Sequencing of Anti-CD19 Therapies in the Management of Diffuse Large B-Cell Lymphoma

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ABSTRACT

Several second- and third-line immunotherapeutic options for patients with relapsed or refractory diffuse large B-cell lymphoma ineligible for autologous stem cell transplant are directed against the B-cell antigen cluster of differentiation 19 (CD19). The anti–CD19 monoclonal antibody tafasitamab, paired with the immunomodulator lenalidomide, mediates antibody-dependent cellular toxicity and phagocytosis; the antibody–drug conjugate loncastuximab tesirine delivers the DNA cross-linking agent tesirine via CD19 binding and internalization; and CD19-directed chimeric antigen receptor T-cell therapy (CAR-T) products are engineered from autologous T cells. Although CD19 expression is assessed at diagnosis, clinically relevant thresholds of CD19 expression—which may not be detectable using current routine methodologies—have not been defined and may vary between CD19-directed treatment modalities. Determining optimal treatment sequencing strategies for CD19-directed therapy is hampered by the exclusion of patients who have received prior CD19-directed therapies from major clinical trials. Antigen escape, which is attributed to mechanisms including epitope loss and defective cell surface trafficking of CD19, is an important cause of CAR-T failure. Limited data suggest that CD19 expression may be maintained after non–CAR-T CD19-directed therapy, and retrospective analyses indicate that some patients with disease relapse after CAR-T may benefit from subsequent CD19-directed therapy. To date, clinical evidence on the effect of anti-CD19 therapy prior to CAR-T has been limited to small case series. Prospective studies and detailed analyses are needed to understand how pretreatment and posttreatment CD19 expression correlates with clinical responses to subsequent CD19-directed therapy to fully maximize treatment strategies.

Introduction

As immunotherapies have expanded the range of treatment options for diffuse large B-cell lymphoma (DLBCL), the complexity of optimal treatment sequencing has concomitantly increased. Frontline treatment is composed of anthracycline-based combination chemotherapy combined with anti–cluster of differentiation 20 (CD20) monoclonal antibodies, most commonly rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP; refs. 1–6). Although this approach can cure a significant subset of patients, approximately 40% of patients have a disease that fails to respond or subsequently relapses (7). Second-line therapy is informed by the response to frontline treatment. For patients with refractory or early relapsed (<12 months) disease, CD19-directed chimeric antigen receptor (CAR) T-cell therapy (CAR-T) can offer clinical benefits, whereas disease relapse beyond 1 year is managed with platinum-based chemotherapy followed by autologous stem cell transplant (ASCT) in the event of disease response (8). However, many patients are ineligible for CAR-T or ASCT due to comorbidities or logistics or do not respond well to CAR-T or ASCT.

Whatever the reason for looking beyond CAR-T and ASCT, recently approved therapies have broadened the therapeutic armamentarium for these patients to include immunotherapy regimens such as tafasitamab plus lenalidomide, polatuzumab vedotin combined with bendamustine plus rituximab, and loncastuximab tesirine (8). Bispecific antibody (BsAb) is an emerging therapeutic option for patients with relapsed/refractory (R/R) DLBCL, including the recently approved CD20xCD3-directed antibodies glofitamab and epcoritamab (9, 10) and odronextramab, which is currently under regulatory review (11), with CD19xCD3-targeting bispecifics also in development (12). However, response data are currently substantially more mature for CD19-based treatments (13–15).

Many of the recently introduced novel second- and third-line immunotherapies are directed against CD19, a B-cell–specific cluster of differentiation molecules expressed on the surface of native and malignant B cells (16). Unlike CD20, CD19 is expressed throughout all B-cell maturation stages (17), is more homogenous, and is commonly preserved in CD20-negative tumor subsets (18, 19). Data on the potential effect of previous CD19-directed treatment before the initiation of CAR-T are limited, and the optimal sequence of CD19-directed therapies is unclear. CD19-directed therapies are under evaluation in the frontline setting, including the phase III frontMIND study (NCT04824092), which evaluated tafasitamab plus lenalidomide with R-CHOP in high-intermediate– and high-risk DLBCL (20). This suggests that the future therapeutic landscape for DLBCL may include CD19-directed immunotherapies in an earlier line of treatment. Understanding ways to optimally sequence these agents is becoming increasingly relevant.

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Clin Cancer Res 2024;30:2895–904

doi: 10.1158/1078-0432.CCR-23-1962

Although several new B-cell targets for lymphoma-directed therapy have emerged, in this review, we examine potential sequencing considerations for CD19-directed treatment in patients with R/R DLBCL, the effect of CD19-directed therapy on the dynamics of subsequent CD19 expression in malignant cells, the effect of CD19-directed treatment on subsequent CAR-T outcomes, and patient-related factors that may influence CD19-directed treatment selection.

