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Multispecific CAR T Cells Deprive Lymphomas of Escape via Antigen Loss

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Abstract

Chimeric antigen receptor (CAR) modified T cell therapy has transformed the management of relapsed/refractory B cell malignancies. Despite high overall response rates, relapse post CAR T treatment remains a clinical challenge. Loss of target antigen, specifically CD19, is one well-defined mechanism of disease relapse. The mechanism of CD19 loss and which patients are at higher risk of CD19 loss remain poorly understood. To overcome CD19 loss, CARs targeting multiple antigens are being tested in clinical trials. CD19/20 and CD19/22 bispecific CARs demonstrate cytotoxicity against CD19-negative cells in preclinical studies. These CARs have also shown efficacy, safety, and a relatively low rate of CD19-negative relapse in phase I trials. These small studies suggest that multispecific CAR T cells can deprive lymphomas of escape via antigen loss. However, the selection of an ideal target, the right CAR construct, and whether these multispecific CARs can induce long-term remissions are still under investigation.

R/R: relapsed/refractory

scFV: single-chain variable fragment

B-ALL: B cell acute lymphoblastic leukemia

LBCL: large B cell lymphoma

CRS: cytokine release syndrome

ICANS: immune effector cell–associated neurotoxicity syndrome

NHL: non-Hodgkin's lymphoma

INTRODUCTION

The treatment of relapsed/refractory (R/R) B cell malignancies has been revolutionized by advances in cellular therapy. First developed in 1990s, the early chimeric antigen receptor (CAR) T cells were unable to persist in vivo (1). These first-generation CARs were designed by linking the CD3 chain from the CD3 T cell receptor or Fc receptor chain to an external single-chain variable fragment (scFv) and lacked sufficient signaling capacity, durability, and antitumor activity (2). After the introduction of the costimulatory domain CD28 in addition to the T cell receptor, the second-generation CARs demonstrated activity against the prostate-specific membrane antigen (3). This activity was later redemonstrated against leukemia cells in vivo when Brentjens et al. (4) expanded T cells in the presence of CD80 and interleukin-15.

Based on its success in B cell malignancies, anti-CD19 CAR T cell therapy has been approved in the R/R setting by the US Food and Drug Administration for B cell acute lymphoblastic leukemia (B-ALL), diffuse large B cell lymphoma, mantle cell lymphoma, and follicular lymphoma (5–8). More recent data have suggested its efficacy in second-line treatment in patients with R/R large B cell lymphoma (LBCL) (9, 10).

However, despite the astounding clinical efficacy of CAR T cell therapy, several factors limit its use. The adverse effects caused by CAR T cell therapy, such as cytokine release syndrome (CRS), immune effector cell–associated neurotoxicity syndrome (ICANS), infections, and persistent cytopenias, can be life-threatening and limit its application to eligible patients with intact organ function. Also, despite initial high remission rates, the duration of response is limited. In adult B-ALL, a median event-free survival of 3–6 months has been reported, while more than half of the patients with aggressive non-Hodgkin's lymphoma (NHL) relapse within 1 year after therapy (11–14). Even in patients with initial objective responses, the long-term success rate remains unknown, with only limited follow-up to date.

Mechanism of Relapse

Mechanisms of relapse and resistance against CD19 CAR T cell therapy can be based on inherent T cell defects, tumor-specific resistance mechanisms, and the tumor microenvironment (15). Antigen-negative relapse is one of the best-defined causes of relapse after CAR T cell therapy. Most of the data come from B-ALL studies, given the ease of detection of antigen loss. Maude et al. (5) first reported the loss of CD19 antigen in 15 of 61 patients with B-ALL who relapsed after successful treatment with CD19 CAR. Several mechanisms have been reported for the loss of antigen. These include presence of a pre-existing target antigen–negative clone, diminished expression of target antigens, acquired mutations, splicing site variations, or lineage switching–mediated target antigen loss, as well as failure of surface presentation of target antigens (15).

