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REVIEW ARTICLE

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Biomarkers of outcome in patients undergoing CD19 CAR-T therapy for large B cell lymphoma

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Abstract

CD19-directed autologous chimeric antigen receptor T cell (CAR-T) therapy has transformed the management of relapsed/refractory (R/R) large B cell lymphoma (LBCL). Initially approved in the third line and beyond setting, CAR-T is now standard of care (SOC) for second-line treatment in patients with refractory disease or early relapse (progression within 12 months) following primary chemoimmunotherapy. Despite becoming SOC, most patients do not achieve complete response, and long-term cure is only observed in approximately 40% of patients. Accordingly, there is an urgent need to better understand the mechanisms of treatment failure and to identify patients that are unlikely to benefit from SOC CAR-T. The field needs robust biomarkers to predict treatment outcome, as better understanding of prognostic factors and mechanisms of resistance can inform on the design of novel treatment approaches for patients predicted to respond poorly to SOC CAR-T. This review aims to provide a comprehensive overview of clinical, molecular, imaging, and cellular features that have been shown to influence outcomes of CAR-T therapy in patients with R/R LBCL.

INTRODUCTION

Chimeric antigen receptor T cell (CAR-T) therapy has established a new gold standard for the management of refractory or relapsed (R/R) large B cell lymphoma (LBCL). Three CAR-T products, axicabtagene ciloleucel (axi-cel), tisagenlecleucel (tisa-cel), and lisocabtagene maraleucel (liso-cel), were approved based on demonstrated benefit in multiply relapsed patients in third line and beyond,[1–3] and since then, axi-cel (ZUMA-7) and liso-cel (TRANSFORM) have established superiority over salvage chemotherapy followed by autologous stem cell transplant (ASCT) in the second-line setting.[4,5] Real-world data (RWD) have reinforced these findings with comparable effectiveness as those observed in randomized clinical trials (RCTs).[6–13]

Although 80%–90% of patients have an initial response to therapy, only about 40% of patients achieve long-term disease-free survival, with the majority of patients relapsing within 12 months of therapy.[14–17] Accordingly, there is an urgent need to understand the mechanisms underlying resistance and to identify prognostic biomarkers predictive of response and CAR-T failure. Defining predictive biomarkers will pave the way to developing treatment strategies to optimize CAR-T responses that are more personalized to the patient and could aid the selection of alternative approaches. The CAR-T literature is rapidly expanding with a plethora of studies ongoing or in development to address this important question.

Despite the rapidly growing research in this area, there are several important limitations in identifying and integrating biomarkers into clinical practice that require careful consideration.[18] Challenges include small sample sizes in study design, limiting statistical power to detect associations. Biomarker studies may lack generalizability to broader patient populations depending on the derivation cohort; while clinical trials limit representation of real-world patients due to strict inclusion/exclusion criteria, retrospective studies may be influenced by selection bias. Biomarker analysis is often therapy specific and may not apply across different treatment platforms. Additionally, biomarker identification involves complex assays or imaging techniques that are costly and technically challenging. Lastly, single biomarkers may not fully capture disease complexity or predict treatment response accurately, necessitating a multidimensional approach with multiple biomarkers to improve predictive accuracy. Despite the potential to increase predictive value, advanced multidimensional approaches are challenging to implement in real time to support clinical decision-making processes.

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With these limitations in mind, we aim to summarize key biomarkers relevant to response with an understanding that certain biomarkers may be prognostic to all therapies, while others may be predictive to CAR-T (and potentially more specific to cellular immunotherapy) from existing studies. Additionally, we discuss limitations that prohibit their immediate implementation into clinical practice and present a potential path forward (visual summary shown in Figure 1).

OVERVIEW OF CD19-DIRECTED CAR-T FOR LBCL

A comprehensive review of the efficacy and toxicity data for the three CAR-T products is beyond the scope of this review; however, a summary is provided here. The efficacy of three autologous CD19-directed CAR-T therapies was established through phase II trials conducted in the third-line setting, leading to their regulatory approval: axi-cel (CD28 co-stimulation; ZUMA-1), tisa-cel (4-1BB co-stimulation; JULIET), and liso-cel (4-1BB co-stimulation and a 1:1/CD4:CD8 T cell ratio; TRANSCEND).[2,3,19–24] In these trials, durable remission was observed in one-third of patients, a scenario where previously no effective therapeutic options existed. Updated data from ZUMA-1 and TRANSCEND demonstrate sustained durability, with 5-year progression-free survival (PFS) of 51% in ZUMA-1 and 2-year PFS of 41% in TRANSCEND.[25,26] Subsequently, all three products underwent evaluation in large phase III RCTs, comparing their efficacy to that of ASCT in a randomized manner. While axi-cel[24] and liso-cel[23] met their primary endpoint of event-free survival (EFS) (with a survival advantage seen with axi-cel), tisa-cel (BELINDA)[27] did not. As a result, both axi-cel and liso-cel have gained regulatory approval for use in the second-line setting in patients who relapse within 12 months of frontline therapy. Variations in the BELINDA study design may have contributed to the discordant EFS results, including the allowance of bridging chemotherapy, an increased duration from randomization to CAR-T infusion due to manufacturing logistics, and the inclusion of a higher proportion of patients with high-risk characteristics.[27] In terms of toxicity, allowing for cross trial comparison, axi-cel had higher rates of severe grade ≥3 cytokine release syndrome (CRS) (6%) compared to the 1% observed with liso-cel. Similarly, serious neurological events were reported in 21% of patients receiving axi-cel, in contrast to 4% for liso-cel, a finding corroborated in RWD.[6,28] These increased toxicity rates are likely associated with the faster rates of CAR-T expansion, a property linked to signaling through the CD28 co-stimulatory domain.[4] Nevertheless, it is important to note that direct head-to-head comparisons between the CAR-T products are lacking, and their comparative effectiveness remains untested.

Besides variations in trial design that may explain differences in efficacy, the three CAR-T products vary in several aspects, including the CAR construct, the viral vector for transduction, the manufacturing process, and the dosage. These attributes, alongside the quality of the collected T cells, are likely significant contributors to efficacy and toxicity, alongside patient and disease-related factors. Thus, comparing the phenotypic composition of CAR-T cells and conducting biomarker analysis directly across the three products is crucial to understand whether such distinct characteristics are accountable for their response.

PATHOLOGICAL FEATURES AND MOLECULAR MARKERS

Conventional tumor-related prognostic features associated with inferior outcomes in the frontline setting include cell of origin (COO), double- or triple-hit rearrangements, that is, high-grade B cell lymphoma (HGBL), and dual expressor status. However, these factors have not retained prognostic value in patients treated with CAR-T and appeared to have derived similar benefit in subgroup analyses of landmark RCTs,[2,3,29–32] findings corroborated in subsequent RWD.[12,33–36] Although it may be possible that CAR-T may overcome these adverse factors, it is important to acknowledge that the existing