CD19-Directed Therapies

Currently available CD19-directed therapies for the treatment of R/R DLBCL include tafasitamab, loncastuximab tesirine, and CAR-T products [axicabtagene ciloleucel (axi-cel), tisagenlecleucel (tisa-cel), and lisocabtagene maraleucel (liso-cel)], all of which have different mechanisms of action (**Fig. 1**; **Table 1**).

Tafasitamab is an Fc-enhanced, humanized, anti–CD19 monoclonal antibody that functions as an immunotherapeutic agent by mediating antibody-dependent cellular toxicity and antibody-dependent cellular phagocytosis (21). Tafasitamab is administered in combination with the immunomodulator lenalidomide (21), which has antineoplastic, antiangiogenic, and proerythropoietic properties (22). In the phase II L-MIND study, patients receiving tafasitamab achieved an overall response rate (ORR), complete response (CR) rate (CRR), and a median progression-free survival (mPFS) of 60%, 43%, and 12.1 months, respectively (23). The updated 5-year analysis showed similar ORR, CRR, and mPFS but demonstrated a median overall survival (mOS) of 33.5 months (13).

Loncastuximab tesirine is an anti-CD19 antibody–drug conjugate (ADC) that delivers the conjugated drug tesirine, a chemotherapeutic agent that cross-links DNA in target cells, after binding and internalization (24, 25). When evaluated in the phase II LOTIS-2 trial, loncastuximab tesirine showed an ORR of 48.3%, with 24% of patients experiencing CR, and an mOS of 9.5 months (26, 27).

CAR-T products are derived from genetically modified autologous T cells transduced by *ex vivo* lentiviral- or adeno-associated viral transgene insertion to express a CAR specific for CD19 (28–31). This process takes 8 to 37 days (28, 30, 32), depending on the specific CAR-T–cell product used (30). Bridging therapy is commonly administered during the CAR-T–cell manufacturing process (8).

Axi-cel is an anti-CD19 CAR-T with a CD28 costimulatory domain. The phase II ZUMA-1 trial demonstrated, after 5 years of follow-up, an ORR with axi-cel, a CRR, a mOS, and a median duration of CR of 83%, 58%, 25.8 months, and 62.2 months, respectively (33, 34). The phase III ZUMA-7 trial that compared axi-cel with standard of care (SOC) chemotherapy followed by ASCT in patients with R/R DLBCL showed improved event-free survival (EFS) in patients receiving axi-cel versus SOC (8.3 vs. 2 months), mPFS (14.7 vs. 3.7 months), and mOS (not reached vs. 31.1 months; ref. 35).

Tisa-cel is an anti-CD19 CAR-T with a 4-1BB costimulatory domain. In the phase II JULIET study, tisa-cel demonstrated an ORR, a CRR, and an mOS of 52%, 40%, and 8.3 months, respectively (29), with similar results in the long-term JULIET analysis (15). In BELINDA, a phase III trial, tisa-cel was found not to be superior to SOC, with both groups showing an mEFS of 3 months (36).

Liso-cel is an anti-CD19 CAR-T with a 4-1BB costimulatory domain, which, in the phase II TRANSCEND trial, achieved an ORR, a CRR, and a mPFS of 73%, 53%, and 6.8 months, respectively (37). An updated analysis of the BELINDA study at 2 year follow-up showed similar response rates, a median response duration of 26.1 months, and an mOS of 27.3 months (38). In the phase III TRANSFORM study, liso-cel, as compared with SOC, showed improvements in EFS (not reached vs. 2.4 months), CRR (74% vs. 34%), mOS (not reached vs. 29.9 months), and mPFS (not reached vs. 6.2 months; ref. 39).

All three randomized trials conducted to compare the effect of CAR-T on salvage chemotherapy followed by ASCT in patients with primary refractory or early relapsing large B-cell lymphoma (LBCL; ZUMA-7, BELINDA, and TRANSFORM) had similar objectives but yielded discordant results (35, 36, 39). Acknowledging the limitations of cross-trial comparisons, differences in trial design, definitions of EFS, CAR-T manufacturing timelines, and participant heterogeneity may have contributed to these discrepancies. The BELINDA and TRANSFORM studies permitted bridging chemotherapy, whereas the ZUMA-7 study permitted only steroids, potentially introducing selection bias for less aggressive lymphoma in the ZUMA-7 cohort. In the two studies that permitted crossover, BELINDA and TRANSFORM, the former allowed crossover only after progressive disease following two lines of salvage therapy, whereas the latter required progressive disease after only one line of salvage chemotherapy, potentially introducing a selection bias of more heavily pretreated patients in the CAR-T arm of BELINDA. Regarding the intertrial variability in defining EFS, the BELINDA study was the only trial that did not include the initiation of new lymphoma therapy as an event, and all three trials assigned a different response assessment time point as event-defining. Although the TRANSFORM study did not report the median time from randomization to CAR-T infusion, there was a marked difference in the ZUMA-7 study compared with the BELINDA study (median, 29 vs. 52 days, respectively). The CAR-T manufacturing challenges in the BELINDA study may have led to participants with higher tumor burden and declining functional status at the time of CAR-T infusion, two clinical characteristics associated with lower CAR-T efficacy (40–42).

