at high antigen densities on B-cell ALL blasts.[37,38] However, its expression is more variable in B-NHL. Certain types of lymphoma, such as diffuse LBCL or follicular lymphoma, can show diminished surface levels of CD19 and significant interpatient variability.[39,40]

CD22 is also almost always expressed in B-cell ALL with the exception of a proportion of patients with infant ALL.[41] In B-cell ALL, treatment with CD22 CAR T cells alone has shown robust expansion and high complete remission (CR) rates.[10,11,42,43] However, high rates of relapse due to downregulation of CD22 expression were observed, unless used as a bridge to allogeneic hematopoietic stem cell transplantation.[10,11] This suggests that the ability of CD22 CAR T cells to recognize targets with low antigen density may be critical. In the B-NHL patient population, single-targeting CD22 CAR T cells have also been explored.[22] However, CD22 expression seems to be more variable in the range of 60% to 85% CD22-positive cases depending on histology,[44] and this could potentially impact efficacy.

CD20 is another possible target, expressed on most B-NHL and ~40% to 50% of B-cell ALL. CAR T cells targeting CD20 have been developed for B-NHL.[19-21] Importantly, although CD20-targeted therapy (rituximab) is used throughout B-NHL therapy, malignant cells rarely seem to lose or downregulate CD20.[45]

Several trials are underway using different manufacturing methods with CAR T cells targeting CD19 and CD22, or CD19 and CD20, which are reviewed herein.[46,47] Indeed, some groups are exploring targeting all 3 antigens, and preclinical xenografted leukemia and lymphoma models have shown superior activity with this trispecific approach.[48]

Strategies for delivery of dual-targeting CAR T cells

There are currently 4 main strategies to deliver dual-targeting CAR T cells to patients (Figure 1): coadministration, cotransduction, use of bicistronic vectors, and bivalent tandem CARs. Each has different advantages and disadvantages, summarized in Table 3.

Coadministration. Two separate single antigen–targeting CAR T-cell products are generated and infused into patients. Two different vectors are used (1 encoding a CD19, the other a CD22 or CD20 CAR) and transduced into T cells separately. Then, the 2 products can be pooled together,[50] infused separately on the same day,[51] on sequential days,[52-54] or >1 month apart.[55-57]

Cotransduction. T cells are transduced with 2 different vectors at the same time, generating a mixed population of single- and dual-targeting CAR T cells.

Bicistronic vector. T cells are transduced with 1 single bicistronic vector with antigen-binding domains for both antigens. This results in a homogeneous population of CAR T cells with 2 separate CARs expressed at an equimolar concentration on their surface.

Bivalent tandem CAR. In this case, T cells are transduced with a bivalent vector that generates 1 single CAR on the surface of the cell. It has 2 binding domains, and the variable light and heavy chains of the single-chain variable fragment (scFv) can be set up in a sequential or loop design.[47]

Review of current trials using dual targeting for relapsed/refractory B-cell ALL

The major studies are summarized in Table 4.

CAR constructs and manufacture

Multiple CAR designs and strategies have been applied for B-cell ALL. For example, Wang et al[53] applied third-generation CARs with both 4-1BB and CD28 as costimulatory molecules, and Cordoba et al[49] used humanized scFvs in their bicistronic vector. Ghorashian et al[60] used the previously reported[5] CAT CAR backbone, designed with lower affinity to the CD19 antigen in combination with a novel CD22 CAR based on the 9A8 binder, which recognizes the target's expression of CD22 at low antigen densities.[67] Tandem CARs have generally used the murine anti-CD19 FMC63 scFv and the human anti-CD22 m971 scFv, with variations in the arrangements of light and heavy chains. Because of these differences in CAR design, it is difficult to generalize observed differences in outcomes between the varying dual-targeting strategies above or to attribute these specifically to the approach used.

CAR T cells were manufactured using both closed[49,62,66] (such as the CliniMACS Prodigy system) and open[50,57,60] processing procedures, variable sources of activation beads (CD3/CD28

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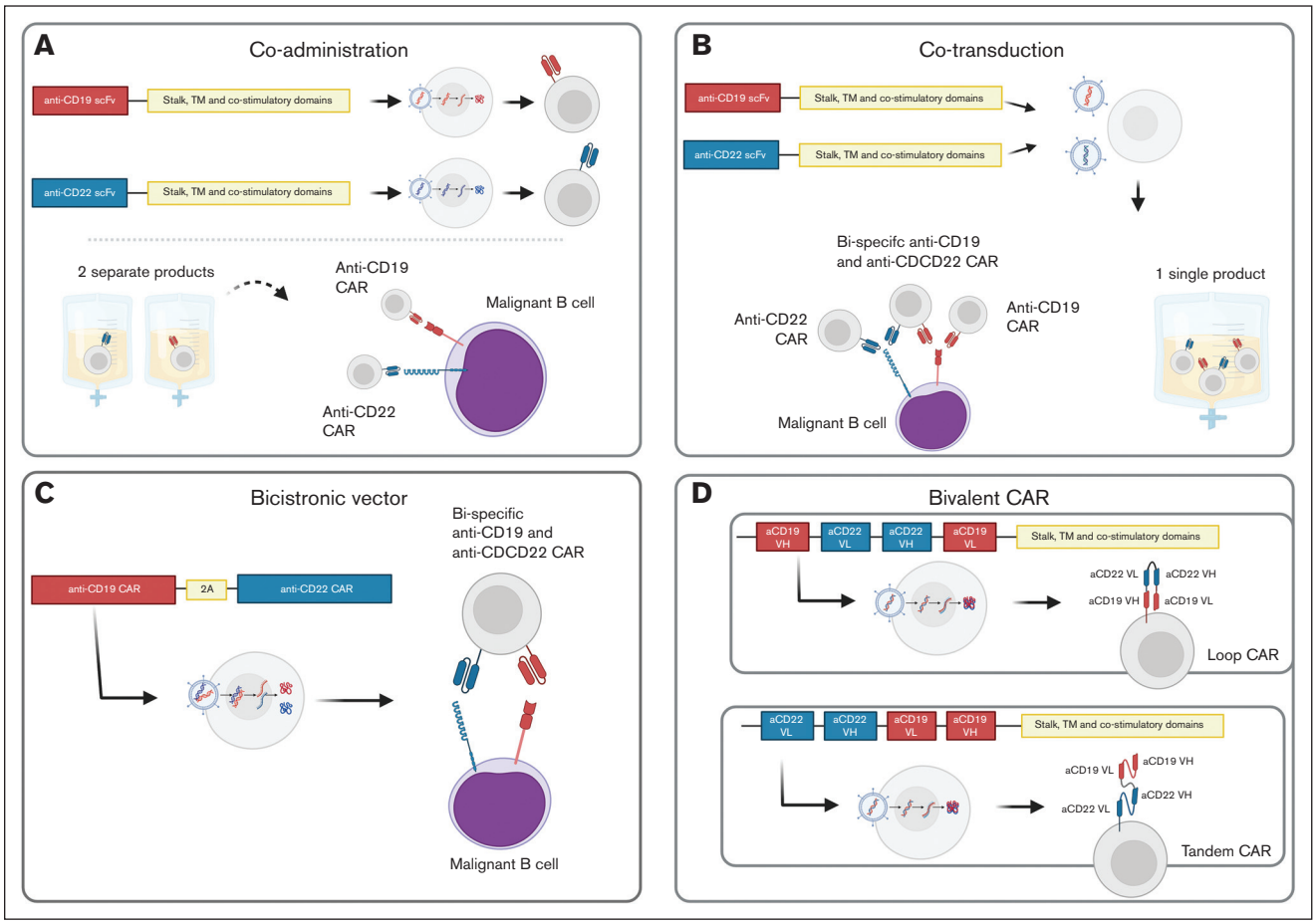