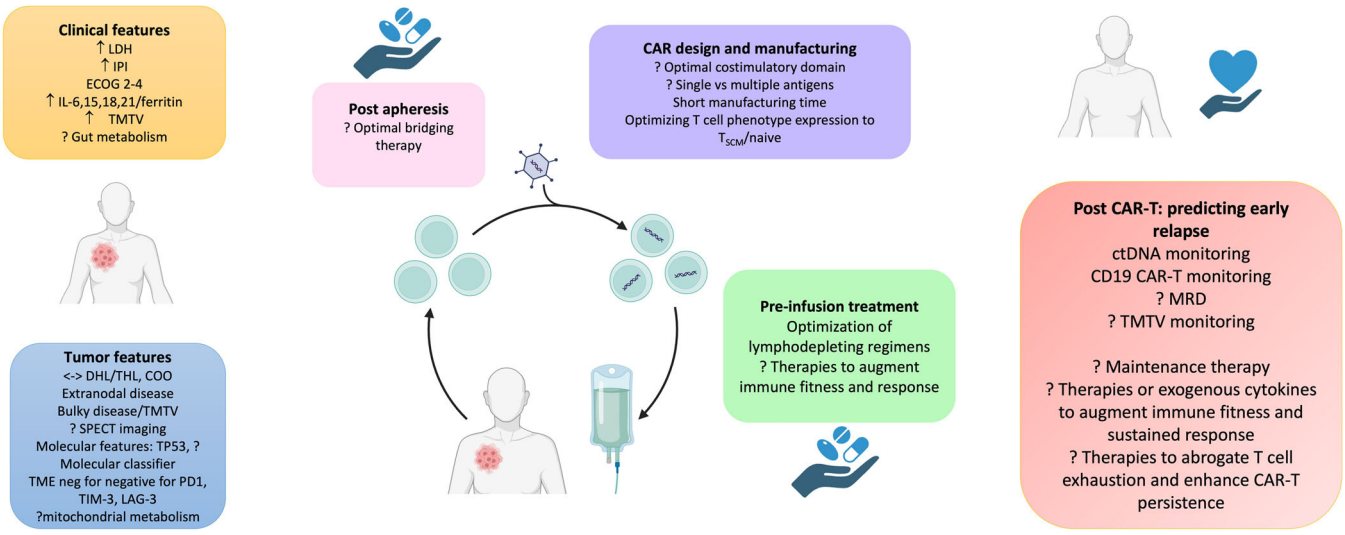

FIGURE 1 The efficacy of chimeric antigen T cell receptor therapy (CAR-T) for large B cell lymphoma is influenced by pre- and postinfusion biomarkers. Despite the revolutionary advancement, the optimal therapeutic approach for various stages of CAR-T therapy, such as postapheresis bridging therapy, CAR manufacturing, and postinfusion maintenance/consolidation therapy, remains to be determined to enhance current responses and long-term outcomes. Image created with https://www.BioRender.com. COO, cell of origin; ctDNA, circulating tumor DNA; DHL, double-hit lymphoma; ECOG, Eastern Cooperative Oncology Group; IL, interleukin; IPI, International Prognostic Index; LAG-3, lymphocyte activation gene 3; LDH, lactate dehydrogenase; MRD, minimal residual disease; PD1, programmed cell death protein 1; SPECT, single-photon emission computed tomography; THL, triple-hit lymphoma; TIM-3, T cell immunoglobulin and mucin-domain containing-3; TME, tumor microenvironment; TMTV, tumor metabolic tumor volume; T_{SCM}, T stem cell memory subset.

data have several limitations. While there's limited statistical power to identify variations in the subgroup analysis of landmark RCTs, retrospective RWD is limited by selection bias. In this case, patients who ultimately receive the cell products may already be predisposed to more favorable outcomes, regardless of their pathological characteristics.

Recent molecular analyses reported that TP53 alterations were predictive of inferior outcomes to CAR-T (Table 1).[50,51,56] Furthermore, Shouval et al. showed that patients harboring TP53 mutations who received a CAR-T product with the 4-1BB co-stimulatory domain had inferior PFS and overall survival (OS) as compared to those with a CD28 co-stimulatory domain (1-year PFS 10% vs. 34% and 1-year OS 36% vs. 51%).[32] Other genetic alterations that have been associated with CAR-T resistance include APOBEC mutational signatures, genomic damage from reactive oxygen species, and recurrent chromosomal deletion of the RHOA tumor suppressor.[52] Finally, while genomic classifiers have identified distinct genetic subtypes of LBCL,[57] whether these classifiers are prognostic of CAR-T response is unknown.

The application of many of these tests remain limited in routine clinical practice, even in major academic institutions. The assessment of COO continues to be commonly conducted through surrogate methods using immunohistochemistry. Additionally, basic genomic analyses are frequently performed retrospectively rather than in real time for clinical application.

Nevertheless, the above findings would benefit from additional validation in larger studies as RCTs were not powered to detect subgroup differences, and existing RWD were limited by sample size. If significant differences in outcomes are identified in well-validated series, it may lead to clinical utility as decisions around patient management may be made. Studies are underway to investigate whether patients with high likelihood of treatment failure with standard chemoimmunotherapy (even using simple clinical tools such as the IPI; ZUMA-23 NCT05605899) may derive benefit from frontline CAR-T therapy at the outset.

PATIENT AND LABORATORY FACTORS

Clinical factors predictive of response have been studied in pivotal trials and RWD (Table 1). However, patient enrollment in RCTs is selective and homogenous, limiting the identification of demographic variables and comorbidities predictive of CAR-T outcomes. Accordingly, RWD are crucial to inform clinical factors associated with CAR-T response as these studies expanded the patient population who could receive CAR-T.[6–13]

While age is a major limitation to the broad application of ASCT, this may not be the case for CAR-T therapy. Outcomes of CAR-T in older patients, defined as those ≥65 years, have been evaluated in trials and RWD, which suggests no association between older age and inferior outcomes.[9,15,58–60] Furthermore, the recent report by Berning et al. reported no significant difference in nonrelapse mortality between patients <70 and those >70 years old undergoing CAR-T therapy.[39] To the contrary, one study reported that younger than (<60 years old) had worse outcomes,[12] which was further corroborated by a recent study by the Center for International and Marrow Transplant Research, indicating that patients ≥65 years old age who received axi-cel had higher response rate than their younger counterparts.[61] The biological basis for this is unclear and may be related to selection bias (in which older patients with higher risk disease may not be selected for CAR-T treatment). This observation, though interesting, is counterintuitive and requires further confirmatory analysis across the various CAR-T platforms.

Performance status has been a well-established predictor of poor outcomes, although it may reflect features driven by lymphoma or other noncancer-related comorbidities. Baseline Eastern Cooperative Oncology Group (ECOG) performance status ≥2 has been consistently shown as an independent predictor of inferior outcomes across several RWD reports, including the largest RWD analysis to date by Jacobson and colleagues,[10,12,33,38] highlighting the importance of functional status assessments during CAR-T decision-making process.[12,40] Although specific comorbidities associated with inferior outcomes could not be determined from pivotal trials, recent RWD analyses suggest their prognostic impact. Hepatic, renal, and respiratory diseases have been associated with inferior response to CAR-T.[9,62] A Cumulative Illness Rating Scale (CIRS) comorbidity score of ≥7 has been reported to be independently associated with OS.[38] In the largest RWD reporting on comorbidity, Shouse et al. developed a novel model based on CIRS components (Severe4), which was predictive of PFS and OS.[40] Although management decisions are not likely to be altered based on comorbidity indices at present, they do inform discussions with patients.