In a large study of 628 patients with R/R B-ALL, 17% had >1% CD19-negative blasts before any immunotherapy, while 7% had low CD19 expression at the RNA level and 24% had low-normal CD19 expression. Mutations within the CD19 domain, as well as alternative splicing especially at exon 12, have been described as the cause of antigen-negative relapse after CAR T cell therapy (5, 16, 17). Lineage switching from lymphoid to myeloid phenotype has also been described in B-ALL leading to CD19-negative relapse (18, 19) (**Figure 1**).

Although not as well defined as in B-ALL, several mechanisms of antigen loss have also been described in lymphomas (20). CD19 loss occurred in 30% of the patients with LBCL treated with CD19 CAR in the ZUMA-1 trial. This is likely due to selection of a pre-existing CD19-negative clone. Other potential mechanisms including alternative exon splicing or point mutations, which, although well described in B-ALL, have not been reported to alter the expression of CD19 significantly in LBCL. Another recently described mechanism of antigen escape is trogocytosis, which

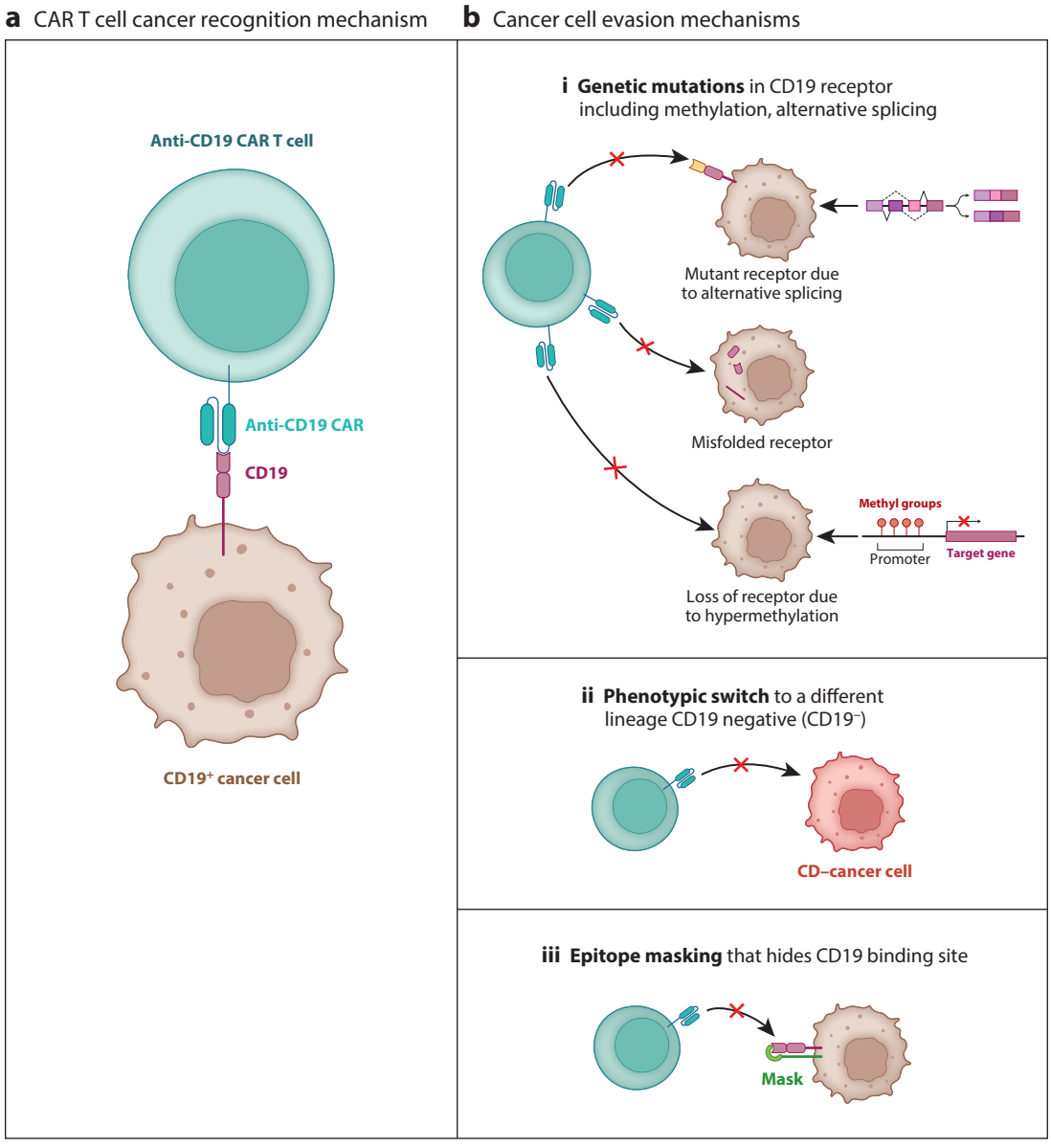

Figure 1

Mechanism of antigen loss and cancer cell evasion from chimeric antigen receptor (CAR) T cells. (*a*) CAR T cell cancer recognition mechanism. (*b*) Cancer cell evasion mechanisms include (*i*) genetic mutations, (*ii*) phenotype switching, and (*iii*) epitope masking. Figure adapted from CAR T for CD19$^+$ Cancer Cells by BioRender.com (2022), retrieved from **https://app.biorender.com/biorender-templates**.

results in target antigen transfer to T cells (21, 22). This leads to autoreactivity of the CAR T cells and fratricide resulting in antigen-low tumor relapse (**Figure 1**). Although the factors associated with CD19-negative relapse are not well understood, the risk of relapse may be higher in patients with low baseline CD19 expression before therapy (23).

Relapse due to antigen loss is not restricted to CD19 antigen (24, 25). In a phase I trial among patients with R/R B-ALL, CD22 expression was diminished or absent in seven of eight patients who had initially responded and subsequently relapsed after CD22 CAR T cell therapy (24). An interesting observation reported by Plaks et al. (20) is persistence of other B cell antigens, including CD20, CD22, and CD79a, in patients who had a CD19-negative relapse. This observation provides the rationale for using multispecific CAR T cells to minimize CD19-negative relapse (20).