Figure 1. Strategies for delivery of dual-targeting CAR T cells. CD19 and CD22 are shown as an example of antigenic targets. (A) Coadministration: 2 independent products are generated and infused into patients. (B) Cotransduction: T cells are transduced with 2 different vectors, generating 1 single product with a mixed population of single antigen–targeted and bispecific CAR T cells. (C) Bicistronic vector: 1 single vector with binding domains for 2 different antigens is used. The vector is then cleaved and generates CAR T cells with 1 CAR for each antigen on their surface. (D) Bivalent tandem CAR: 1 vector generates 1 single CAR on the surface of the cell. That CAR has binding domains for 2 different antigens. TM, transmembrane; VH, variable heavy chain; VL, variable light chain.

Table 3. Summary of advantages and disadvantages of the different strategies

Variants	Advantages	Disadvantages
Coadministration	- Minimal optimization: allows for combination of 2 single CAR constructs - Dose can be adjusted for each single CAR product	- High manufacturing cost - Coordination and regulation around 2 infusions of 2 different products
Cotransduction	- Minimal optimization: allows for combination of 2 single CAR constructs	- High manufacturing cost - Heterogeneity in product composition may result in uneven expansion in vivo
Bicistronic vector	- Only 1 vector (lower cost) - Homogeneous product - Single activation signal	- Large vector size can result in lower transduction efficiency - Impact of increased CAR density/signaling uncertain
Bivalent CAR	- Only 1 vector (lower cost) - Homogeneous product - Single activation signal	- Optimization of construct to ensure efficient targeting of both antigens challenging

Adapted from Cordoba et al[49] and Xie et al.[47]

dynabeads or TransAct), variable cytokines (eg, Cordoba et al[49] adding interleukin-7 [IL-7] and IL-15 and Ghorashian et al adding no cytokines[60]), and durations of manufacture. These variables may impact the phenotype of the final CAR-T product, which may in turn affect persistence (see "Summary and future directions").

Toxicity

Toxicity observed in trials in B-cell ALL is summarized in Table 5. In general, the published data do not suggest increased toxicity with the addition of a CD22-targeting construct. CAR-related toxicities were mild to moderate (grade 1-2) in most patients. The rate of grade 3 to 4 cytokine release syndrome ranged from 0% to 28.4% and from 0% to 17.6% for neurotoxicity (immune effector cell–associated neurotoxicity syndrome), which is comparable to single targeting. Previously reported[70] immune effector cell–associated hemophagocytic lymphohistiocytosis–like syndrome after single antigen–targeted CD22 has not been widely observed, except in the series of Spiegel et al,[62] in which 2 cases of immune effector cell–associated hemophagocytic lymphohistiocytosis-like syndrome were observed using a tandem construct.

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Table 4. Main trials using dual-targeting CAR T cells for CD19 and CD22 in B-cell ALL

Reference	Trial phase	CAR construct	n*	In vivo expansion	Rate of CR	Relapse phenotype				Persistence	EFS/OS
						$CD19^+$ $CD22^+$	$CD19^+$ $CD22^-$	$CD19^-$ $CD22^+$	$CD19^-$ $CD22^-$		
Wang et al, 2020[53],† Wuhan, China	1	Coadministration Third-generation CAR Sequential, day 0-4	51 (age 9-62 y)	–	48/51 (96%) on day 30	23/24	0	0	1/24 ($CD19^-$/$CD22^{dim}$)	Short persistence (4 mo median time to recovery of bone marrow B-cell hematogones)	53% 12 mo RFS
Pan et al, 2021[55] Beijing, China	1	Coadministration Sequential, separated by 1.65 mo, once CAR19 undetectable	20 (age 1-16 y)	–	20/20 (100%) MRD-negative on day 28	1/3 (downregulation)	0	2/3	0	Good persistence (17/20 patients showed >1 y CAR T-cell persistence)	80% 18 mo RFS
Liu et al, 2021[56] Beijing, China	1	Coadministration Sequential, separated by at least 1 mo	27 infusion 1 21 infusion 2 (age 1.6-55 y)	Similar expansion after CD19 product and CD22	23/27 CR after infusion 1 20/21 CR after infusion 2	4/21	0	2/21	0	B-cell aplasia (median): 10 mo 75% lost CD22 CAR T cells on day 60 50% had CD19 CARs on day 60	65% 18 mo EFS 84% 18 mo OS
Wang et al, 2022[50] Shanghai, China	2	Coadministration Second-generation CAR Pooled 1:1 7-d manufacture	225 (<20 y)	Earlier and more robust expansion for CD19-CAR T cells	192/194 (99%) MRD-negative on day 28	24/43	0	16/43	1/43	B-cell recovery: - median 74 d –60% by 6 mo	74% 12 mo EFS 88% 12 mo OS
Zhang et al, 2022[54] Tianjin, China	1	Coadministration Sequential, days 1 and 2. HIB22 CD22 CAR	4 (age 18-40 y)	Peak 14-21 d	4/4 (100%) MRD-negative on day 28	2/4	0	0	1/4 ($CD19^-$/CD22dim)	9 mo CAR T-cell presence in peripheral blood of 2 patients alive and without HSCT. Both relapsed with CD19 and CD22 expression.	25% 18 mo EFS 50% 18 mo OS
Pan et al, 2023[57] Beijing, China	2	Coadministration Sequential, separated by 39 d CD19 murine CD22 humanized	81 (79 received both infusions) (age 1-18 y)	CD19: peak at 9 d CD22: peak at 12 d Peak not related to dose or bone marrow burden	79/81 (98%) MRD-negative or CRi at 3 mo	11/79	0	2/79	1/79	20% B-cell recovery at 12 mo 40% CAR T-cell loss at 12 mo (as undetectable CAR transgene)	79% 18 mo EFS 96% 18 mo OS
Gardner et al, 2018[58] (PLAT-05, SCRI-CAR19x22v1) Seattle, Washington	1	Cotransduction aCD19(FMC63)-4-1BBz aCD22(m971)4-1BBz	7	Selective expansion of CD19 components - CD19 9.1% - CD22 1.2% - CD19/CD22 2.4%	4/7 (57%) MRD negative on day 21	1/4	0	2/4	1/4	–	No follow-up time reported
Annesley et al, 2021[59] (PLAT-05, SCRI-CAR19x22v2) Seattle, Washington	1	Cotransduction	12	Product skewed toward CD22. In vivo expansion mostly CD22	11/12 (91%) MRD negative	–	–	–	–	–	No follow-up available yet

CR, complete remission; CRi, complete remission with incomplete recovery; EMD, extramedullary disease; HSCT, hematopoietic stem cell transplantation; MRD, minimal residual disease; OS, overall survival; qPCR, quantitative polymerase chain reaction; RFS, relapse-free survival; VH, variable heavy chain; VL, variable light chain.