Dynamics of CD19 Expression

Understanding the effects of CD19-directed therapeutic modalities on CD19 expression is important for developing rational treatment strategies across therapeutic lines. However, the level of tumor cell CD19 expression required for the clinical response to CD19-directed therapy is unclear and may differ among drugs. Furthermore, data on clinical activity in R/R DLBCL using sequential CD19-directed therapies are limited because several pivotal trials leading to regulatory approval excluded patients who had received prior CD19-directed therapy (23, 29, 33, 35, 43).

CD19 detection and expression level assessment

DLBCL treatment guidelines recommend testing for CD19 expression as part of the diagnostic process (8, 44). The two methods routinely used in clinical practice to assess CD19 include immunohistochemistry (IHC) and flow cytometry. Although valuable for initial screening, the applicability of these methods for monitoring CD19 expression after CD19-directed therapy and guiding subsequent treatment strategies is unclear (45–47).

A small, growing body of literature, including retrospective studies and subset analyses from three prospective studies, indicates that pretreatment CD19 expression is not strongly correlated with the efficacy of various CD19-directed therapies (48). Retrospective studies evaluating CD19 expression levels, as assessed by

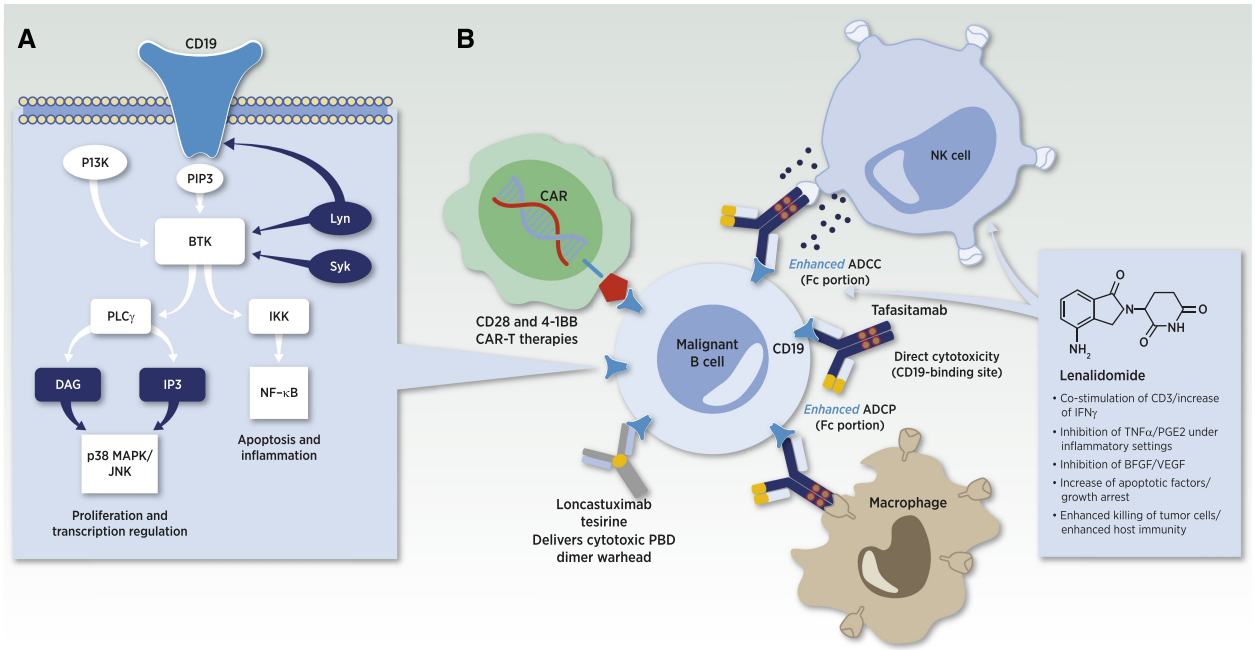

Figure 1.

Mechanisms of action in lymphoma. **A,** CD19 signaling pathway. **B,** Interaction of treatments with a lymphoma cell. ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; NK, natural killer; PBD, pyrrolobenzodiazepine.

quantitative immunofluorescence analysis and IHC, have broadly shown that the ORR of CD19-directed therapies is independent of baseline CD19 expression, irrespective of the CD19-directed treatment modality, including CAR-T. In the phase II JULIET and ZUMA-2 trials, CD19 expression was assessed using quantitative immunofluorescence and IHC, respectively; these studies showed equivalent ORRs in patients with unequivocal CD19 expression and those with pretreatment low/negative CD19 expression (29, 49). Similarly, a phase II LOTIS-2 study evaluated CD19 expression (method not described) and reported that responses to loncastuximab tesirine were observed at all levels of CD19 expression in patients (50).