Lactate dehydrogenase (LDH) measured at therapy selection, apheresis, and pre-lymphodepletion has been shown to be a negative predictor of CAR-T response across RCTs and RWD.[37,41–43,63,64] However, pre-lymphodepleting LDH may be more important than LDH measurement at apheresis,[2,12] underscoring the importance of obtaining serial measurements to ascertain the timepoint(s) that are most relevant to inform outcomes. The reason for the association of LDH with outcomes may be twofold, reflecting high tumor burden and proliferative activity, and an immunosuppressive tumor microenvironment (TME) that inhibits CAR-T function.[65]

Preinfusion inflammation has emerged as a key determinant of CAR-T resistance across both trial and real-world settings. An in-depth analysis of correlative samples from the ZUMA-1 trial by Locke et al. found that the presence of elevated cytokines and suppressive myeloid cells (expression of monocytic myeloid-derived suppressor cells) was associated with reduced CAR-T cell expansion and lower efficacy.[63] Routinely available proinflammatory markers associated with poor outcomes include c-reactive protein (CRP), ferritin, and albumin.[3,9,33,37,63,66]

Taken together, inflammation, ECOG performance status (>=2), elevated LDH, and comorbidity burden are well-described risk factors of poor outcomes. There is currently no validated risk score that systematically assesses the relative significance of these variables on CAR-T outcomes. The IPI and the age-adjusted IPI (aaIPI) are two prognostic indices validated for predicting survival outcomes in patients with DLBCL, both in the frontline setting and before ASCT.[67,68] Whether IPI retains prognostic value following CAR-T therapy has not been extensively studied, apart from a small retrospective cohort of 60 patients, whereby it did maintain prognostic value in predicting PFS and OS.[69] Several groups have developed predictive models to evaluate outcomes following CAR-T therapy, such as the CAR-HEMATOTOX and the Endothelial Activation and Stress Index scores. However, these models were designed to predict hematotoxicity, CRS, or immune effector cell-associated neurotoxicity syndrome, rather than predicting efficacy outcomes.[70,71] For instance, while the CAR-HEMATOTOX model, comprising inflammation markers and baseline cytopenias, was predictive of hematotoxicity, the derivation cohort was not significantly associated with PFS or OS,[71] though it was predictive in a subsequent separate cohort.[72] Recently, Faramand et al. developed and validated a model utilizing preinfusion CRP and ferritin to predict efficacy outcomes.[66] Future well-structured large data sets are necessary to develop and validate risk stratification tools utilizing established preinfusion risk factors readily available in routine clinical practice. Such tools would help

TABLE 1 Summary of select studies reporting on association of baseline clinical, imaging, and molecular markers associated with outcomes for patients with large B cell lymphoma treated with CAR-T. (continued on next page) (continued on next page)

Study	Country	Publication year	No. of patients	Median age	CAR-T product	Subtypes of LGBL	ECOG	IPI	Disease progression		Overall survival	
Vercellino et al.[37]	France	2020	116	NR	Axi-cel (n = 49), tisa-cel (n = 67)	DLBCL, PMBCL, TFL	0–2	0–5	LDH at apheresis	HR 2.0 (95% CI 1.2–3.5)		
									EN sites ≥2	HR 2.5 (95% CI 1.4–4.5)	EN sites ≥2	HR 3.6 (95% CI 1.6–8.4)
									CRP	HR 1.1 (95% CI 1.1–1.2)	CRP	HR 1.1 (95% CI 1.1–1.2)
									Pre-LD high TMTV >80 mL	HR 2.2 (95% CI 1.2–3.9)	Pre-LD high TMTV >80 mL	HR 3.4 (95% CI 1.4–8.3)
Nastoupil et al.[12]	USA	2020	209	60	Axi-cel	DLBCL, PMBCL, TFL	0–4	0–5	Pre-LD LDH	HR 1.9 (95% CI 1.3–2.9)	Pre-LD LDH	HR 3.0 (95% CI 1.7–5.4)
									Male	1.8 (95% CI 1.2–2.7)	Male	1.7 (95% CI 1.0–2.7)
									Age <60 years	HR 1.6 (95% CI 1.1–2.3)		
Jacobson et al.[10]	USA	2022	1297	62	Axi-cel	DLBCL, PMBCL, TFL, HGBL	0–2+	-	Age >65 years	HR 0.9 (95% CI 0.7–1.0)	Age >65 years	HR 1.1 (95% CI 0.9–1.3)
									ECOG	HR 2.6 (95% CI 1.9–3.6)	ECOG ≥2	HR 3.3 (95% CI 2.4–4.5)
									LDH at diagnosis	-	LDH at diagnosis	HR 1.4 (95% CI 1.1–2.0)
									Chemo-resistant prior to infusion	HR 1.5 (95% CI 1.2–1.8)	Chemo-resistant prior to infusion	HR 1.4 (95% CI 1.2–1.8)
Kittai et al.[38]	USA	2021	130	63	Axi-cel (n = 94), tisa-cel (n = 36)	DLBCL, HGBL	0–2+	-	Baseline CIRS ≥7 or CIRS-3+	HR 1.7 (95% CI 0.9–3.1)	Baseline CIRS ≥7 or CIRS-3+	HR 2.4 (95% CI 1.1–5.2)
									Age (10 years)	HR 0.9 (95% CI 0.96–1.1)	Age (10 years)	HR 0.8 (95% CI 0.7–1.0)
									ECOG	HR 1.4 (95% CI 1.0–2.1)	ECOG	HR 1.6 (95% CI 1.1–2.5)
Berning et al.[39]	Europe	2024	172	65	Axi-cel (n = 113), tisa-cel (n = 59)	DLBCL, TFL	0–3+	-	Age <70 vs. >70 years	HR 1.0 (95% CI 0.65–1.6)	Age <70 vs. >70 years	HR 1.1 (95% CI 0.67–1.8)
									ECOG 3+	HR 1.8 (95% CI 0.9–3.5)	ECOG	HR 2.7 (95% CI 1.4–5.4)
									Axi-cel vs. tisa-cel	HR 1.6 (1.0–2.5)	Axi-cel vs. tisa-cel	HR 1.2 (0.76–2.0)
Shouse et al.[40]	USA	2023	577 (learning); 218 (validation)	63	Axi-cel (n = 393), tisa-cel (n = 120)	DLBCL, TFL, HGBL	0–4	0–5	Baseline Severe4 score	HR 2.2 (95% CI 1.5–3.0)	Baseline Severe4 score	HR 1.9 (95% CI 1.4–2.8)
Kwon et al.[28]	Spain	2022	307	61	Axi-cel (n = 152), tisa-cel (n = 155)	DLBCL, TFL, HGBL	0–3	0–5	LDH at apheresis	HR 2.2 (95% CI 1.3–3.7)	LDH at apheresis	HR 1.9 (95% CI 1.1–3.0)
									ECOG at apheresis	HR 0.7 (95% CI 0.2–2.6)	Pre-LD LDH	HR 1.3 (95% CI 0.6–3.0)
									ECOG at LD	HR 5.4 (95% CI 2.4–12.6)	LDH at infusion	HR 1.2 (95% CI 0.5–2.9)
											ECOG at apheresis	HR 2.1 (95% CI 1.1–4.0)
											ECOG at LD	HR 4.3 (95% CI 1.8–10.1)

TABLE 1 (Continued)