MULTISPECIFIC CAR T CELL THERAPY

One way to overcome CD19-negative relapse is to target multiple B cell antigens at the same time. This could theoretically prevent CD19-negative relapse by additionally targeting B cells that have no or low CD19 expression (26). Several trials are ongoing to assess the efficacy and safety of multispecific CAR T cell therapies.

Approaches to Multispecific CAR T Cell Therapy

There are several design options for CAR therapies to target multiple antigens concurrently (27):

1. Coadministration of two or more CAR T cell populations against various targets.
2. Infusion of T cells with 2 different CARs expressed on the same cell.
3. Cotransduction of different CAR vectors on the same T cell, which will generate dual and single CAR-expressing T cell subsets.
4. Generation of two CAR domains connected to the same receptor as a tandem CAR T cell.

In addition, the CAR T cells can be bispecific or trispecific, i.e., they can target two or three lymphoma cell antigens at the same time.

Choosing a Target and Optimizing Design

In order to increase efficacy and limit on-target off-tumor adverse effects, target selection is of immense importance. Several factors can influence this selection, including specificity to prevent on-target off-tumor effects and stability to prevent relapse (28).

In addition, bispecific CARs should exert their cytotoxic effect in the presence of either antigen alone or both antigens simultaneously. In the initial studies of bispecific CARs against CD19 and HER-2 antigens, the bispecific CARs showed lower activity in the presence of $CD19^{-}$/$HER\text{-}2^{+}$ cells versus $CD19^{+}$/$HER\text{-}2^{+}$ targets. This would defeat the purpose of preventing antigen-negative relapse (29). Therefore, it is important to select an appropriate second target, in addition to CD19, to make the multispecific CAR safe and effective. Several candidate antigens are being studied as the second target for CARs, including CD20, CD22, and other B cell markers.