*Showing the final number of patients who received infusions.

†Showing results for the B-cell ALL cohort only.

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Table 4 (continued)

Reference	Trial phase	CAR construct	n*	In vivo expansion	Rate of CR	Relapse phenotype				Persistence	EFS/OS
						$CD19^+$ $CD22^+$	$CD19^+$ $CD22^-$	$CD19^-$ $CD22^+$	$CD19^-$ $CD22^-$		
Ghorashian et al, 2024[60] (CARPALL study) London, UK	1	Cotransduction aCD22-9A8-4-1BBz aCD19-CAT-4-1BBz	12 (<24 y)	Balanced expansion of all 3 components	10/12 (83%) MRD-negative at 2 mo (molecular MRD)	5/10	0	0	0	qPCR in blood (median): - CD19 CAR-T: 135 d - CD22 CAR-T: 105 d Less persistence than equal CD19 CAR product	60% 12 mo EFS 75% 12 mo OS
Cordoba et al, 2021[49] London, UK (AMELIA study)	1	Bicistronic vector Humanized CAR (AUTO3)	15 (age 4-16 y)	Kinetics of expansion like tisa-cel	13/15 (86%) MRD-negative at 2 mo	6/13	0	2/13	1/13	119 d median time to last detection in blood (lower than tisa-cel)	32% 12 mo EFS
Dai et al, 2020[61] Beijing, China	1	Tandem CAR	6 (age 17-44 y)	Peak at 2 wk	6/6 (100%) MRD-negative at 1 mo	2/6			1/6 ($CD19^-/CD22^{dim}$)	5/6 patients <6 mo persistence	
Spiegel et al, 2021[62,†] Stanford, California	1	Tandem CAR	17 (age 25-78 y)	Peak at 10-14 d Higher expansion of CD8 compared with CD4	15/17 (88%) MRD-negative at 6 mo (10^{-4} sensitivity)	4/15 (1 no CD22 status reported)	0	4/15	0	All CAR-T present at day 60. No measurements undertaken thereafter.	33% 6 mo EFS
Hu et al, 2021[63] Hangzhou, China	1	Tandem CAR Universal CRISPR/ Cas9-engineered	6 (age 26-56 y)	Peak at 10-14 d	5/6 (83%) MRD-negative on day 28	0	1/6 ($CD19^+/CD22^{dim}$)	0	0	Patients with ongoing remission (2 patients) persistent CAR T cells >90 d Relapsed patient lost CAR T cells <60 d	-
Cui et al, 2023[64] Suzhou, China	½	Tandem CAR CD22 VL – CD19 VH, VL – CD22 VH – 4-1BB	47 (age 6-56 y)	–	40/47 (85%) MRD-negative on day 28	10/47	0	2/47	0	35 patients (75%) underwent consolidative HSCT at median of 2 mo from CAR T-cell infusion	69% 24 mo RFS 74% 24 mo OS
Niu et al, 2023[65] Shanghai, China	1	Tandem CAR CD19 VL – CD22 VH – VL – CD19 VH – 4-1BB	15 (age 23-70 y) First-line MRD-positive patients and relapsed MRD-positive patients	Peak at 10 d. Higher in patients with sustained remission than in those who relapsed.	14/15 (94%) MRD-negative on day 28	4/15	0	1/15	0	3 patients with CAR T-cell persistence >90 d	77% 12 mo RFS 86% 12 mo OS
Shalabi et al, 2022[66] Bethesda, Maryland	1	Tandem CAR	20 (age 5-34 y)	Lower expansion than CD22 CAR alone	16/20 (80%) MRD-negative at 1 mo (but 4 patients residual or progressive EMD)	3/12 ($CD19^+$, no CD22 status reported)	0	0	1/12 ($CD19^-$, no CD22 status reported)	Less persistence compared with patients receiving CD22 CAR alone (median 28 vs 88 d)	58% 12 mo RFS in responders

CR, complete remission; CRi, complete remission with incomplete recovery; EMD, extramedullary disease; HSCT, hematopoietic stem cell transplantation; MRD, minimal residual disease; OS, overall survival; qPCR, quantitative polymerase chain reaction; RFS, relapse-free survival; VH, variable heavy chain; VL, variable light chain.

*Showing the final number of patients who received infusions.

†Showing results for the B-cell ALL cohort only.

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Table 5. Toxicity profile of main dual-targeting CAR products for B-cell ALL

Reference	Grading system used	n	CRS					Neurotoxicity (ICANS)				
			Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Wang et al[53]	CTCAE[68]	89 (B-cell ALL + B-NHL)	66 (74%)	–	15 (17%)	3 (3%)	1 (1%)	11 (12%)	0	0	1 (1%)	0
Pan et al[55]		20 Cycle 1 20 Cycle 2	17 (85%) 15 (75%)		1 (5%) 0		0	3 (15%) 3 (15%)	0	1 (5%) 0	0	0
Liu et al[56]		27 first 21 second	3 (11%) 8 (38%)	13 (48%) 3 (11%)	5 (19%) 0	1 (4%) 0	1 (4%) 0	1 (4%) –	1 (4%) –	1 (4%) –	–	–
Wang et al[50]		225	133 (59%)		64 (28%)		1 (0.4%)	36 (16%)		9 (4%)	2 (0.8%)	
Zhang et al[54]	ASTCT[69]	4	2 (50%)	0	1 (25%)	0	–	1 (25%)	0	0	0	–
Pan et al[57]		81 cycle 1 79 cycle 2	60 (74%) 54 (68%)		12 (15%) 2 (3%)	1 (1%) 2 (3%)	–	19 (23%) 13 (16%)	3 (4%) 1 (1%)	1 (1%) 0		–
Gardner et al[58]		7	5 (71%)	–	–	–	–	2 (29%)	0	0	0	–
Annesley et al[59]		12	5 (42%)	–	–	–	–	4 (33%)	0	1 (8%)	0	–
Ghorashian et al[60]		12	5 (42%)	6 (50%)	–	–	–	4 (33%)	1 (8%)	0	1 (8%)	–
Cordoba et al[49]		15	11 (73%)	1 (7%)	–	–	–	4 (27%)	0	0	0	–
Dai et al[61]		6	4 (67%)	2 (33%)	0	0	–	0	0	0	0	–
Spiegel et al[62]		17	5 (29%)	7 (41%)	–	1 (6%)	–	1 (6%)	1 (6%)	2 (12%)	1 (6%)	–
Hu et al[63]		6	3 (50%)	2 (33%)	1 (17%)	0	–	0	0	0	0	–
Cui et al[64]		47	33 (70%)		8 (17%)		–	1 (2%)	0	0	0	–
Niu et al[65]		15	2 (13%)	2 (13%)	0	0	–	1 (7%)	0	0	0	–
Shalabi et al[66]		20	7 (35%)	–	3 (15%)	–	–	0	0	1 (5%)	0	–

ASTCT, American Society for Transplantation and Cellular Therapy; CRS, cytokine release syndrome; CTCAE, Common Terminology Criteria for Adverse Events; ICANS, immune effector cell–associated neurotoxicity syndrome.