Study	Country	Publication year	No. of patients	Median age	CAR-T product	Subtypes of LGBL	ECOG	IPI	Disease progression		Overall survival	
Sermer et al.[41]	USA	2020	69	63	Axi-cel (n = 47), tisa-cel (n = 22)	DLBCL, PMBCL, TFL, HGBL	0–2+		Pre-LD LDH	HR 1.7 (95% CI 1.2–2.7)	LDH	HR 2.1 (95% CI 1.4–3.2)
									Tumor bulk >10 cm	HR 1.8 (95% CI 1.2–2.9)	Tumor bulk >10 cm	HR 1.7 (95% CI 1.1–2.7)
Iacoboni et al.[8]	Spain	2021	75	60	Tisa-cel	DLBCL, TFL, HGBL	0–2	0–5	Pre-LD LDH	HR 2.5 (95% CI 1.4–4.4)	LDH	HR 2.8 (95% CI 1.4–5.4)
											ECOG	HR 2.8 (95% CI 1.1–7.1)
Iovino et al.[42]	USA	2022	60	62	Axi-cel	DLBCL, PMBCL, TFL, HGBL	0–4	-	Pre-LD LDH	HR 5.1 (95% CI 1.1–23.9)	Pre-LD LDH	HR 10.5 (95% CI 2.0–55.0)
Hirayama et al.[43]	USA	2019	65	69	NR	DLBCL, TFL, HGBL, mantle cell, follicular	-	0–4	Pre-LD LDH	HR 1.4 (95% CI 1.1–1.6)		
									MCP-1	HR 0.3 (95% CI 0.1–0.9)		
									IL-7	HR 0.9 (95% CI 0.8–1.0)		
Galtier et al.[44]	France	2022	160	63	Axi-cel (n = 95), tisa-cel (n = 65)	DLBCL, PMBCL, TFL	0–4	0–3+	Pre-LD TMTV	HR 2.1 (95% CI 1.2–3.5)	Pre-LD TMTV	HR 4.5 (95% CI 2.5–8.1)
									LDH	HR 1.9 (95% CI 1.0–3.4)	LDH	HR 2.2 (95% CI 1.1–4.4)
									DS 5	9.5 (95% CI 5.0–18.0)	D5	HR 6.8 (95% CI 3.2–14.6)
									Tisa-cel	1.9 (95% CI 1.0–3.3)	Tisa-cel	HR 1.7 (95% CI 0.8–3.3)
									Pre-LD TMTV	HR 2.6 (95% CI 1.2–5.7)		
									Baseline TMTV (≥25 cm^3)	HR 3.4 (95% CI 1.2–10.1)	Baseline TMTV (≥25 cm^3)	HR 6.3 (95% CI 0.8–47.9)
Voltin et al.[45]	Germany	2022	47	61	Axi-cel (n = 4), tisa-cel (n = 43)	DLBCL, TFL, PMBCL, HGBL	0–4	1–5	Pre-LD TMTV	HR 2.6 (95% CI 1.2–5.7)		
Iacoboni et al.[46]	Spain	2021	35	58	Tisa-cel or investigational product	DLBCL, TFL, HGBL	0–2+	0–5	Baseline TMTV (≥25 cm^3)	HR 3.4 (95% CI 1.2–10.1)	Baseline TMTV (≥25 cm^3)	HR 6.3 (95% CI 0.8–47.9)
Bethge et al.[47]	Germany	2022	356	60	Axi-cel (n = 173), tisa-cel (n = 183)	DLBCL, PMBCL, TFL	0–2+	0–3+	Bridging therapy nonresponder	HR 2.3 (95% CI 1.5–3.5)	Bridging nonresponder	HR 2.3 (95% CI 1.3–3.9)
									Pre-LD LDH	HR 1.4 (95% CI 1.1–1.9)	Pre-LD LDH	HR 1.4 (95% CI 1.0–2.0)
									ECOG	HR 1.2 (95% CI 0.99–1.4)	ECOG	HR 1.4 (95% CI 1.1–1.7)
									Tisa-cel	HR 1.5 (95% CI 1.1–1.9)		
									Age	HR 0.9 (95% CI 0.8–0.9)		
Roddie et al.[48]	UK	2023	171	59	Axi-cel (n = 130), tisa-cel (n = 41)	DLBCL, PMBCL, TFL, HGBL	0–1	0–3+	Bridging responder	HR 0.6 (95% CI 0.4–0.9)		
									Pre-LD LDH	HR 2.1 (95% CI 1.3–3.20		
									EN site ≥3	HR 2.5 (95% CI 1.5–4.3)		

TABLE 1 (Continued)

Study	Country	Publication year	No. of patients	Median age	CAR-T product	Subtypes of LGBL	ECOG	IPI	Disease progression		Overall survival	
Dean et al.[49]	Germany	2023	57	59	Axi-cel	DLBCL, PMBCL, HGBL	0–2	-	Pre-LD low TMTV	HR 0.17 (95% CI 0.03–0.71)	Pre-LD TMTV	HR 0.19 (95% CI 0.04–0.80)
									Low ctDNA	HR 0.11 (95% CI 0.04–0.3)	Low ctDNA	HR 0.13 (95% CI 0.04–0.39)
Shi et al.[50]	China	2022	84	49	Axi-cel	DLBCL	0–2+	1–5	TP53	HR 3.0		
Shouval et al.[51]	USA	2021	153	66	Axi-cel (n = 77), tisa-cel (n = 49), liso-cel (n = 27)	DLBCL, TFL, HGBL	-	-	TP53	HR 3.8 (95% CI 1.3–11)	TP53	HR 4.6 (95% 1.6–12.8)
Jain et al.[52]	USA	2022	49	65	Axi-cel (n = 49), tisa-cel (n = 2), liso-cel (n = 2)	DLBCL, TFL, CLL	0–3	1–5	Genomic drivers	HR 4.3 (95% CI 1.1–14.2)		
									TMTV	0.76 (0.3–1.8)		
Locke et al.[53]	Global	2024	175	58	Axi-cel	DLBLC, HGBL	0–2	0–3+	Baseline TMTV based on threshold determined through exploratory analysis	2.0 (HR 1.2–3.6)		
									IPI 2–3 vs. 0–1	1.2 (0.54–2.6)		
									Age 65+ vs. <65 years	0.77 (HR 0.54–1.2)		
									Baseline LDH	0.89 (HR 0.40–2.0)		
Frank et al.[54]	USA	2021	72	62	Axi-cel	DLBCL, PMBCL, TFL, HGBL	-	-	Pre-LD ctDNA	HR 5.4 (95% CI 1.8–16.7)		
Iacoboni et al.[55]	Spain	2023	439	61	Axi-cel (n = 254), tisa-cel (n = 185)	DLBCL, PMBCL, TFL, HGBL	-	0–5	Bendamustine within 9 mns	HR 1.8 (95% CI 1.2–2.7)	Bendamustine within 9 mns	HR 2.1 (95% CI 1.3–3.4)

Abbreviations: CAR-T, chimeric antigen T cell receptor therapy; CI, confidence interval; CRP, c-reactive protein; ctDNA, circulating tumor DNA; DLBCL, diffuse large B cell lymphoma; DS, Deauville score; ECOG, Eastern Cooperative Oncology Group; EN, extranodal; HGBL, high-grade B cell lymphoma including double hit/triple hit; HR, hazard ratio; IPI, International Prognostic Index; LD, lymphodepletion; LDH, lactate dehydrogenase; OS, overall survival; PFS, progression-free survival; PMBCL, primary mediastinal B cell lymphoma; TFL, transformed follicular lymphoma or other indolent lymphoma; TLG, total lesion glycolysis; TMTV, total metabolic tumor volume.

guide decision-making processes, including optimizing disease burden with bridging therapy (BT) before CAR-T infusion (details discussed below).

While postinfusion CRS and neurotoxicity were initially proposed to be correlated with CAR-T expansion and efficacy, subsequent reports have not shown a relationship between CRS severity and outcomes.[28,33,73] Moreover, the use of tocilizumab and corticosteroids has not been associated with inferior outcomes across trials and RWD.[3,28,63]

IMAGING PARAMETERS

While response assessment using imaging is the standard of care in LBCL, advanced quantitative imaging metrics (radiomics) and the application of artificial intelligence represent an active area of research and may lead to development of new imaging-based, prognostic biomarkers for stratifying CAR-T response and outcome. The parameters examined in studies based on fludeoxyglucose-18 positron emission tomography with a computed tomography (18-FDG PET/CT) included SUVmax, Deauville score (DS), and innovative measures such as total tumor metabolic volume (TMTV) and total lesion glycolysis (TLG).