CD20 is expressed exclusively on B cells, and given its specificity as well as its predictive role in B cell survival, CD20 is an attractive target in lymphoma (30). In fact, rituximab, the most commonly used drug in lymphoma, is a monoclonal antibody against CD20 (31). Many other CD20 monoclonal antibodies have since been developed and are used in NHL treatment (32, 33). Like CD20, CD22 is also expressed almost exclusively on B cells and plays an important role in B cell survival, B cell receptor and Toll-like receptor signaling, and generation of memory B cells, making CD22 another suitable antigen for CAR T cells (34). In fact, CD22 CAR T cells have already demonstrated favorable outcomes in patients with CD19-negative disease (24). Other potential markers that are being investigated include CD123, CD79b, CD38, and CD37 (35–38). In addition to choosing the right target, the design of the construct and methodology of dual targeting can impact its success. Gardner et al. (39) reported preliminary findings with a cotransduced CD19/22 CAR product in pediatric B-ALL. While feasibility and efficacy were in

Table 1 Preclinical studies with multispecific chimeric antigen receptors

Reference	Disease	Vector	Antigen	Pattern
40	Leukemia/lymphoma	Lentivirus	CD19/20	Tandem
63	ALL/NHL	Lentivirus	CD19/20	Tandem
41	NHL	Lentivirus	CD19/20	Tandem
42	ALL	Retrovirus	CD19/20	Tandem
46	ALL	Lentivirus	CD19/22	Tandem
47	Leukemia	Lentivirus	CD19/22	Tandem
54	ALL	Lentivirus	CD19/123	Coadministration/ bicistronic/tandem
55	ALL	Lentivirus	CD19/123	Tandem
57	NHL	Lentivirus	CD19/79b	Tandem
56	NHL	Retrovirus	CD19/38	Coadministration
59	NHL	Lentivirus	CD19/37	Tandem
64	B-ALL	Lentivirus	CD19/20/22	Tandem
63	ALL/NHL	Lentivirus	CD19/20/22	Tandem/bicistronic
61	NHL	Lentivirus	CD19/20/22	Coadministration

Abbreviations: ALL, acute lymphoblastic leukemia; B-ALL, B cell acute lymphoblastic leukemia; NHL, non-Hodgkin's lymphoma.

line with single-targeted CD19 CAR products, there was preferential in vivo expansion of the CD19 CAR T cells, with limited expansion of the CD22 CAR T cells or CD19/CD22 CAR T cells, demonstrating the challenges of optimizing dual-targeting CARs (39).

CD19/20 Bispecific CAR T Cell Therapy

Preclinical studies. Several preclinical studies have evaluated the functionality and safety of the bispecific CD19/20 CARs (**Table 1**). In a study by Zah et al. (40), CD19/20 CAR T cells demonstrated cytotoxicity against both CD19-positive and CD19-negative lymphoma cells in vitro. The T cell growth, differentiation, exhaustion, and tumor lysis profile were comparable in vitro among the CD19, CD20, and bispecific CARs. Another important observation in this study was the significance of CAR construct and spacer length: The CAR with the short spacer and CD20–19 configuration with a proxy spacer that projects the CD20 scFv away from the T cell membrane demonstrated cytokine production in the presence of CD19 or CD20 antigens comparable to either CAR alone (40).

The importance of the CAR construct was further demonstrated in another study that compared eight different tandem CAR constructs (TanCAR1–8) based on the order of CD19 and CD20 targeting scFv, as well as the type of linker between the two (41). TanCAR7 showed stronger antitumor activity than a single-antigen CAR despite lower cytokine production. TanCAR7 also demonstrated robust antitumor activity through better intratumoral infiltration in a solid tumor xenograft mouse model.

Martyniszyn et al. (42) reported improved avidity of the CD20-CD19 bispecific CAR to double antigen-positive target cells compared to monospecific CAR T cells, in particular at low antigen densities, and successfully eliminated pediatric ALL cells with a mixed $CD19^{+}CD20^{+}/CD20^{-}$ phenotype in mice.

Clinical studies. Multiple phase I studies have demonstrated the efficacy and safety of CD19/20 CAR as well as prevention of antigen loss in patients with lymphoma (**Table 2**).