Expansion of CAR T cells

Regardless of strategy, most clinically tested dual-targeting CAR T-cell products have shown initial expansion kinetics and peak levels broadly similar to those observed with tisa-cel.[7,71] A 2022 study from Shanghai[50] pooled 2 different CAR T-cell populations together at a 1:1 ratio and saw an earlier and higher peak expansion of CD19 CAR T cells compared with CD22 CAR T cells.

With the cotransduction method, expansion of different CAR T-cell populations can vary widely. During manufacture, T cells are exposed to 2 lentiviral vectors and therefore have different transduction efficiencies. Products can therefore be balanced or skewed toward a certain CAR component. Ghorashian et al[60] reported a product composition with predominantly CD19/22-CAR expressing cells (median, 54.4%) with lower but balanced CD19-CAR (13%) and CD22-CAR (11.6%) components. After infusion, early in vivo expansion reflected the phenotype of the product with predominant engraftment of CD19/22 double-transduced T cells and balanced but lower engraftment of CD19 and CD22 single-positive populations. In contrast, early reports from the PLAT-05 study showed a skewed in vivo expansion of the CD19-CAR component using the CAR19x22v1 product.[58] In view of this, the manufacturing methodology was altered to favor the CD22 CAR T cells in the product. However, when this was infused, in vivo expansion was then skewed toward the CD22-CAR component.[59]

The use of bicistronic vectors does not seem to impact early expansion, with Cordoba et al[49] reporting expansion similar to that of tisa-cel.[7,71] However, data from studies by the National Cancer Institute and Stanford[62,66] indicated limited expansion and shorter persistence of their tandem CD19/22 CARs compared with their single antigen–targeted CD22 CAR.

Response

All studies showed minimal residual disease–negative CR or CR with incomplete recovery rates >80%, mirroring the clinical experience with CD19-directed CAR T-cell therapy so far. The only product with lower rates of reported response (57%) was the first product tested in the PLAT-05 study using a cotransduction approach.[58] Coadministration strategies showed particularly favorable responses, with CR rates >90%. Given that bridging chemotherapy is generally used before lymphodepletion, we cannot attribute responses to CAR T cells alone. However, given the refractory nature of the durability of responses in many such patients, it is unlikely that bridging therapy contributes significantly to response rates.

Relapse incidence and phenotype

Regardless of the strategy used, antigen-positive relapse has been the predominant cause of treatment failure observed following dual-targeting CAR T-cell therapy, reflecting poor persistence across a substantial number of dual-targeting CAR products.[49,50,57,60,62,66]

Antigen-negative relapse has still been observed in most studies of dual-targeting CAR T cells in B-cell ALL (see Table 4, column "Relapse phenotype"). $CD19^-$ relapse with ongoing CD22 positivity is the main phenotype, perhaps reflecting the poor performance of the CD22 CAR across the different strategies, shorter

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persistence in coadministration,[50] and stronger selective pressure on the CD19 compared with the CD22 target in bicistronic and tandem CARs.[49,62] Consequently, CD22 negativity is rarely observed. It is important to highlight that because prolonged selective pressure is needed for outgrowth of antigen-negative clones, poor persistence may limit our ability to assess the real prevalence of antigen-negative relapse.

Outcomes

Clinical outcomes with dual-targeting CAR T cells in B-cell ALL have generally been equivalent to those reported with the single-targeting CD19 CAR.[4-6,12]

The most encouraging results have been achieved with coadministration of CD19 and CD22 CAR T cells. One of the 2 largest studies[50] of this approach reports a 12-month event-free survival (EFS) of 74%. They used a short manufacture time (7 days) and infused a fresh, 1:1 pooled product of CD19 and CD22 CAR T cells to 225 patients. Although these results appear superior to data on tisa-cel reported in the ELIANA trial[6] and real-world data,[12,72] it should be noted that the patient characteristics in this study were more favorable, with 32% of patients being minimal residual disease–negative before infusion. Pan et al[57] have also shown impressive outcomes, with an 18-month EFS of 79%. In this study, CD19 CAR T cells were infused first, followed 30 days later by a CD22 CAR T-cell infusion for patients in complete remission and without ongoing toxicities. Interestingly, CD22 CAR T cells expanded and persisted despite eradication of disease with the previous CD19 CAR T cells. Further, disease surveillance presumably relied on the CD22 CAR T cells given that many patients lost their CD19 CAR T cells after receiving a second cycle of lymphodepleting chemotherapy.

Using a cotransduction approach, the CARPALL cohort 3 study by Ghorashian et al[60] reported a 12-month EFS of 60%. Although data need to be interpreted with caution because of the small sample size, antigen-negative relapse was not observed. This may in part reflect the use of CD22 CAR based on the 9A8 binder, which effectively targets tumor cells at low CD22 antigen density. Initial and sustained response was observed in 2 out of 3 patients who had CD19-negative disease on enrollment, demonstrating effective CD22 CAR activity. Additionally, single antigen–targeted CD22 CAR T cells were detectable in blood for longer (median of 7 vs 5 months) than their single CD19 and double CD19/CD22–targeting CAR T-cell counterparts.

Cordoba et al[49] reported a lower EFS using CAR T cells transduced with a bicistronic CD19-22 CAR vector (AUTO3), with a median EFS of 5 months and 12-month EFS of 32%. They observed a high rate of antigen-positive relapses associated with CAR T-cell loss, and short persistence was considered to be the main factor for these poor results. The authors postulate that this replicated a differentiated phenotype of the CAR T-cell product, which in turn may reflect the production methodology used. However, it is also possible that signaling through 2 CARs in a single cell may predispose to activation-induced cell death and/or exhaustion.[33]

Using a tandem CD19-22 CAR, Spiegel et al[62] reported a median EFS of 5.8 months and Shalabi et al[66] a 12-month EFS of 58% in responding patients. These somewhat disappointing outcomes mirror the issues that both groups encountered with the functionality of CD22 targeting in the context of a tandem CAR structure. In the adult cohort of Spiegel et al,[62] they showed that CD19/22 tandem CAR T cells had reduced cytokine polyfunctionality following stimulation with CD22-positive targets compared with T cells transduced with a CD22 CAR alone. Shalabi et al[66] showed suboptimal CD22-targeting activity of the tandem CAR construct, both in vitro, with reduced cytokine secretion against CD19-CD22$^+$ Nalm6 cell lines, and in vivo, with poor antileukemic activity in a xenogeneic CD19-negative, CD22-positive model of B-cell ALL. These data indicate decreased functionality of the CD22 CAR moiety when incorporated into a tandem structure.

Cui et al[64] reported better results in a cohort of 47 patients (24-month EFS of 69%) using a tandem CAR construct, but these results need to be interpreted with caution given that 75% of patients underwent consolidative allogeneic hematopoietic stem cell transplantation at 2 months.