IHC and immunofluorescence are less sensitive and quantitative than conventional flow cytometry analysis, which offers a larger linear dynamic range for the determination of CD19 antigen expression than IHC (51, 52). A small single-center study found that a quantitative analysis of CD19 expression using flow cytometry did not identify a correlation between CD19 expression prior to CD19 CAR-T and outcomes (48). More studies are needed to verify these findings. However, flow cytometry is limited to liquid biopsies and is not routinely used for solid tissue diagnostics, such as lymphoma specimens, as the majority of diagnoses are performed on formalin-fixed, paraffin-embedded blocks. Additionally, these methodologies are difficult to clinically validate and are not routinely available in clinical laboratories.

In the context of CAR-T–cell responses, a limited number of preclinical studies have shown that the activity of CAR-T cells is dependent on target antigen density. CD19 antigen density can be specifically measured using calibration beads for flow cytometry with known fluorescent molecules per bead, as well as the number of fluorescent molecules conjugated to each antibody. For instance, CD19–CD28ζ CAR-T–cell activity was found to be highly dependent on CD19 antigen density (53). Of note, in preclinical models, CD19–CD28ζ CARs were more efficient at targeting CD19-low tumor cells than CD19-4-1BBζ CARs (53).

Treatment impact on expression levels

Changes between pretreatment and posttreatment CD19 expression in tumor biopsies may offer further insight into the dynamics of CD19 expression following CD19-directed therapy. A small retrospective exploratory analysis of the phase II L-MIND study used IHC, whole exome DNA, and exome RNA sequencing to quantify pretreatment and posttreatment CD19 expression and mutations. Five of six patients treated with tafasitamab retained comparable CD19 expression at both time points, with no evidence of *CD19* genomic alterations. Of the six patients, three experienced an objective response (54). Similar data were reported for tafasitamab in an analysis of patients with R/R chronic lymphocytic leukemia, in which CD19 expression was maintained pretreatment and posttreatment (55, 56). A small single-center study examining patients who received axi-cel found that 10 of the 16 patients with LBCL who developed progressive disease after treatment with axi-cel had no or low CD19 expression (48). However, this is not the case for all CD19-targeted therapies in all relevant diseases; for example, DLBCL that relapses after CAR-T can be CD19 negative (48, 57–60).

A case series that indicates the time point at which a tumor biopsy is collected following CD19-directed therapy may confound accurate assessment of posttreatment CD19 expression through temporary antigen masking (61). Two patients with $CD19^{+}$ non-Hodgkin lymphoma (NHL) treated with tafasitamab underwent two posttreatment tumor biopsies within 1 and 3 to 4 weeks following the final tafasitamab dose. Interestingly, CD19 expression by IHC and flow cytometry was negative in the immediate

Table 1. Summary of approved CD19-directed therapies for R/R DLBCL.[a]

Regimen	Mechanism of action	Trial	Trial design	Cancer types (*n*)	Median follow-up, months	ORR, %	CRR, %	Median PFS or EFS, months	Median DOR in patients with response, months	Median OS, months
Axicabtagene ciloleucel	Anti-CD19 CAR-T + CD28 CS domain	ZUMA-1 (33)	Phase I/II	DLBCL (77), PMBCL (8), and TFL (16)	15.4	82	54	5.8 (PFS)	NR	NR
		ZUMA-1, extended follow-up (110)	Phase I/II, extended follow-up	DLBCL (77), PMBCL (8), and TFL (16)	63.1	83	58	5.9 (PFS)	62.2	25.8
		ZUMA-7 (35)	Phase III, axi-cel vs. SOC (chemoimmunotherapy followed by ASCT) as second-line therapy for R/R LBCL	DLBCL (126), HGBCL (31), not confirmed (18), and other (5)	24.9	83	65	14.7 (PFS) and 8.3 (EFS)	26.9	NR
		ZUMA-7, extended follow-up (14)	Phase III, axi-cel vs. SOC (chemotherapy + ASCT) as second-line therapy for R/R LBCL, extended follow-up	DLBCL (126), HGBCL (31), not confirmed (18), and other (5)	47.2	83	61	14.7 (PFS) and 10.8 (EFS)	41.7	NR
Tisagenlecleucel	Anti-CD19 CAR-T + 4-1BB CS domain	JULIET (29)	Phase II	DLBCL (88), TFL (21), and other (2)	14	52	40	Not reported	NR	8.3
		JULIET, extended follow-up (15)	Phase II, extended follow-up	DLBCL (92), HGBCL (17), and TFL (21)	40.3	53	39	2.9 (PFS) and 2.8 (EFS)	Not estimable	11.1
		BELINDA (36)	Phase III, tisa-cel vs. SOC as second-line therapy for R/R aggressive B-cell lymphoma	DLBCL (101), HGBCL (39), PMBCL (12) FL (5), and other (5)	10	46.30	28.40	3 (EFS)	Not reported	Not estimable
Lisocabtagene maraleucel	Anti-CD19 CAR-T + 4-1BB CS domain	TRANSCEND NHL 001 (37)	Phase I/II	DLBCL (215), HGBCL (36), PMBCL (15) and FL (3)	18.8	73	53	6.8 (PFS)	NR	21.1
		TRANSCEND, extended follow-up (38)	Phase I/II, extended follow-up	DLBCL (276), HGBCL (44), PMBCL (21), and FL (4)	24	73	53	6.8 (PFS)	26.1	27.3
		TRANSFORM (39)	Phase III, liso-cel vs. SOC (chemotherapy + ASCT) as second-line therapy for R/R LBCL, extended follow-up	DLBCL (66), HGBCL (22), PMBCL (8), THRBCL (1), and FL (1)	17.5	87	74	NR (EFS and PFS)	NR	29.9
Tafasitamab + lenalidomide	Anti–CD19-induced ADCC and ADCP	L-MIND (23)	Phase II	DLBCL (81)	13.2	60	43	12.1 (PFS)	21.7	NR
		L-MIND, extended follow-up (13)	Phase II, extended follow-up	DLBCL (81)	44	57.50	41.20	11.6 (PFS)	NR	33.5
Loncastuximab tesirine	Anti-CD19 ADC	LOTIS-2 (26)	Phase II	DLBCL (127), HGBCL (11), and PMBCL (7)	7.8	48.30	24	4.9 (PFS)	10.3	9.9
		LOTIS-2, extended follow-up (27)	Phase II, extended follow-up	DLBCL (127), HGBCL (11), and PMBCL (7)	7.8	48	25	4.9 (PFS)	NR	9.5