Table 2 Outcomes of CD19/CD20 bispecific CAR T cell therapy in non-Hodgkin's lymphoma

Reference	Target	Pattern	Total patients	Median age	Dose (cells per kg)	Response	Adverse events	Antigen-negative relapse
43	CD19/20	Tandem	22	57	2.5×10^5–2.5×10^6	ORR 82% CRR 64%	CRS 64%, ICANS 32%	0 CD19 loss, 1 CD20 loss
44	CD19/20	Tandem	87	50	0.5–8×10^6	ORR 78% CRR 70%	CRS 80%, ICANS 17%	25% (1/4) CD19 loss

Abbreviations: CAR, chimeric antigen receptor; CRR, complete remission rate; CRS, cytokine release syndrome; ICANS, immune effector cell–associated neurotoxicity syndrome; ORR, overall response rate.

A first-in-human trial of bispecific, tandem lentiviral anti-CD20, anti-CD19 (LV20.19) CAR T cells demonstrated low toxicity and high efficacy in patients with R/R B cell malignancies (43). This trial was done in two phases: dose escalation and dose expansion. In the dose escalation phase, doses ranging from 2.5×10^5 to 2.5×10^6 of LV20.19 CAR T cells/kg were infused in 22 patients with B cell malignancies. Given no toxicity at higher dosages, a dose of 2.5×10^6 cells/kg was chosen for an expansion cohort. Among all patients, the overall response rate (ORR) at day 28 was 82%, with 64% achieving complete remission (CR). Dual targeting did not worsen CAR-associated toxicities. There were no deaths reported due to LV20.19; 64% of patients had CRS (5% being grade 3–4), while ICANS was present in 32% of patients (14% being grade 3–4), and all achieved full neurological recovery. Importantly, among relapsing patients, CD19 loss was not identified, suggesting that downregulation of target antigen did not contribute to CAR failure. Another important finding of this study was lower response in patients who had relapsed after prior CD19 CAR T cell therapy. This could be due to lower LV20.19 expansion, as well as to a lower transduction in vivo, which may be secondary to immune-mediated rejection of LV20.19 (43).

The efficacy and safety of the CD19/20 CAR T cells were also demonstrated by Tong et al. (41). In this phase I/II single-arm study, 28 patients with R/R NHL received CD19/20 CAR T cells. In the later updated analysis (44), the best ORR was 78%, with 70% patients achieving CR; median progression-free survival (PFS) was 27.6 months, while the overall survival (OS) was not reached. Patients with aggressive lymphomas and higher stages also demonstrated an ORR and complete remission rate (CRR) of 75% and 68%, respectively, and PFS was 27.6 months. Three treatment-related deaths were reported in the study, which were due to pulmonary infection from myelosuppression, CRS-related pulmonary injury, and pulmonary infection with secondary multiple organ failure (44). CRS was reported in 80% of patients (10% of those being grade 3), while 17% had neurotoxicity (2 patients grade 3). In contrast to the results reported by Shah et al. (43), seven of nine patients who relapsed after CD19 CAR responded to the bispecific CAR, and no differences were observed in duration of response compared to other patients. Antigen loss was reported in only one of four patients who relapsed after CD19/20 CAR.

ORR: overall response rate

CR: complete remission

PFS: progression-free survival

OS: overall survival

CRR: complete remission rate

CD19/22 Bispecific CAR T Cell Therapy

Preclinical studies. Similar to CD19/20 targeting, varying approaches to the targeting of CD19 and CD22 antigens simultaneously have been studied in preclinical models (**Table 1**). In a study by Qin et al. (45), mice injected with a mixture of $CD19^-$, $CD22^-$, and parental NALM6 ($CD19^+/CD22^+$) ALL cells received CD19 CAR. This was followed by CD22 CAR at the time of CD19-negative relapse. However, this sequential infusion was unsuccessful in preventing progression of ALL. Although coinfusion (simultaneous administration) of CD19 and CD22 CAR had better outcomes, this still resulted in CD19-negative relapse, demonstrating the challenges in preventing antigen downregulation.