Review of current trials using dual targeting for relapsed/refractory LBCL

The major studies are summarized in Table 6 (CD19/CD20 CARs) and Table 7 (CD19/CD22 CARs).

CAR constructs and manufacture

Constructs used for B-NHL are more homogeneous than those used in B-cell ALL. Tandem CARs targeting CD19 and CD20 used sequences derived from the murine scFv regions Leu-16 for CD20 and FMC63 for CD19.[51,73,74,76] As for CD19 and CD22, the studies on coadministration from Wuhan[52,53,78] all applied a third-generation CAR with 4-1BB and CD28 as costimulatory molecules. Roddie et al[79] used 2 humanized scFv regions in a bicistronic vector: LT22 for CD22 and HD37 for CD19, the same product (AUTO3) Cordoba et al[49] used for B-cell ALL. Tandem CARs targeting CD19 and CD22 use the same scFv as described for B-cell ALL, FMC63 for CD19 and m971 for CD22, in a second-generation backbone.[62,80,81]

In terms of manufacturing, as with B-cell ALL, processing procedures varied across studies. Larson et al[73] specifically enriched the apheresis product for CD62L to obtain a higher yield of naïve and memory T cells. They extended the expansion period to 12 to 16 days before cryopreserving the final product. Manufacturing times varied from 8 to 14 days. Although shortened manufacturing methodologies, such as the T-Charge platform, have been used with CD19-directed CAR T cells,[83] they have not yet been applied to dual-targeting CAR T cells.

Toxicity

The toxicity profile across the reviewed trials for B-NHL is summarized in Table 8. There does not seem to be any increased toxicity when adding CD20 or CD22 antigen recognition. Grade 3 to 4 cytokine release syndrome occurred in 0% to 28.5% of cases, and grade 3 to 4 immune effector cell–associated neurotoxicity syndrome was reported in 0% to 13.6% of cases across all studies. Larson et al[73] reported a relatively low incidence of adverse events in their trial. They noted low peak cytokine levels while maintaining clinical efficacy of their CAR T-cell product. This could be explained by the skewed naïve/memory T-cell phenotype

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Table 6. Main trials using CD19/CD20 dual-targeting CAR T cells for B-cell lymphomas

Reference	Trial phase	CAR construct	n*	Diseases	Patient characteristics	Response	In vivo expansion	Persistence	Progression/ relapses (and relapse phenotype if available)	EFS/OS
Sang et al, 2020[51] Xuzhou, China	2	Coadministration, same day - aCD19 scFv – 4-1BB - aCD20 scFv – 4-1BB	21 (age 23-72 y)	DLBCL: 21	Refractoryv: 15 Previous autologous HSC: 1 Previous CAR-T: none Bridging: none	ORR: 17/21 (81%) CR: 11/21 (52%) At day 90	Higher expansion in patients with response. No difference between CD19 and CD20 peak.	Not reported for the full cohort. Persistence around 6 mo	9/21 (43%) patients No CAR T cells detected in relapsed patients. 5/9 patients had B-cell recovery.	25% 12 mo PFS 30% 12 mo OS
Larson et al, 2023[73] UCLA, Los Angeles, California	1	Tandem CAR CD20 VL CD20 VH CD19 VH CD19 VL – 4-1BB	10 (age 29-70 y)	MCL: 1 FL: 3 DLBCL: 1 tFL: 3 PM LBCL: 1 DH HGBCL: 1	Refractory: 4 Previous autologous HSC: 1 Previous CAR-T: none Bridging: 9/10 (90%)	ORR: 9/10 (90%) CR: 7/10 (70%) At day 60	Peak at 14 d	All responders remained in B-cell aplasia at time of data cutoff. 6 patients >12 mo B-cell aplasia	PD: 2/10 Relapse: 1/10	40% 18 mo PFS 70% 18 mo OS
Shah et al, 2020[74] Updated by Zurko et al[75] in 2022 Milwaukee, Wisconsin	1	Tandem CAR CD20 – CD19 – 4-1BB 15 patients received fresh noncryopreserved products	22 (age 38-72 y)	DLBCL: 11 MCL: 7 CLL: 3 FL: 1	Previous autologous HSC: 8 Previous allogeneic HSCT: 3 Previous anti-CD19 CAR-T: 1 Bridging: 7/22 (32%)	ORR: 18/22 (82%) CR: 14/22 (64%) At day 28	Higher expansion in patients with response. Peak at 7-12 d.	For patients with early CR, B-cell recovery was 42% at 6 mo and 56% at 9 mo.	PD: 8/22 Relapse: 5/22 All had biopsies and there was no CD19 or CD20 antigen loss.	Updated data for 16 patients who received target dose: 44% 24 mo PFS 69% 24 mo OS
Tong et al, 2020[76], extended by Zhang et al,[77] Beijing, China	1-2	Tandem CAR (TanCAR7) CD20 VH CD20 VL CD19 VL CD19 VH – 4-1BB Fresh noncryopreserved product in all infusions.	87 (age 16-70 y)	DLBCL: 58 FL: 13 tFL: 6 PMBCL: 5 CLL: 2 Small lymphocytic lymphoma: 2 MCL: 2 MALT: 1	Previous autologous HSC: 12 Previous anti-CD19 CAR: 9 Bridging: none	ORR: 68/87 (78%) CR: 61/87 (70%) At month 3	Peak 7-14 d. Higher levels in patients who achieved response.	Median around 100 d. Up to 400 d in 30 patients with ongoing CR. No difference in CAR T-cell levels between patients with ongoing response and relapse at days 21-40 and 41-60	Relapse: 16/87 PD: 18/87 Biopsy available in 12 relapsed patients: –1 patient had CD19 and CD20 loss. –7 patients did not have detectable CAR T cells in tumor tissue or peripheral blood	Median PFS 27.6 mo 61% 12 mo PFS 79% 12 mo OS

CLL, chronic lymphocytic leukemia; CR, complete remission; DH HGBCL, double-hit high-grade B-cell lymphoma; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; HSC, hematopoietic stem cell; HSCT, hematopoietic stem cell transplantation; MALT, mucosa-associated lymphoid tissue lymphoma; MCL, mantle cell lymphoma; ORR, objective response rate; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PMBCL, primary mediastinal B-cell lymphoma; PM LBCL, primary mediastinal large B-cell lymphoma; tFL, transformed follicular lymphoma; UCLA, University of California, Los Angeles; VH, variable heavy chain; VL, variable light chain.

*Showing number of final infused patients.