The prognostic impact of these parameters was investigated both at baseline and following CAR-T infusion. TMTV, the cumulative volume of lesions on 18-FDG PET/CT, has been increasingly recognized as a prognostic factor in LBCL.[74] Patients with baseline high TMTV before CAR-T infusion exhibited higher risk of progression and inferior PFS and OS compared to patients with low TMTV, which is consistent across the pivotal RCT JULIET[2] and RWD reports (Table 1), though the strength of the association differs across these populations.[37,44,45,63,74–79] An exception to this is the study by Sesques et al., which did not report an association between baseline TMTV and outcomes. However, their report demonstrated that an increased TMTV between apheresis and infusion of CAR-T was prognostic, suggesting the importance of measuring TMTV kinetics.[80] The recent report from ZUMA-7 evaluating the prognostic role of baseline TMTV indicated that median TMTV was not associated with EFS (hazard ratio [HR] 1.3, 95% confidence interval [CI] 0.89–2.0), while exploratory analysis defined a higher threshold that was associated with EFS (HR 2.0, 95% CI 1.2–3.6).[53] While SUVmax is a standard PET parameter, its prognostic role has not been rigorously studied. A limited study of 48 patients revealed an association between SUVmax >17 at the time of CAR-T decision and poor OS.[81]

Following CAR-T infusion, response and disease control is a dynamic process, wherein improvements in response can persist in the short term without the need for additional therapy. However, the precise moment at which the peak response is achieved and the risk of relapse is highest remains undefined, and imaging may help define the inflection point. While PET evaluation at 1-month following CAR-T therapy is routine practice, patients showing partial response or stable disease (SD) on imaging constitute a diverse patient group, with clinical uncertainty regarding which individuals will progress to complete remission (CR) and who may face progressive disease (PD)/early relapse. For instance, in a study of 206 patients, those with Day 30 PD or SD by Lugano criteria on PET who subsequently converted to CR had similar 90-day and 1-year median PFS as those who achieved CR on Day 30 (Table 2).[83] In this study, SUVmax ≥10 predicted subsequent PD with high sensitivity. Similarly, a recent study by Lutfi et al. showed that SUVmax modelled as a continuous variable was correlated with PFS and OS.[79] Kuhnl et al. confirmed the role of 1-month PET using DS for response and outcomes, with a stepwise decline in PFS and OS as DS increased, while 3-month DS did not show prognostic utility.[82] Lastly, Guidetti et al. integrated 30-day DS with SUV, and their findings indicated that patients with DS 4–5 and lower SUV exhibited comparable outcomes to those with DS 1–3. Conversely, patients with DS 4–5 and elevated SUV had worse PFS, suggesting the combination of DS and SUV could provide better stratification.[84]

Taken together, although PET parameters show potential as prognostic biomarkers, limitations exist that need to be addressed before their incorporation into clinical practice. For instance, even though TMTV holds great promise as a biomarker, no standardization for TMTV quantification nor interpretation currently exists. Furthermore, the range of TMTV reported differs across data sets, highlighting variability in the methodologies that underlie TMTV analysis, and a discriminatory threshold distinguishing between high and low values is currently lacking. Additionally, studies comparing different PET parameters are scarce. Future research should employ larger data sets measuring a spectrum of PET parameters, including standardized measurements of DS, SUVmax, and TMTV/TLG, and over multiple time points. This approach may yield parameters and time points with prognostic value for CAR-T failure, with the goal of informing therapeutic strategies to improve upon CAR-T outcomes. These strategies may include consolidative radiation therapy (RT) for patients with PET-positive disease after CAR-T[85] or the utilization of alternative treatments, such as bispecific antibodies, alternative cell therapy products, or other novel approaches.

Lastly, advanced functional imaging techniques such as single-photon emission computed tomography may contribute to a deeper mechanistic understanding of tumor resistance; however, these techniques are still at early stages of development.[86]

CIRCULATING TUMOR DNA

Circulating tumor DNA (ctDNA) is a promising prognostic noninvasive biomarker that may predict response to CAR-T.[54,87,88] Despite its anticipated clinical utility as a biomarker, there are several limitations that treating oncologists should be mindful of. Currently, there is no gold standard tool for quantifying ctDNA, and methodologies include polymerase chain reaction, whole-genome sequencing, clonotype sequencing, and cancer personalized profiling by deep sequencing (CAPP-seq). Furthermore, the prognostic timepoints for measurement are yet to be clarified, and the actionable implications upon the detection of ctDNA remain unclear.

The prospective multicenter study led by Frank et al. serially measured ctDNA by clonotype sequencing was one of the initial studies to underscore the utility of ctDNA as a biomarker. Their study highlighted two important principles: (1) pre-lymphodepletion ctDNA correlated with PFS; (2) the prognostic value of Day 28 ctDNA for subsequent relapse, whereby patients with detectable ctDNA had median PFS and OS of 3 and 19 months, respectively, compared to not reached for those with undetectable ctDNA (Table 2). Moreover, patients with relapse had detectable ctDNA either on or before evidence of radiological relapse. These findings support the role of ctDNA as a potential minimal residual disease monitoring tool.[54] A recent comprehensive RWD study conducted by Sworder et al. corroborated the importance of dynamically measured ctDNA (CAPP-seq) as predictive of outcomes (Table 2). They identified that ctDNA levels 4 weeks after infusion reported as the most robust predictor of outcomes.[64] The integration of ctDNA concentrations with imaging parameters such as TMTV requires further investigation. Dean et al. demonstrated that ctDNA levels did not correlate with TMTV at 1 month but correlated at 3 months, which may support the use of ctDNA in conjunction with 1-month PET.[49]

While ctDNA is a promising tool for disease surveillance, further studies are required to validate its utility in clinical practice. Moreover, ctDNA integration into clinical trials is essential for designing therapies to prevent relapse after CAR-T.

TABLE 2 Summary of select studies reporting on biomarkers associated with outcomes following CAR-T infusion.

Study	Country	Publication year	No. of patients	Median age	CAR-T product	Subtypes of LGBL	ECOG	IPI	Disease progression		Overall survival	
Breen et al.[77]	USA	2022	69	61	Axi-cel	DLBCL, PBMCL, TFL	-	-	1-mn TMTV	HR 2.7 (95% CI 1.3–5.4)	1-mn TMTV	HR 4.7 (95% CI 2.1–10.4)
									1-mn TLG	HR 3.3 (95% CI 1.4–7.7)	1-mn TLG	HR 2.4 (95% CI 1.0–5.6)
									1-mn SUVmax >10	HR 2.8 (95% CI 1.2–6.2)	1-mn SUVmax >10	HR 2.4 (95% CI 1.0–5.6)
Kuhnl et al.[82]	UK	2022	171	59	Axi-cel ($n = 130$), tisa-cel ($n = 41$)	DLBCL, HGBL	0–2	-	1-mn DS 3 vs. DS 1–2	HR 2.5 (95% CI 1.0–6.1)		
									1-mn DS 4 vs. DS 1–2	HR 3.5 (95% CI 1.5–8.4)		
									1-mn DS 5 vs. DS 1–2	HR 19.8 (95% CI 7.8–49.7)		
Al Zaki et al.[83]	USA	2022	206	62	Axi-cel	DLBCL, HGBL	0–4	0–5	Day 30 SUVmax ≥10	Correlated with subsequent progression ($p = 0.001$)		
Roddie et al.[48]	UK	2023	171	59	Axi-cel ($n = 130$), tisa-cel ($n = 41$)	DLBCL, PMBCL, TFL, HGBL	0–1	0–3+	1-mn DS	DS 3 HR 2.5 (95% CI 1.0–6.1) DS 4 HR 3.5 (95% CI 1.5–8.4) DS 5 HR 20 (95% CI 7.8–49.7)		
Frank et al.[54]	USA	2021	72	62	Axi-cel	DLBCL, PMBCL, TFL, HGBL	-	-	Undetectable 1-week ctDNA	70% responders vs. 13% progressed ($p < 0.001$)	Day 28 detectable ctDNA vs undetectable	19 mns vs. not reached
									Day 28 detectable ctDNA vs. undetectable	3 mns (all ctDNA + at time or before relapse) vs. not reached		
Sworder et al.[64]	USA	2023	138	58	Axi-cel	DLBCL, PMBCL, TFL, HGBL	-	0–5	Week 1 ctDNA	HR 2.2	Pre-LD ctDNA	HR 2.1
									Week 4 ctDNA	HR 2.3	Week 1 ctDNA	HR 1.7
									LDH	HR 1.3	Week 4 ctDNA	HR 2.5
									Week 1 cfCAR19	HR 0.55	LDH	HR 1.5

Abbreviations: CAR-T, chimeric antigen T cell receptor therapy; cfCAR19, cell-free CD19 CAR-T cells; CI, confidence interval; ctDNA, circulating tumor DNA; DLBCL, diffuse large B cell lymphoma; DS, Deauville score; HGBL, high-grade B cell lymphoma including double hit/triple hit; HR, hazard ratio; mn, month; mns, months; OS, overall survival; PFS, progression-free survival; PMBCL, primary mediastinal B cell lymphoma; TFL, transformed follicular lymphoma or other indolent lymphoma; TLG, total lesion glycolysis; TMTV, total metabolic tumor volume.