ADCC, antibody-dependent cell-mediated cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; CS, costimulatory; DOR, duration of response; FL, follicular lymphoma; HGBCL, high-grade B-cell lymphoma; NR, not reached; PMBCL, primary mediastinal large B-cell lymphoma; TFL, transformed follicular lymphoma; THRBCL, T-cell/histiocyte-rich large B-cell lymphoma.

[a]This summary is provided for ease of reference to the applicable studies only. The populations and methodology are substantially different in each study, and outcomes should not be directly compared.

posttreatment biopsy but was detected on the subsequent biopsy obtained at a later time point. These patients proceeded with CD19-directed CAR-T in later therapy lines. In this case, the half-life of tafasitamab may have resulted in persistent binding to CD19 at the time of the 1-week posttreatment biopsy, obscuring CD19 detection. To mitigate potential CD19 antigen masking, a suitable washout period may prove valuable for determining an accurate CD19 expression status following progression on CD19-directed therapy.

Although the data are intriguing, these studies included heterogeneous populations, used different mechanisms of assessing CD19 expression levels, lacked a uniform definition of CD19 positivity, and assessed posttreatment CD19 levels at varying time points. Further studies are required to determine the most clinically relevant mechanism and time points for assessing CD19 expression, particularly following CAR-T, because this area has not been adequately explored.

CD19 Expression following CAR-T-Cell Therapy Relapse

Although approximately 40% of patients experience a durable response to CAR-T, the remaining patients require subsequent lymphoma-directed treatment. Outcomes in the CAR-T refractory setting are poor, with mPFS and mOS of 2.8 and 5 to 9 months, respectively (62, 63). Well-established guidelines for optimal salvage treatment strategies are necessary.

Antigen escape

Loss of CD19 expression after CD19-directed CAR-T occurs in approximately 30% of patients with DLBCL (48, 57–59) and is an important cause of therapeutic failure that would affect subsequent CD19-directed therapy. In a small study, 9 of 15 patients whose disease progressed following CAR-T had decreased surface CD19 levels, as measured using quantitative flow cytometry, and five of those nine patients had no detectable surface CD19 expression (<3,000 molecules per cell; ref. 48). The current understanding of CD19-directed antigen escape mechanisms is largely derived from studies in patients with B-cell acute lymphoblastic leukemia undergoing CAR-T and includes CD19 mutation(s) and splice variants and defective trafficking of CD19 to the cell surface membrane. CD19 mutations or splice variations (e.g., in exon 2 of the *CD19* gene) can lead to a conformational change and loss of the CAR-T–cell CD19 epitope (64). Epitope mapping of the CD19 extracellular domain confirmed that the antigen-recognition domains of CAR-T cells (based on the FMC63 antibody) and tafasitamab (based on the 4G7 antibody) have partially overlapping conformationally sensitive epitopes dependent on spatially adjacent loops coded by exons 3 and 4, and splice variations in exon 2 could lead to epitope loss for CAR-T and tafasitamab (65, 66). Defective trafficking of CD19 to the cell surface as an antigen escape mechanism was reported in a patient with a CD19-negative acute lymphoblastic leukemia relapse after treatment with blinatumomab. Molecular evaluation showed the absence of CD81, a protein that regulates CD19 protein maturation and cell surface trafficking (67).