Table 7. Main trials using CD19/CD22 dual-targeting CAR T cells for B-cell lymphomas

Reference	Trial phase	CAR construct	n*	Diseases	Patient characteristics	Response	In vivo expansion	Persistence	Progression/relapses (and relapse phenotype if available)	EFS/OS
Wang et al, 2020[53]† Wuhan, China	1	Coadministration (third-generation sequential, day 0-4)	38 (age 9-71 y)	DLBCL NOS: 23; DH HGBL: 4; HGBL NOS: 3; FL: 3; Burkitt lymphoma: 2; PMBCL: 1; Others: 2	Refractory: 15 First relapse: 11 Second relapse: 4 ≥third relapse: 8 Bridging: allowed, but no data	ORR: 26/36 (72%) CR: 18/36 (50%) at month 3	NR	NR	18/38 (7 were biopsied, showed $CD19^+$/$CD22^+$ disease)	50% 12 mo PFS 55.3% 12 mo OS
Cao et al, 2021[52] Wuhan, China	1	High-dose chemotherapy with aHSCi, followed by aCD22 then aCD19 coadministration (days 2 and 3)	42 (age 24-61 y)	DLBCL NOS: 30 tFL: 7 DH HGBL: 2 Others: 3	PR: 10/42 PD: 23/42 SD: 9/42 Bridging: high-dose chemotherapy with aHSCi	ORR: 38/42 (91%) CR: 34/42 (81%) at month 3	Peak at 1 wk	Median time to B-cell recovery 8.2 mo	7/42 (5 were biopsied, showed $CD19^+$/$CD22^+$ disease)	83% 24 mo PFS 83% 24 mo OS
Wu et al, 2021[78] Wuhan, China	1	High-dose chemotherapy with aHSCi followed by sequential CD19 and CD22 CART infusion for CNS	13 (age 23-65 y)	DLBCL with CNS involvement: 8 Primary CNS DLBCL: 4 ILBCL: 1	Refractory: 1 PR: 2 PD: 3 CNS relapse: 7 Bridging: permitted, no data available	ORR: 9/11 (82%) CR: 6/11 (55%) at month 3	Peak at 1 wk	Median persistence <3 mo	3/11	75% 12 mo PFS 83% 12 mo OS
Roddie et al, 2023[79] London, UK (ALEXANDER study)	1	Auto 3 **Bicistronic vector** Humanized CAR + pembrolizumab	52 (age 27-83 y)	DLBCL: 36; tFL: 10; PM LBCL: 1; t nodal MZL: 1; HGBL: 3	Previous autologous HSC: 16 Bridging: 37/51 (73%)	ORR: 31/47 (66%) CR: 23/47 (49%) at month 1	Median peak at 12 d	Median of 4.2 mo persistence	33/52 13 had biopsy: -Majority $CD19^+$ -7/13 $CD22^{lo/-}$ -2 cases of clear $CD19^-$ (H-score heat mapping)	26% 12 mo EFS 54% 12 mo OS
Spiegel et al, 2021[62]† Stanford, California	1	Tandem CAR (CD19VH – CD22 VL – CD22 VH – CD19 VL – 4-1BB)	21 (age 25-78 y)	DLBCL: 14 tFL: 4 PMBCL: 2 Richter: 2	Previous autologous HSC: 4 Previous CAR: none Bridging: permitted, no data available	ORR: 13/21 (62%) CR: 6/21 (29%) at month 3	Peak at 10-14 d CD8 > CD4 expansion	NR	Relapse: 1/21 PD: 15/21 14 biopsied at progression: 4 patients $CD19^{-/lo}$	25% 12 mo PFS 65% 12 mo OS
Wei et al, 2021[80] Hangzhou, China	1	Tandem (VL-VH-VL-VH)	16 (age 23-68 y)	DLBCL: 13 B-LLy: 2 Burkitt lymphoma: 1	Previous autologous HSCT: 1 Bridging: none	ORR: 14/16 (87.5%) CR: 10/16 (62.5%) at month 1	Peak at 5-10 d	8/16 ongoing B-cell aplasia at 10 mo 13/16 ongoing B-cell aplasia at 6 mo	Relapse: 3/16 PD: 7/16 (2 were biopsied, showed $CD19^+$/$CD22^+$ disease)	40.2% 12 mo PFS 77.3% 12 mo OS
Zhang et al, 2021[81] Suzhou, China	1	Tandem (CD22VL – CD19 VL – CD19 VH – CD22 VH – 4-1BB)	32 (no age range given) <60 y: 24 ≥60 y: 8	DLBCL: 27 tFL: 2 PMBCL: 1 HGBL: 2	Primary refractory: 5 Previous autologous HSC: 4 Bridging: no data available	ORR: 22/29 (76%) CR: 10/29 (34%)	Peak 10-14 d Responders had higher expansion	Median 92 d persistence in peripheral blood (min, 13; max, 763)	10/29 PD No biopsy performed at time of progression.	40% 12 mo PFS 63% 12 mo OS
Zhang et al, 2023[82] Suzhou, China	2	Tandem + tislelizumab	16 (age 19-70 y)	DLBCL: 13 Richter: 2 Burkitt lymphoma: 1	Previous autologous HSC: 4	ORR: 14/16 (88%) CR: 11/16 (69%)	Peak at median of 12 d	CAR T cells present in 50% of patients at 6-mo follow-up	Relapse: 2/16 PD: 3/16	69% 12 mo PFS 81% 12 mo OS

aHSCi, autologous hematopoietic stem cell infusion; B-LLy, B-cell lymphoblastic lymphoma; CAR, chimeric antigen receptor; CNS, central nervous system; CR, complete remission; DH HGBL, double-hit high-grade lymphoma; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; HGBL, high-grade B-cell lymphoma; HSC, hematopoietic stem cell; HSCT, hematopoietic stem cell transplantation; ILBCL, intravascular large B-cell lymphoma; MZL, marginal-zone lymphoma; NOS, not otherwise specified; NR, not reported; ORR, objective response rate; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PMBCL, primary mediastinal B-cell lymphoma; PM LBCL, primary mediastinal large B-cell lymphoma; PR, partial remission; SD, stable disease; tFL, transformed follicular lymphoma; VH, variable heavy chain; VL, variable light chain.

*Showing the final number of patients who received infusions.

†Showing results for the B-cell lymphoma cohort only.

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Table 8. Toxicity profile of main dual-targeting CAR products for B-cell lymphoma

Reference	Grading system used	Total n	CRS					Neurotoxicity (ICANS)				
			Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Sang et al[51]	ASTCT	21	15 (71%)		6 (29%)		–	3 (14%)		2 (10%)		–
Larson et al[73]	ASTCT	10	6 (60%)	0	0	0	–	0	0	0	0	–
Shah et al[74]	ASTCT CTCAE*	22	13 (59%)		1 (4%)		–	4 (18%)		3 (14%)		0
Tong et al[76] Zhang et al[77]	ASTCT	87	39 (45%)	13 (15%)	8 (9%)	1 (1%)	–	11 (13%)	2 (2%)	2 (2%)	0	–
Cao et al[52]	ASTCT CTCAE*	42	26 (62%)	12 (29%)	2 (5%)	0	–	5 (12%)	2 (5%)	2 (5%)	0	0
Wu et al[78]	ASTCT	13	9 (69%)	2 (15%)	0	0	–	2 (15%)	0	1 (8%)	0	–
Roddie et al[79]	ASTCT	52	11 (21%)	7 (13%)	1 (2%)	0	–	2 (4%)		2 (4%)		0
Spiegel et al[62]	ASTCT CTCAE*	21	12 (57%)	3 (14%)	1 (5%)	0	–	5 (24%)	3 (14%)	1 (5%)	0	0
Wei et al[80]	ASTCT	16	4 (25%)	11 (69%)	0	1 (6%)	–	0	0	0	0	–
Zhang et al[81]	CTCAE	32	14 (44%)	6 (19%)	5 (16%)	3 (9%)	1 (3%)	1 (3%)	0	4 (13%)	0	0
Zhang et al[82]	CTCAE	16	7 (44%)	0	1 (6%)	0	0	0	0	0	0	0

ASTCT, American Society for Transplantation and Cellular Therapy; CRS, cytokine release syndrome; CTCAE, Common Terminology Criteria for Adverse Events; ICANS, immune effector cell–associated neurotoxicity syndrome.