As with CD19/20 CAR, the design of the CAR construct plays a considerable role in its potency and cytotoxic activity. In the above study (45), the tandem CARs were unable to demonstrate in vitro or in vivo activity when a loops structure was used, whereas the CD19/22 CAR with a short linker between CD19 and CD22 variable chains demonstrated optimal in vitro and in vivo activity against both CD19 and CD22 antigens as well as CD19-negative cells.

On the other hand, Zanetti et al. (46) demonstrated that tandem CD22-CD19 CAR showed expansion and efficacy similar to CD19 CAR with superior long-term control against patient-derived B-ALL xenografts despite a lower transduction rate. Wei et al. (47) used a different bispecific CAR construct, i.e., CD19/22 CAR, and demonstrated its efficacy in preclinical models. These CD19/22 CAR T cells exhibited higher cytotoxicity due to more granzyme B production during both in vitro and in vivo studies.

Clinical studies. CD19/22 CAR, which demonstrated preclinical efficacy in the study by Wei et al. (47), was studied in an early-phase clinical trial and exhibited safety and efficacy in 16 patients with R/R NHL. ORR was 87.5% while CRR was 62.5%, and patients who achieved CR had a favorable PFS (**Table 3**). The rates of severe toxicity with this CAR were low (grade 3 or higher CRS in 6%, 0% neurotoxicity), which may be due to lower cytokine levels in the blood, indicating the possible greater cytotoxic effect of granzyme B (47). CD19/CD22 CAR was also able to prevent relapse due to antigen loss. Among the three patients who relapsed, none exhibited loss of CD19 antigen.

The loop CD19/CD22 CAR used in the preclinical setting by Qin et al. (45) was assessed in patients with LBCL by Spiegel et al. (23). In 22 patients with R/R LBCL, this bispecific CAR demonstrated an ORR and CRR of 40% and 33% at 3 months, respectively, and a median PFS and OS of 3.2 and 22.5 months, respectively, at a 10-month follow-up. While there were no dose-limiting toxicities, CRS occurred in 77%, neurotoxicity in 45%, and macrophage-activating syndrome in two patients. Antigen-negative relapse was reported after treatment with the CD19/22 CAR. Four of the 14 patients who progressed after the bispecific CAR had either no or low CD19 expression. However, this CAR product had higher CD4 T cell content than the standard CD19 CAR. These cells also demonstrated a higher expression of CD39 and PD-1 markers, which are associated with CAR T cell exhaustion.

A different CD19/22 CAR with a loop construct demonstrated an ORR of 79.3% and a CRR of 34.5% in 32 patients with NHL (48). The PFS was 6.8 months; OS was not reached. About one-third of patients relapsed, but whether the relapse was due to antigen loss was not reported. The toxicity profile was comparable to other bispecific CARs: neutropenia, anemia, and thrombocytopenia were the most common grade 3 or higher adverse events. CRS occurred in 90% of patients, and 28% had grade 3 or higher; one patient died from severe CRS-associated kidney injury. Neurotoxicity occurred in 15% of patients, with 12% being grade 2 or higher.

An interesting combinatorial approach involved the use of pembrolizumab with autologous transduced bicistronic CAR T cells expressing both anti-CD19 and anti-CD22 CARs (AUTO3) in 23 patients with R/R diffuse large B cell lymphoma (49). The role of pembrolizumab is to prevent T cell exhaustion by inhibiting PD-1, thus potentially augmenting CAR activity and persistence. ORR and CRR were 69% and 56%, respectively, in the 16 patients who received a higher dose of cells; this improved further to 75% and 63%, respectively, in patients who received pembrolizumab at day 1. Although neutropenia, thrombocytopenia, anemia, and hypophosphatemia were common, none of the patients had severe CRS, and only 33% had mild CRS. Neurotoxicity was reported in 9% of patients and occurred in the setting of relapsed disease with little to no presence of CAR T cells in the blood.

Hu et al. (50) demonstrated efficacy of bispecific CD19/22 CAR, which incorporated CD19 and CD22 single-chain variables in a single CAR construct in preclinical as well as clinical settings.