CD19-independent mechanisms of CAR-T failure

Using various genetic techniques, several groups have demonstrated that tumor features before CAR-T can help predict therapy outcomes, irrespective of pretreatment CD19 expression or *CD19* genetic alterations. It has been reported that tumors with complex genomics have inferior responses to CD19 CAR-T (68, 69). Additionally, specific mutational patterns, such as those in *RHOA* and *TMEM30A*, were shown to affect outcomes in patients with these tumors (69, 70). Exploring how these features affect CD19-directed antibody therapies requires further investigation.

In addition to antigen escape, other mechanisms such as immunosuppression by the tumor microenvironment (TME), tumor metabolic volume at treatment, and intrinsic CAR-T–cell dysfunction have been shown to be involved in CAR-T failure in LBCL (71). Similar to patient endogenous T cells, CAR-T cells can also exhibit exhaustion, as measured by increased coinhibitory receptors and metabolic dysfunction (72). A CD8 T-cell exhaustion signature, as measured by single-cell RNA sequencing on CAR-T cells 7 days postadministration, was associated with a poor outcome in a study of 24 patients (73). Additionally, a CD8 memory phenotype in the infused CAR-T product was associated with superior outcomes. T-cell exhaustion has been proposed to account for the modest effect (27% ORR) of pembrolizumab, a programmed death-1 inhibitor, in a small prospective study of patients with DLBCL in whom CAR-T had failed (74). Additionally, the TME contains suppressive immune cell subsets such as regulatory T cells and myeloid-derived suppressor cells that can inhibit infiltrating CAR-T–cell function (75, 76) through nutrient and metabolite modulation as well as growth factor sequestration. T-cell metabolism and attempts to manufacture CAR-T cells to function in this inhospitable environment are beyond the scope of this publication but have been examined in several other reviews (77–81).

CD19-directed therapy post–CAR-T relapse

CAR-T–cell persistence, T-cell exhaustion, and an immunosuppressive TME are likely prominent drivers of CAR-T failure, and limited data indicate that antigen escape is a less common etiology (82). Thus, subsequent CD19-directed therapy with an alternative mechanism of action is a rational approach for patients whose disease relapses after CAR-T. Data on the clinical utility of CD19-directed therapy for R/R DLBCL in the post–CAR-T setting are limited and primarily derived from real-world retrospective studies. A retrospective analysis of patients with CAR-T–refractory B-cell NHL treated with tafasitamab plus lenalidomide after CAR-T progression reported ORRs of 33% and 17% in the first- ($n = 6$) and second-line ($n = 6$) post–CAR-T treatment settings, respectively (63). A multicenter observational study reported an ORR of 17% and 10% for tafasitamab plus lenalidomide and loncastuximab tesirine, respectively (60), in patients with post–CAR-T R/R disease. In the LOTIS-2 study, an ORR of 42.9% was reported in 14 patients with post–CAR-T progression who subsequently received loncastuximab tesirine (27). Although these data indicate a potential role for CD19-directed therapy in the post–CAR-T setting, prospective studies with larger cohorts are necessary to guide clinicians.

CD19-Directed Treatment before CAR-T–Cell Therapy

The CAR-T–cell manufacture time is a key consideration for CAR-T administration. Bridging therapy is often necessary (8) and commonly consists of chemotherapy, radiation, steroids, or novel agents. Currently, data on CD19-directed antibodies, or ADCs, as a bridging therapy is lacking.

Limited data evaluating the efficacy of CAR-T in patients previously exposed to CD19-directed antibodies or ADCs are available.

Preclinical evidence from a mouse xenograft model of mantle cell lymphoma indicates that tafasitamab has no detrimental effect on the efficacy of subsequent CAR-T–cell treatment (83). Using a CAR-T–cell construct similar to tisa-cel, mice treated sequentially with tafasitamab followed by CAR-T showed improved tumor control, later and stronger T-cell expansion, and a higher probability of survival than mice treated with saline followed by CAR-T. A second preclinical study indicated that pretreatment with tafasitamab before CD19 CAR-T improved efficacy while attenuating cytokine release syndrome (84). In a case study from the L-MIND trial, a patient whose disease relapsed following treatment with tafasitamab plus lenalidomide subsequently received axi-cel and experienced a CR (85). A retrospective analysis combining patients with R/R DLBCL from the phase I and II studies of loncastuximab tesirine who subsequently proceeded to CD19-directed CAR-T after disease relapse ($n = 14$) reported an ORR of 50% [CR = 6, partial response (PR) = 1] at the 3-month assessment (86). The median time between loncastuximab tesirine treatment and CAR-T was 120 days. Ten patients were evaluated for CD19 expression using IHC, and all showed retention of CD19 expression following loncastuximab tesirine treatment. The four patients with unknown posttreatment CD19 status following loncastuximab tesirine treatment had a CR with CAR-T, indicating that they also retained CD19 expression at progression. The phase III TRANSCEND trial investigating liso-cel in patients with R/R DLBCL included 12 patients with prior CD19-directed therapy [anti–CD19 monoclonal antibodies ($n = 3$), ADCs ($n = 8$), and bispecific T-cell engagers ($n = 1$)] and biopsy-confirmed persistent CD19 expression (87). A *post hoc* analysis of this subset showed that 11 of 12 patients (92%) achieved an objective response, five of whom had a response duration of ≥9 months and four were ongoing at the time of data cutoff.