BRIDGING THERAPY

BT during the CAR-T manufacturing period may be important, particularly for patients with high tumor burden, as loss of disease control during this period could lead to patients failing to reach CAR-T infusion. There are currently no evidence-based guidelines around BT, and the choice is typically based on physician discretion.[89] Moreover, insights into optimal BT use are unlikely to be gleaned from RCTs as its allowance and utilization was inconsistent. BT was not allowed in ZUMA-1,[3] while 59% and 92% of patients in TRANSCEND[1] and JULIET[2] received BT, respectively. Similarly, with the exception of corticosteroids, BT was not allowed in ZUMA-7, raising concern that patients with more aggressive disease at enrollment may have been excluded as a result.[32] In contrast, both TRANSFORM[23] and BELINDA[27] allowed receipt BT including platinum-based chemotherapy.

In RWD reports, the use of BT was as high as 80%,[28,47] including multi-institutional use of BT before axi-cel despite this being disallowed on trial. While earlier reports suggested that receipt of BT was associated with poor outcomes,[12,90] this is likely a surrogate for more aggressive or higher burden disease as opposed to adverse effects of BT. Given that the association between preinfusion high tumor burden and unfavorable outcomes has been consistently shown, BT aimed at reducing tumor burden may play an important role in optimizing CAR-T response (Table 1).[91,92] Though the data are limited, BT may lead to CR in some patients, with favorable outcomes who proceed to CAR-T therapy while in CR.[93,94] Conversely, the study by Bethge et al. showed that BT nonresponders per Lugano criteria had inferior PFS and OS.[47] Roddie et al. corroborated these findings, reporting that responders to BT (PR/CR per Lugano criteria) had a 42% reduction in disease progression compared to nonresponders.[95] Interestingly, they showed that the prognostic impact of BT may vary between axi-cel and tisa-cel. All BT nonresponders who subsequently received tisa-cel experienced relapse within 12 months of therapy. The potential reasons behind these observations are unclear; no disparities in baseline characteristics were identified among BT non-responders receiving tisa-cel compared to axi-cel. This finding may have important implications for CAR-T selection and, importantly, emphasizes the importance of conducting biomarker discovery and validation studies that distinguish between different CAR-T products.

While these data may support the role of BT in patients with high disease burden, it is unclear whether the disease reduction itself or response to BT is a surrogate of better disease biology and responsiveness to CAR-T. Indeed, outcome differences in the BT versus no BT group were no longer significant following propensity score matching based on seven patient and disease-related factors.[92] Nevertheless, as achieving PR/CR with BT is associated with improved outcomes, determining which patients are likely to respond to BT and identifying the optimal BT is an important area to be clarified. Systemic BT options include steroids, traditional chemotherapy, and targeted therapies such as lenalidomide, ibrutinib, or polatuzumab vedotin-based therapies. Bridging RT is an encouraging approach for disease control during the manufacturing period. Although lacking prospective evidence, RWD suggests that RT is associated with improved response rates, local disease control, and may prolong disease-free intervals in patients with bulky disease.[48,96,97] This may be related to a RT-induced abscopal effect based on preclinical studies.[98] RT and polatuzumab-based therapy are associated with higher rates of PR/CR, and the optimal BT regimen remains uncertain.[48,92,99,100] Nevertheless, the decision for BT needs a careful balance between the potential benefits and pitfalls from BT such as myelosuppression, infectious complication, or organ toxicity, which can delay definitive CAR-T therapy. Ultimately, the decision to proceed with BT should be individualized for each patient based on tumor burden, prior lines of therapy, and preinfusion risk factors of poor CAR-T response.

PHARMACOLOGY OF CAR-T

The CAR-T manufacturing process follows the collection of peripheral blood mononuclear cells from the patient and the isolation of T cells from this apheresis product.[101] The clinical efficacy of CAR-T is likely contingent on the quality of the apheresis material, the CAR construct, the composition of CAR-T (and possibly the uninfected) cell subsets, and the expansion and persistence of the cell product following infusion.

T CELL SUBSETS

T cell subsets are categorized based on their functional characteristics, and recent research delved into their distinct roles in CAR-T expansion and persistence.[102,103] These subsets include naïve T (T_N) cells, T memory (T_M) cells such as T central memory (T_{CM}), T stem cell memory (T_{SCM}), and T effector memory (T_{EM}) cells, all with varying degrees of self-renewal potential, and T effector (T_E) cells that are fully differentiated but exhibit limited expansion and exhaustion phenotype.[104,105]

CHARACTERIZATION OF THE APHERESIS PRODUCT

Several studies have attempted to characterize the cellular composition of the apheresis product that serves as the starting material for the CAR-T manufacturing process. Currently available data suggest that the properties of the collected cells destined for CAR-T production impact the overall quality of the infusion product and are associated with clinical response.

Multiparameter flow-activated cell sorting has shown that the presence of CD8+ CD45RA+ CD27+ T cells in the apheresis product, but not the engineered CAR-T product, is associated with response to therapy.[106] The phenotype of the CD8+ subset of the T cells in the apheresis product has also been shown to be predictive of CAR-T efficacy.[107] The levels of self-renewal competent T_{SCM} (TCF7+, LEF1+, CCR7+, and CD27+) were associated with durable responses in patients with LBCL. Similarly, apheresis product T cells harboring a T_N phenotype (CCR7+ and CD45RA+) with CD27 and CD28 expression were associated with improved EFS and PFS.[108] In these studies, it appears that higher levels of less differentiated T cells (as compared to terminally differentiated T_E cells) lead to the generation of superior CAR-T products. When compared to healthy controls, patients with DLBCL had a higher frequency of differentiated CD3+ CD27− CD28− cells, which was associated with inferior outcomes.[109] Analysis of the ZUMA-1 axi-cel product samples found that infused products enriched for CCR7+ CD45RA+ T cells were associated with durable responses[63]; the improved outcomes were very recently confirmed in the analysis of axi-cel product samples in ZUMA-7.[110] Collectively, CAR-T infusion products containing cells with T_N, T_{SCM}, and T_{CM} phenotypes showed the highest expansion and proliferation, likely underpinning clinical responses, while cells with T_E or exhausted phenotypes have been associated with resistance. These studies suggest the potential importance of using T cells with stem-like phenotypes as opposed to terminally differentiated T cells for CAR-T manufacturing. The advantage of apheresis products containing

populations of T cells with stem or memory phenotypes may be due, in part, to their proliferative capacity and their ability to effectively expand upon encountering the CAR cognate antigen (generally CD19 in lymphoma). These cells also tend to persist longer in patients and lack markers of functional exhaustion, which have been shown to be negative indicators of efficacy.[111]

In the study by Wang et al., it was determined that the presence of CD14+ monocytes in the apheresis product, above a defined threshold, had deleterious effects on the efficacy of the resulting CAR-T product.[112] The prevalence of CD14 cells was disease-specific and highest in non-Hodgkin's lymphoma patients as compared to myeloma or acute lymphoblastic leukemia. The authors reasoned that these cells may allude to the presence of myeloid-derived suppressor cells, which are known to inhibit immunity and may impair CAR-T function. Furthermore, these monocytes, by way of their phagocytic activity, were shown to engulf the antibody-conjugated beads used to activate the T cells during the CAR-T manufacturing process, thereby impeding T cell activation and expansion ex vivo. Taking advantage of the adhesive properties of the monocytes, they were able to effectively reduce the numbers of these cells in the apheresis product by simple adhesion to plastic culture dishes followed by removal of the nonadherent T cells. This simple enrichment process markedly improved the properties of the engineered cell product.