Table 3 Outcomes of CD19/CD22 bispecific CAR T cell therapy in non-Hodgkin's lymphoma

Reference	Target	Pattern	Total patients	Median age	Dose (cells per kg)	Response	Adverse events	Antigen-negative relapse
23	CD19/22	Tandem	22 (LBCL)	69	$1–3 \times 10^6$	ORR 62% CRR 29%	CRS 77% ICANS 45%	28.5% (4/14) CD19 loss/low
50	CD19/22	Tandem	16	NA	NA	ORR 87.5% CRR 62.5%	CRS 100% ICANS 0	NR
48	CD19/22	Tandem	32	NA	$3.69–3.285 \times 10^9$	ORR 79.3% CRR 34.5%	CRS 90% ICANS 15% Deaths 1	NR
47	CD19/22	Tandem	16	52	$4.9–9.4 \times 10^6$	ORR 87.5% CRR 62.5%	CRS 100% ICANS 0	None
51	CD19/22	Sequential	36	47	CD19 CAR $5.1 \pm 2.1 \times 10^6$, CD22 CAR $5.3 \pm 2.4 \times 10^6$	ORR 83% CRR 58.3%	CRS 100% ICANS 13.2%	None (in 7/18 patients who had biopsy after relapse)
52	CD19/22	Sequential	36	36 (in both groups)	CD22 CAR $5.28 \pm 2.44 \times 10^6$, CD19 CAR $5.14 \pm 2.06 \times 10^6$	ORR 72.2% CRR 50%	CRS 100% ICANS 13.2	None (in 3/8 who had biopsy after relapse)
53	CD19/22	Sequential, followed by ASCT	42	41	CD22 4.0×10^6, CD19 4.1×10^6	ORR 90.5% CRR 81%	CRS 95% ICANS 21%	None (in 5/7 who had biopsy after relapse)

Abbreviations: ASCT, autologous stem cell transplant; CRR, complete remission rate; CRS, cytokine release syndrome; ICANS, immune effector cell–associated neurotoxicity syndrome; LBCL, large B cell lymphoma; NA, not applicable; NR, not reached; ORR, overall response rate.

Similar to previous reports, the bispecific CAR produced a greater amount of granzyme B. Among 16 patients with R/R aggressive B cell lymphoma, ORR was 87.5%, and 62.5% achieved CR. The 2-year OS and PFS rates were 77.3% and 40.2%, respectively, and PFS was found to be higher in patients who had previously received two or fewer lines of therapy than in those who had received more than two lines. Although all patients had CRS, grade 3 CRS was reported in only one patient, while no cases of neurotoxicity were reported. Relapse rate after the bispecific therapy was not reported.

Targeting CD19 and CD22 simultaneously by sequential administration of CD19 and CD22 CARs was reported in 38 patients with R/R NHL (51). The median PFS and OS were 9.9 months and 18 months, respectively. CRS was reported in all patients with neurotoxicity in 13.2%. Postrelapse biopsy was available in 7 of the 18 patients, and among them, none had loss of CD19 or CD22 antigen. Sequential administration of third-generation CD19 CAR and CD22 CAR was also reported in another study in 38 patients with NHL (52). The ORR and CRR were 72% and 50%, respectively, in data available in 36 patients. All patients had CRS (21% grade 3 or higher), while neurotoxicity occurred in 13.2%; these effects were all reversible. Of the eight patients (26.7%) who relapsed, three underwent rebiopsy, and none had CD19 or CD22 antigen loss.

Although the role of autologous transplant consolidation after bispecific CAR T cell therapy is unclear, a small study including 42 patients with NHL reported favorable outcomes in patients who underwent autologous transplant after sequential CD19 and CD22 CAR infusions (53). ORR was 90.5% and CRR was 81%, while median PFS and OS were not reached at a median follow-up of 24.3 months. CRS was reported in 95% of patients (5% being grade 3), while 21% had neurotoxicity (5% being grade 3). Among the five patients who underwent postrelapse biopsies, none demonstrated CD19- or CD22-negative disease.