The half-lives for tafasitamab and loncastuximab tesirine are ~16 (88) and ~21 (89) days, respectively, indicating that only 2% and 5% of antibodies will remain 90 days posttreatment, respectively. In addition to residual binding of CD19 from immediate pre–CAR-T, the recycling and production of new CD19 antigens following cessation of CD19-directed therapy may influence targetable antigen levels. However, these dynamics are not well characterized. There are no data to indicate a washout period between CD19-directed therapy and subsequent CAR-T. Further clinical studies are required to evaluate the optimal timing of sequential CD19-directed therapies.

Patient-Related Factors Affecting CD19-Directed Treatment Selection

Although much focus is placed on disease-related characteristics when selecting lymphoma-directed therapy, patient-related factors have an important role and include performance status, comorbidities, prior treatment, access to cellular therapy centers, psychosocial factors, and patient preference. Although only 50% of patients with R/R DLBCL are eligible for ASCT, the proportion of patients eligible for CAR-T remains undefined because of the lack of consensus regarding patient eligibility criteria (90). Using the ZUMA-1 inclusion/exclusion criteria, a single-center retrospective analysis performed in Sweden found that 49/60 (82%) patients with R/R DLBCL would have been eligible for CAR-T (91). This proportion stands in contrast to real-world findings in a multicenter retrospective analysis of axi-cel used as SOC for R/R LBCL, in which only 57% of 298 patients who received treatment met the ZUMA-1 criteria (42). In this study, 20% of patients did not meet the ZUMA-1 criteria because of an Eastern Cooperative Oncology Group performance score (ECOG PS) of >2, and the rest likely had medical comorbidities or were aged >65 years, which would have excluded them, although these data are not reported. However, despite not meeting the ZUMA-1 criteria, patients receiving axi-cel still experienced comparable safety and efficacy. Another real-world retrospective study found that only 49% of 1,500 patients met the ZUMA-1 criteria (92). In this study, the primary reason for patients not meeting the ZUMA-1 criteria was age >65 years in 38% of patients. An additional study investigating 129 patients whose disease progressed after frontline therapy estimated that 65% of patients would likely be eligible for second-line CAR-T if the trial inclusion criteria were expanded to an ECOG PS ≤2 (93). Considering patients aged >65 years and permitting an ECOG PS ≤2, ~70% to 75% of patients in the R/R DLBCL setting have an ECOG PS of 0 to 1; thus, it is likely that a majority of patients with R/R DLBCL are eligible for CAR-T (94, 95).

ECOG PS is a key prognostic indicator in R/R DLBCL, and notably, patients with an ECOG PS of ≥2 were excluded from the three CAR-T registrational studies. The median age of patients in ZUMA-1, JULIET, and TRANSCEND was <60 years, whereas real-world data show that CAR-T is administered to and has comparable efficacy in patients aged 65 to 74 years, with poorer outcomes in those aged ≥75 years (96, 97). Retrospective real-world experience has also reported poorer outcomes in patients with an ECOG PS of ≥2 (42, 98, 99). For this patient population, CAR-T may not be the optimal CD19-directed therapy. Earlier treatment lines may influence the number and quality of autologous CAR-T cells. In particular, growing evidence suggests prior bendamustine-containing regimens negatively impact T-cell numbers and composition at apheresis and CAR-T–cell expansion, potentially reducing the efficacy of CAR-T–cell treatment (43, 100).

Unequal access to health care and logistical challenges further limit the use of CAR-T. At present, CAR-T is administered at a limited number of academic centers, often necessitating prolonged commutes, hospitalization, and caregiver support for extended periods, which are features that may limit this therapy for a subset of patients (101). Currently, 96.8% of CAR-T cases occur in urban hospitals, and although 19% of patients live in rural areas, only 14.7% of patients receiving CAR-T are from rural areas (102). This disparity is likely because of the need for close access to a cellular therapy center. Although over a third of patients live >2 hours away from a CAR-T center, patients living >60 miles from a CAR-T center were less likely to undergo therapy than those living <60 miles (103). Geographical concerns are also at play; in the United States, patients in the South were less likely to have access to CAR-T than those in the Northeast (OR = 0.284; ref. 104). Income level and insurance status also affect access to CAR-T. Patients with higher income levels are more likely to receive CAR-T, with one study showing that only 7.3% of patients who receive CAR-T come from neighborhoods with a median income of less than $40,000. Similarly, patients receiving CAR-T are less likely to have Medicare or be uninsured, which limits access to treatment because of the high cost of therapy (103).