*Neurotoxicity graded by CTCAE in these studies.

achieved during production, or the thorough preclinical construct optimization,[84] leading to increased clinical efficacy and consequently allowing for a lower CAR T-cell dose (median of 55 × 10^6 cells).

Expansion of CAR T cells

Despite using more complex constructs, CAR T cells expand well and peak at ~2 weeks, with a tendency toward greater expansion in patients who show a response.[74,76,81] Persistence, however, has been reported to be very short in the B-NHL cohort. CAR T cells are lost earlier compared with the B-cell ALL population, with most trials reporting 3- to 6-month persistence.[75,77,79,81] As observed with single antigen–targeting CAR T cells, it is not clear if a shorter persistence correlates with relapse in the B-NHL cohort. An early, greater expansion might be more significant for durable remission in lymphoma[75] compared with B-cell ALL.

Response

Overall response rates range from 60% to 90% across different trials, whereas CRs range from 29% to 81%. These numbers do not differ significantly from the responses observed with single antigen–targeting CAR-T trials.[26] Deep initial responses with dual-targeting CAR T cells seem to correlate with durable remissions,[53] as has also been observed with single antigen targeted CAR T-cell therapy.[26] Shah et al[74] report a trend toward a greater proportion of naïve and central memory phenotypes in the apheresis products of patients who showed good clinical response. Although bridging therapy is frequently used in B-cell ALL, its use in B-NHL has varied historically in pivotal trials, and also varies across dual-targeting studies, with some studies not giving any,[51,76,80] others permitting its use at each center's discretion,[50,62,78] and some reporting its use as part of the study protocol.[73,74,79] Roddie et al[79] commented on the role of effective bridging in debulking disease before CAR T-cell infusion and how low disease burden was a predictor of response to their product, AUTO3. Conversely, Zurko et al[75] found inferior survival in patients who required bridging therapy, which may reflect higher disease burden on recruitment.

Relapse incidence and phenotype

In lymphoma, a biopsy is needed to assess antigen expression on tissues, often with patchy lymphoma involvement, which makes representation of CD19 and CD20/CD22 expression at baseline and relapse more challenging. Modalities to assess pre- and postrelapse antigen expression include the H-score[62,85] and flow-based assessment of fine-needle aspiration material.[62] From the available data,[62,74,79,85] relapses seem to follow the same phenotype as with B-cell ALL. Most relapses retain expression of CD19 and CD22/CD20, as has been the case with CD19-targeted products. For example, in the ZUMA-1 trial, one-third of LBCL relapse cases after axi-cel administration were due to antigen loss, and two-thirds of cases relapsed with ongoing CD19 expression.[13] In most patients with lymphoma, CAR T cells do not persist long-term, and this may account for antigen-positive relapse in some cases. However, other factors may also be contributory. Certainly, T-cell fitness and the functionality/expansion potential of CAR T cells in vivo play important roles in the achievement of clinical response. Thus, antigen-positive relapse is more likely when the CAR T-cell product is intrinsically unfit because of prior chemotherapy. Moreover, endogenous immune and tumor microenvironment-associated factors may impede T-cell function in vivo and contribute to the risk of antigen-positive relapse.[29]

Despite dual targeting, there are still some observed cases of suspected clonal escape with downregulation of CD19 and CD20/CD22 antigen expression.[77,79] Given that exhaustion is another of the proposed mechanisms of CAR T-cell treatment failure, some studies have attempted adding checkpoint inhibitors after CAR T-cell infusion. Results are mixed. Roddie et al[79] observed no clear benefit in adding pembrolizumab on day 14 after the administration of dual CAR T cells, in line with the ZUMA-6 results.[86] However,

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Zhang et al[82] reported improved response rates and progression-free survival with addition of the PD-1 inhibitor tislelizumab on day 1 after infusion.

Outcomes

Results varied regarding outcomes, with some studies reporting lower EFS and others superior EFS compared with the pivotal trials, as depicted in Tables 4 and 5.

The study by Cao et al[52] using high-dose therapy with autologous hematopoietic stem cell infusion followed by CD19 and CD22-targeted CAR T cells showed a 24-month EFS and overall survival of 83%, which exceeds the outcomes of high-dose therapy alone (~30%-40%)[87] or those reported in the CD19-directed studies.[18,23,25,88] It should be noted, however, that the patient population in this study was predominantly aged <50 years (73%) and transplant-naïve. Additionally, it is a complex approach that requires 2 apheresis procedures (one with stem cell mobilization) and involves toxic myeloablative conditioning.

Roddie et al[79] used a bicistronic vector targeting CD19 and CD22, and they encountered issues similar to those reported in the B-cell ALL cohort with short persistence (perhaps reflecting the differentiated phenotype of the product), leading to a lower EFS of 25% at 12 months. However, effective CD22 targeting can be inferred because 7 out of 13 cases downregulated CD22 at relapse.

With tandem products, Spiegel et al[62] reported an EFS of 25% at 12 months in their B-NHL cohort, and the potential reasons for these poor outcomes have been discussed in "Outcomes" of "Review of current trials using dual targeting for relapsed/refractory B-cell ALL". Larson et al[73] produced CD19-20 tandem CAR T cells through bead-based enrichment of CD62L expression, generating a final product skewed toward naïve and memory T cells. They reported an EFS of 40% at 18 months. Activity of the tandem construct against the 2 antigens did not seem to be impaired, with reports of high overall responses and CAR T-cell persistence over 6 months.

A group in Wisconsin[74,75] also designed a CD19-20 tandem construct and reported outcomes equivalent to those of single-antigen targeting, with an EFS of 44% at 24 months. In CAR-naïve patients with diffuse LBCL, EFS increased to 50%. For patients who showed an initial CR and then relapsed (6/12), the relapses occurred late (>180 days), which is not the usual pattern observed with tisa-cel[23] or axi-cel.[13] Early expansion seems to correlate with durable responses, as suggested by this study,[75] data from the CD19 National Institutes of Health (NIH) product with a CD28 costimulatory domain,[24] and data from ZUMA-1 with axi-cel.[88] Regarding patterns of resistance, Shah et al[74] highlighted a patient who relapsed with detectable circulating CAR T cells and available relapse biopsy material. When cocultured in vitro, frozen CAR T cells were able to kill $CD19^+/CD20^+$ Raji cells, but did not show any activity against bright $CD19^+/CD20^+$ biopsy material. This suggests other mechanisms of resistance in the tumor microenvironment in B-NHL beyond antigen loss or downregulation.