CARs Against Other Antigens

CD22 and CD20 are not the only targets being investigated as potential partner targets with CD19 for CAR T cell therapy in B cell malignancies. Multiple preclinical studies have reported the efficacy of these bispecific CARs, but clinical data are lacking. Ruella et al. (54) used different mechanisms to construct CD19/123 CAR T cells, i.e., sequential administration of CD19 CAR and CD123 CAR, tandem CD19/123 CAR, and bicistronic CAR, all showing efficacy in mouse models. The tandem CD19/123 CAR was also studied by Yan et al. (55) and showed a robust antileukemic effect against both leukemia and lymphoma cells expressing CD19, CD123, or both.

Similarly, CD19/79b, CD19/37, and CD19/38 CARs have demonstrated antitumor activity in vitro as well as in vivo in mice and have delayed tumor progression (56–59).

Trispecific CARs

Targeting the three main B cell antigens (CD19, CD20, and CD22) may, theoretically, be the most effective approach in preventing relapse due to antigen loss. However, data on the efficacy and safety of trispecific CARs are scarce in comparison to bispecific. A study using a duoCAR with a tandem CD19 and CD20 targeting binder, linked by the P2A self-cleaving peptide to a second CAR targeting CD22, tested this hypothesis in the preclinical setting (60). This CD19/20/22 duoCAR displayed similar persistence but a higher cytokine release and antitumor activity when compared to single CARs in mice containing CD19/20/22-positive as well as CD19-, CD20-, and CD22-negative variants of both ALL and NHL.

Meng et al. (61) demonstrated enhanced expansion and more robust antitumor effects with sequential administration of CD19, CD20, and CD22 CAR T cells both in vivo and in vitro.

Although trispecific CARs have not yet been tested in the clinic, targeting these antigens by sequentially administering CD19, CD20, and CD22 CARs has been reported (61, 62).

LIMITATIONS OF MULTISPECIFIC CAR T CELL THERAPY

Multispecific CAR T cell therapy has shown promise in preventing antigen-negative relapse, but the results to date are mixed, limited to small phase I trials that lack a comparator arm. While rates of CD19 antigen loss appear to be lower in these dual-targeting trials than reported in earlier single-targeting CD19 CAR trials, CD19 loss still occurs in a small subset of patients, demonstrating that even multispecific targeting may not completely eliminate target antigen loss. The role of bispecific and trispecific CAR T cell therapy in the era of immunotherapy, including bispecific antibodies and antibody drug conjugates like blinatumomab, loncastuximab, and inotuzumab, also needs to be addressed. These drugs can alter antigen expression and give rise to a disease with heterogenous phenotype, thereby impacting the response rates of CAR T cell therapy. Ultimately, larger phase II studies and longer follow-up are needed to assess the safety and determine the durable efficacy of multispecific CARs and their ability to preventing antigen loss in patients with R/R lymphomas.

SUMMARY POINTS

1. Disease relapse in patients with R/R lymphoma after CAR T cell therapy presents a treatment conundrum. One of the main mechanisms of relapse after CD19 CAR T cell therapy is loss of target antigen and clonal escape of a CD19-negative malignant population.
2. Targeting multiple antigens can limit antigen loss through coinfusion or sequential infusion of multiple CARs, bispecific CARs, or trispecific CARs.
3. Various B cell antigens are being investigated to increase the cytotoxicity and mitigate the side effects of on-target off-tumor effects, the most common being CD20 and CD22.
4. CD19/20 and CD19/22 bispecific CARs have shown safety and efficacy in preclinical and clinical studies, leading to lower rates of antigen-negative relapse than previously reported.
5. More recently, trispecific CD19/20/22 CAR has shown favorable cytotoxicity against antigen-negative lymphoma cells in preclinical studies.
6. The data regarding targeting multiple antigens are sparse, and many issues remain to be resolved, including the ideal second target, the appropriate CAR construct, and the long-term efficacy and safety of multispecific CAR. The data so far, however, have been encouraging, and further clinical trials are ongoing.

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