Altogether, these data suggest that a careful analysis of the apheresis product (or peripheral blood close to the time of apheresis) and subsequent manipulation via enrichment of beneficial or depletion of deleterious cell types may be a useful strategy to improve the quality of the starting material prior to their entry into the CAR-T engineering process. While the implementation of additional selection strategies would add another step and time to the already cumbersome manufacturing protocols, the current data indicate that it may be beneficial to optimize the characteristics of the apheresis material as it could pay dividends with regards to improving the quality of the CAR-T infusion product and subsequently the durability of response to this therapy.

PHARMACOKINETICS OF CAR-T

All three CAR-T products are administered as a single dose infusion (axi-cel CD28/CD3ζ, 2×10^6 cells/kg; tisa-cel 4-1BB/CD3ζ, $0.6–6 \times 10^8$ cells; liso-cel 4-1BB/CD3ζ, 1×10^8 cells), after which rapid proliferation and expansion ensues upon encountering antigen-expressing target cells.[4] The pharmacokinetic profile of the CAR-Ts following infusion is similar irrespective of the costimulatory domain, reaching peak levels in the periphery within 7–14 days.[101] While the infusion dose does not appear to influence response, the proliferation and expansion kinetics are key determinants of CAR-T success. Higher CAR-T expansion and area under the curve (AUC) cellular concentration within 28 days of infusion correlates with clinical response, observed in both trial and real-world settings.[1,3,113,114]

While it may be conceivable that clinical variables may affect the apheresis product and subsequent expansion in vivo, available data in this area are limited. In a RWD report, CAR-T expansion does not appear to be influenced by clinical variables such as age, stage, tumor burden, pathological features, or prior therapies.[80] However, CAR-T expansion may be hindered by proinflammatory markers through interferon signaling as shown in analysis of ZUMA-1 samples.[63,115] The CD3+ lymphocyte count at the time of apheresis may serve as a surrogate marker of T cell quality and predict CAR-T expansion, whereby higher number of CD3+ lymphocytes (>553 μL) was associated with improved PFS and OS.[116] Congruent with this, a recently published report comprising 439 patients revealed that patients treated with bendamustine before apheresis exhibited lower CD3+ cells at apheresis, as well as lower CAR-T peak and AUC concentrations.[55] These bendamustine-exposed patients had inferior PFS and OS compared to bendamustine-naïve patients. Moreover, the use of bendamustine with <3-month washout period may impair manufacturing success.[117] These observations may speak to the fitness of the T cells themselves, the status of the microenvironment(s) in which they expand, or likely, a combination of the two. Lastly, data suggest that optimizing the lymphodepletion regimen is likely crucial in the kinetics of CAR-T cells, as studies have demonstrated that fludarabine and cyclophosphamide can enhance proliferation, expansion, and persistence.[43,118,119]

CAR PHARMACODYNAMICS

CAR-T products approved for LBCL are second-generation constructs featuring an intracellular domain CD3ζ, coupled with a costimulatory domain: either CD28 (axi-cel) or 4-1BB (liso-cel and tisa-cel).[120] Recent data suggest that the costimulatory domain has an impact on T cell subsets within the CAR-T product; however, the optimal costimulatory domain remains to be clarified.[121] The available data thus far indicate that there are functional distinctions between various T cell subsets concerning in vivo proliferation and activity, which consequently impacts clinical outcomes.

While CD28 imparts higher cytotoxic activity and a T_E phenotype,[122] 4-1BB exhibits greater persistence and a T_M phenotype.[123–125] Despite the greater persistence with 4-1BB, recent RWD indicates that axi-cel may be superior to tisa-cel for PFS (Bethge et al.: tisa-cel vs. axi-cel HR 1.5, 95% CI 1.1–1.9; Bachy et al. axi-cel: vs. tisa-cel HR 0.6, 95% CI 0.5–0.8) and OS (Bachy et al.: axi-cel vs. tisa-cel HR 0.6, 95% CI 0.5–0.9).[6,28,47] Regardless of the co-stimulatory domain, expression of T_N and T_M CAR-T cells has been correlated with in vivo expansion. Higher proportion of CD8 T_N and T_{SCM} cells was associated with durable responses in ZUMA-1,[63] corroborated by Monfrini et al. showing that CD8 T_{CM} CAR-T cells were associated with increased expansion and improved PFS.[114] Similarly, CAR-T cells with memory gene expression signatures were associated with improved responses while exhaustion gene signatures were associated with poor clinical response.[111,126] In line with this, two independent studies concurrently demonstrated that enhancing expression of the FOXO1 transcription factor resulted in increased T_{CM} subsets, corresponding to augmented CAR-T efficacy,[127,128] further supporting novel bioengineering strategies aimed at enhancing CAR-T potency.

An important consideration in CAR-T manufacturing is the optimal ratio of CD4:CD8 CAR-T cells. Liso-cel adopts a 1:1 ratio, while axi-cel and tisa-cel do not follow a specific ratio. Despite lacking a defined CD4:CD8 ratio, both ZUMA-1 and JULIET found no difference in outcomes in relation to CD4:CD8 ratio,[2,3] which argues against its necessity. Moreover, although direct head-to-head comparisons of CAR-T products are lacking, a real-world study comparing the three CAR-T products suggested that axi-cel may be more efficacious than liso-cel, despite the latter adhering to a defined CD4:CD8 ratio.[129]

One of the goals going forward should be to produce the most effective CAR-T cell product with a favorable T cell phenotype to achieve optimal efficacy. The enrichment of CAR-T cells with memory signatures and absence of exhaustion signatures in the preinfusion products are anticipated to enhance clinical response.[130–132] T cell exhaustion is a dynamic process and serves as an important target for immunotherapies to prevent differentiation toward exhaustion.[133] Finally, an area of active investigation is the interplay between CAR-T cells and the TME, whereby features of senescence/exhaustion such

as the expression of programmed cell death protein 1, T cell immunoglobulin and mucin-domain containing-3, and lymphocyte activation gene 3 have been correlated with CAR-T resistance.[14,134,135] Therapeutic strategies to enhance the TME and counter immunosuppressive programs to achieve optimal CAR-T responses are needed.