BsAb therapies are also gaining approval for R/R DLBCL. Although these products are "off-the-shelf" and do not require manufacturing for individual patients, there are limitations to their widespread adoption in clinical practice. Similar to CAR-T, these treatments require hospitalization for administration or step-up dosing, which limits their use in certain settings. However, an ongoing clinical trial is challenging the role of inpatient step-up dosing for epcoritimab (NCT05451810). Although long-term data from CAR-T–cell trials such as ZUMA-1 have shown a flattening in progression-free survival/overall survival curves, indicating that

~40% of patients may be cured, long-term data on BsAbs are lacking. For patients with either disease- or patient-related factors that are less suitable for the logistical and toxicity challenges of CD19 CAR-T or BsAbs, more readily available "off-the-shelf" CD19-directed therapies administered in the outpatient setting, such as tafasitamab plus lenalidomide and loncastuximab tesirine, represent an alternative approach (105). These regimens often have fewer burdensome side effects and have promising clinical efficacy.

Expert Recommendations to Support Therapeutic Decision Making

As approved indications for CD19-directed therapies expand, clinicians will be increasingly faced with the dilemma of optimal use. Several factors influence optimal treatment sequencing. For patients with a suitable degree of fitness and access to limited centers that provide immune effector cell therapy, CAR-T will likely be the initial CD19-directed therapy. For more frail patients, those with rapidly progressive disease, or those without access to tertiary referral centers, monoclonal antibodies, or ADCs, may be the initial CD19-directed therapy.

Defining clinically relevant thresholds and sensitive CD19 expression level quantification methodologies will be paramount for optimizing sequencing. Given the limitations of routinely available CD19 assays and the potential for antigen masking or reductions in CD19 expression following CD19-directed therapy, a suitable washout period may be indicated to obtain an accurate posttreatment disease phenotype. However, no guidelines are available to define the appropriate timeline for a treatment washout, and the urgency of treatment due to patients' disease characteristics may be the primary driver of subsequent treatment selection.

Genomic classification of DLBCL has shown utility in predicting responses to R-CHOP chemotherapy and may have utility in predicting responses to other targeted therapies (106–109). However, these genetic subgroups have not shown utility in predicting outcomes with CD19-directed therapies, and novel predictors for CD19-directed therapies are needed.

In patients whose disease progresses after CAR-T, responses to currently available treatments are suboptimal, and enrollment in a clinical trial is recommended. As approximately 30% of patients with DLBCL experience relapse with CD19-negative disease following CAR-T (57–59), reassessment of CD19 expression should be strongly considered to inform subsequent treatment strategies. Although retrospective data indicate that CD19-directed therapy may offer clinical benefit in a subset of patients following progression after CAR-T with retained CD19 expression (26, 60, 63), prospective studies are needed.

Limited clinical data indicate that CD19-directed therapies do not preclude the future successful application of CAR-T (54, 85–87). There are insufficient data to recommend the use of CD19-directed therapy as a bridging therapy for CAR-T.

In summary, there are limited clinical data to guide definitive decision-making for sequencing CD19-directed therapies in patients with R/R DLBCL. A sufficiently powered and appropriately designed clinical or real-world study, with the involvement of both clinical institutions and pharmaceutical companies, would substantially aid in the determination of the effectiveness of subsequent CAR-T in patients with R/R DLBCL who received prior CD19-directed therapy. For ongoing and future clinical studies, the collection of sequential CD19 expression data for correlation with clinical responses and the likelihood of responses to sequential CD19-directed therapy are urgently required to inform further decision-making to best benefit patients with DLBCL.

Authors' Disclosures

R. Birhiray reports personal fees from Incyte, MorphoSys, GSK, Pharmacyclics, Janssen, AbbVie, BeiGene, Lilly Oncology, Exelixis, Puma, and ADC Therapeutics outside the submitted work. A. Merchant reports personal fees from AbbVie, Novartis, and Oncovalent outside the submitted work. No disclosures were reported by the other authors.

Acknowledgments

Medical writing and editorial support for the development of this article were provided by Stuart Langley of Syneos Health, UK, and funded by MorphoSys AG, in accordance with Good Publication Practice. MorphoSys AG conducted a courtesy review for the scientific accuracy of the manuscript before submission but had no role in the development of the content, selection of the journal for submission, or the decision to submit the manuscript for publication.

Received July 17, 2023; revised December 1, 2023; accepted March 28, 2024; published first April 25, 2024.

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