Finally, a group from Beijing[76,77] performed detailed in vitro screening of different tandem CAR construct candidates by measuring F-actin accumulation at the immunological synapse (IS) and polarization of the microtubule-organizing center.[76] TanCAR7 proved to have the most stable IS and delivered the most effective target cell lysis, and was thus selected for further in vivo studies. In a phase 1 to 2 study of TanCAR7 in 87 patients with B-NHL, they reported an EFS of 61% at 12 months with a median EFS of 27.6 months. Median persistence duration was ~100 days, and no significant difference was observed between patients who relapsed and those who maintained a response. Interestingly, of the 12 patients with available postrelapse biopsy samples, 5 patients still had detectable CAR T cells in the tissue, but only 1 showed CD19 and CD20 antigen loss.

Summary and future directions

In comparison with the experience with single-antigen CD19-targeting CAR T cells, dual-targeting strategies have shown equivalent initial expansion rates and have proven to be a safe approach with an equivalent toxicity profile. To date, the current generation of dual-targeting CAR T-cell studies have not resulted in significantly improved outcomes compared with targeting CD19 alone. This may reflect both the heterogeneity in approaches used and the fact that dual targeting per se does not address other mechanisms of resistance besides antigen escape. Nonetheless, important lessons have been learned.

If a CD22 CAR is used, it needs to target low antigen density

Clinical studies with CD22 CARs alone[10] have shown high rates of relapse associated with CD22 downregulation. A number of studies suggest[49,66] that optimizing the CD22 CAR domain to recognize low antigen density targets and enhancing its potency are important next steps in improving efficacy.

Cotransduction can lead to skewed in vivo expansion

Different transduction efficiencies can lead to heterogeneous products (of CD19, CD22, and CD19/22 CAR T cells) that can further show skewed and unpredictable expansion of the different cellular components in vivo.

Designing a tandem CAR that functions optimally for both targets is challenging

With a variety of possible designs and conformations, it has proven difficult to optimize function against 2 different antigens, perhaps reflecting differences in the distance of the epitopes from the cell membrane. Studies exploring size and rigidity of the CAR construct[84] or the stability of the IS[76] have proven useful in selecting CARs with the most effective target cell lysis, but in vitro assays do not necessarily recapitulate functionality in vivo.

It is possible that expression of 2 CARs on the surface could trigger cell death

The clinical application of bicistronic vectors has led to products with a differentiated T-cell phenotype and a high proportion of early CAR T-cell loss. It is possible that expressing 2 CARs on a single cell could accelerate activation-induced cell death and/or exhaustion. Further studies are needed to investigate this possibility: if this is the case, coadministration may be preferable to bicistronic or cotransduction approaches. Indeed, on the basis of the available data at present, coadministration strategies have shown the most promising outcomes in B-cell ALL.

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Evasion mechanisms by malignant cells and their microenvironment could be a major barrier for the success of dual-targeting CAR T cells

Although poorly characterized, studies hint at other mechanism of disease resistance aside from loss of persistence and antigen loss/downregulation. For example, Zhang et al[77] describe 4 patients with relapsed B-NHL and antigen positivity despite persisting CAR T cells in the biopsied tissue. Possible causes for such cases could be the inhibition by regulatory T cells and myeloid-derived suppressor cells in the bone marrow microenvironment,[29] upregulation of immune checkpoint molecules via mutations in the IL-6/JAK/STAT3 signaling pathway,[89] abnormalities in the apoptotic pathway,[90] downregulation of cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) pathway signaling,[91] or production of adenosine by tumor cells.[92]

Poor CAR T-cell persistence remains a key challenge

Several mechanisms underlying poor CAR T-cell persistence have been suggested, such as poor CAR T-cell fitness, exhaustion, and immune rejection of the product.

Regarding CAR T-cell fitness, clones derived from naïve populations (T naïve and T stem cell memory) are considered to play a critical role in long-term functional CAR T-cell persistence.[31,93] Biasco et al[31] showed that stem cell memory T-cell subpopulations contributed the most to the clonal pool at late time points of patients with long-term persisting CAR T cells. Some strategies to improve the functionality of the product include optimizing CAR design by reducing the affinity of CAR-T binding to antigens[5]; using CD3 zeta domains with fewer immunoreceptor tyrosine-based activation motifs (ITAMs)[94]; shortening the duration of ex vivo culture[83,95]; using AKT inhibitors[96,97]; or modifying the culture medium by including IL-21,[98] increasing the potassium concentration,[99] or adding N-acetylcysteine.[100]

Exhaustion has been suggested as a possible mechanism through methylation profiling of CD19 CAR T cells after infusion.[34] Addition of checkpoint inhibitors in the B-NHL population has yielded mixed results. Reinfusion of CAR T cells followed by nivolumab is currently being investigated (NCT05310591), while there are preclinical studies on gene-edited CAR T cells with downregulation of DNMT3A[101] or PRDM1.[102]

Finally, immunogenicity of the CAR product must be considered given that most CAR T cells use an antigen recognition domain derived from murine antibodies. Turtle et al[32] observed no expansion or persistence after CD19-targeted CAR T-cell reinfusion in adult patients with B-cell ALL despite the use of lymphodepleting chemotherapy in 4 out of 5 patients. They were able to demonstrate CAR-specific cytotoxic T-cell responses in an in vitro model and define possible antigenic epitopes within the CAR construct. Immune-mediated rejection may explain the relatively low rate of long-term responses to reinfusion of tisa-cel for early B-cell recovery.[103] Given that dual-targeting products incorporate 2 scFvs and are frequently given after single-antigen targeted CAR T-cell therapies with mostly the same constructs, there is an increased potential for immune-mediated rejection, and immunogenicity should be monitored. Humanization of CARs[104] and optimizing exposure to fludarabine[105,106] are being explored as strategies to reduce CAR T-cell rejection.

Importantly, although there is strong evidence that persistence is key for durable remissions in B-cell ALL,[28,107-109] this is not as well established in B-NHL. Interestingly, most patients with LBCL still relapse with antigen-positive disease following CAR T-cell therapy, which warrants further investigation if products with longer persistence profiles could deliver more durable responses in LBCL.

Future directions

Although dual targeting has not yet fully eradicated CD19$^-$ relapse or improved outcomes, the studies to date have given important insights into the challenges to overcome. Building on these lessons, the next generation of dual-targeting CAR T-cell studies are well placed to fully achieve the potential of this approach. Subsequent studies should use CD22 CARs that recognize low antigen density targets and incorporate strategies to enhance CAR T-cell persistence. For example, in our next study in pediatric B-cell ALL, we plan to combine optimized lymphodepletion with fludarabine therapeutic drug monitoring with the use of CAR T cells transduced with CD19CAR and CD22CAR vectors separately generated with a rapid manufacturing protocol. Such approaches may increase the regulatory complexity and cost of CAR T cells; however, if they achieve sufficiently improved long-term outcomes relative to existing licensed products, this investment will be justified. Moreover, as we move forward, the lessons learned in dual targeting of B-lineage ALL and non-Hodgkin lymphoma may provide important insights on how to best deliver dual-targeting CAR T cells for other malignancies.

Authorship

Contribution: G.d.O.C. prepared the manuscript draft, tables, and figures; C.R. and P.J.A. reviewed and contributed to the final version of the manuscript; and P.J.A. supervised the writing process.

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