EXPLORATORY BIOMARKERS

Additional investigational parameters (not available in clinical practice) associated with reduced efficacy to CAR-T include interleukin-6 (IL-6), IL-7, IL-15, IL-18, IL-21, monocytic myeloid-derived suppressor cells, and monocyte chemoattractant protein 1.[37,43,56,63,136]

Given the now well-established relationship between metabolism and T cell function, metabolomic analyses offer a potentially rich source of information on immune function.[137] Here, elevated levels of acetylated polyamines, secondary metabolites of arginine metabolism, were associated with worse PFS and OS. As the polyamine pathway is regulated by the MYC oncoprotein, the presence of polyamines in patient's plasma may be a marker of aggressive disease.[138]

The gastrointestinal tract microbiome has been postulated to modulate antitumor response.[139] A recent report found that antibiotic exposure within 4 weeks before CAR-T and specific microbiota composition were predictive of inferior PFS and OS.[140]

CONCLUSIONS

In recent years, CD19-directed CAR-T therapy has revolutionized R/R LBCL treatment, showing improved survival in the second-line setting and beyond. RWD has corroborated the efficacy seen in RCTs, extending a curative option to a broader patient population. Clinical trials are underway evaluating its efficacy in the transplant-ineligible population and for frontline use in high-risk treatment-naïve patients. Despite the successes of CAR-T, achieving curability is limited to a subset of patients, necessitating urgent improvement in response and durability.

Biomarker identification at various timepoints around CAR-T infusion is crucial for understanding CAR-T response and resistance. However, the lack of consensus on standardized biomarker analysis, uncertain key timepoints for analysis, technological challenges, sample size, inconsistent differentiation between costimulatory domains, and heterogeneous patient populations between RCTs and real-world studies all pose as barriers to their implementation in clinical practice.

Factors influencing outcomes before infusion include age, specific comorbidities, ECOG, LDH, proinflammatory factors, BT, tumor burden, and T cell composition. Preinfusion factors before CAR-T infusion can inform decisions regarding optimization of BT and lymphodepletion. As CAR-T is a "living drug," it is not surprising that disease progression also depends on postinfusion factors that are not apparent preinfusion, highlighting the importance of dynamic disease evaluation. Postinfusion factors are likely more important for identifying early relapse and guiding consolidation or maintenance therapy to prevent relapse and enhance outcomes. Combining postinfusion biomarkers such as ctDNA and PET parameters holds greater potential than individual biomarkers in isolation.

To conclude, consensus is currently lacking regarding a set of biomarkers validated for routine clinical practice to identify patients prone to relapse. Further comprehensive and collaborative research delineating CAR-T response and resistance mechanisms is crucial for identifying reliable biomarkers. The integration of technologies, including ctDNA, imaging techniques, gene expression profiling, mutational analysis, T cell phenotype analysis, and immune fitness assessment, is needed to develop a personalized treatment approach tailored to individual mechanisms of CAR-T failure. While valuable information can be acquired from the periphery, there are critical aspects of CAR-T biology that cannot be captured with these samples alone. Efforts should be made to collect on-treatment biopsies to better understand the evolution of the lymphoma microenvironment and CAR-T cells in situ. Finally, as more knowledge is gleaned regarding the mechanisms of resistance, the judicious choice of CAR-T for the appropriate patient at the optimal time may become clearer.

AUTHOR CONTRIBUTIONS

Inna Y. Gong, Rob C. Laister, Samuel Saibil, and John Kuruvilla designed the review. Inna Y. Gong, Daisy Tran, and Rob C. Laister conducted the literature search. All authors revised the article, read, and approved the submitted version.

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The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

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REFERENCES

1. Abramson JS, Palomba ML, Gordon LI, et al. Lisocabtagedone maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. *Lancet*. 2020;396(10254):839-852. doi:10.1016/s0140-6736(20)31366-0
2. Schuster SJ, Bishop MR, Tam CS, et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. *N Engl J Med*. 2019;380(1):45-56. doi:10.1056/NEJMoa1804980
3. Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med*. 2017;377(26):2531-2544. doi:10.1056/NEJMoa1707447
4. Westin J, Sehn LH. CAR T cells as a second-line therapy for large B-cell lymphoma: a paradigm shift? *Blood*. 2022;139(18):2737-2746. doi:10.1182/blood.2022015789
5. Westin JR, Oluwole OO, Kersten MJ, et al. Survival with axicabtagene ciloleucel in large B-cell lymphoma. *N Engl J Med*. 2023;389(2):148-157. doi:10.1056/NEJMoa2301665
6. Bachy E, Le Gouill S, Di Blasi R, et al. A real-world comparison of tisagenlecleucel and axicabtagene ciloleucel CAR T cells in relapsed or refractory diffuse large B cell lymphoma. *Nat Med*. 2022;28(10):2145-2154. doi:10.1038/s41591-022-01969-y
7. Casadei B, Argnani L, Guadagnuolo S, et al. Real world evidence of CAR T-cell therapies for the treatment of relapsed/refractory B-cell non-Hodgkin lymphoma: a monocentric experience. *Cancers*. 2021;13(19):4789. doi:10.3390/cancers13194789
8. Iacoboni G, Villacampa G, Martinez-Cibrian N, et al. Real-world evidence of tisagenlecleucel for the treatment of relapsed or refractory large B-cell lymphoma. *Cancer Med*. 2021;10(10):3214-3223. doi:10.1002/cam4.3881

9. Jacobson CA, Locke FL, Ma L, et al. Real-world evidence of axicabtagene ciloleucel for the treatment of large B cell lymphoma in the United States. *Transplant Cell Ther*. 2022;28(9):581.e1-581.e8. doi:10.1016/j.jtct.2022.05.026
10. Jacobson CA, Munoz J, Sun F, et al. Real-world outcomes with chimeric antigen receptor T cell therapies in large B cell lymphoma: a systematic review and meta-analysis. *Transplant Cell Ther*. 2024;30:77.e1-77.e15. doi:10.1016/j.jtct.2023.10.017
11. Kuhnl A, Roddie C, Kirkwood AA, et al. A national service for delivering CD19 CAR-Tin large B-cell lymphoma—the UK real-world experience. *Br J Haematol*. 2022;198(3):492-502. doi:10.1111/bjh.18209
12. Nastoupil LJ, Jain MD, Feng L, et al. Standard-of-care axicabtagene ciloleucel for relapsed or refractory large B-cell lymphoma: results from the US Lymphoma CAR T Consortium. *J Clin Oncol*. 2020;38(27):3119-3128. doi:10.1200/jco.19.02104
13. Sesques P, Ferrant E, Safar V, et al. Commercial anti-CD19 CAR T cell therapy for patients with relapsed/refractory aggressive B cell lymphoma in a European center. *Am J Hematol*. 2020;95(11):1324-1333. doi:10.1002/ajh.25951
14. Shah NN, Fry TJ. Mechanisms of resistance to CAR T cell therapy. *Nat Rev Clin Oncol*. 2019;16(6):372-385. doi:10.1038/s41571-019-0184-6
15. Chihara D, Liao L, Tkacz J, et al. Real-world experience of CAR T-cell therapy in older patients with relapsed/refractory diffuse large B-cell lymphoma. *Blood*. 2023;142(12):1047-1055. doi:10.1182/blood.2023020197
16. Spiegel JY, Dahiya S, Jain MD, et al. Outcomes of patients with large B-cell lymphoma progressing after axicabtagene ciloleucel. *Blood*. 2020;137(13):1832-1835. doi:10.1182/blood.2020006245
17. Di Blasi R, Le Gouill S, Bachy E, et al. Outcomes of patients with aggressive B-cell lymphoma after failure of anti-CD19 CAR T-cell therapy: a DESCAR-T analysis. *Blood*. 2022;140(24):2584-2593. doi:10.1182/blood.2022016945
18. Drucker E, Krapfenbauer K. Pitfalls and limitations in translation from biomarker discovery to clinical utility in predictive and personalised medicine. *EPMA J*. 2013;4(1):7. doi:10.1186/1878-5085-4-7
19. Bao F, Wan W, He T, et al. Autologous CD19-directed chimeric antigen receptor-T cell is an effective and safe treatment to refractory or relapsed diffuse large B-cell lymphoma. *Cancer Gene Ther*. 2019;26(7):248-255.
20. Nastoupil LJ, Jain MD, Spiegel JY, et al. Axicabtagene ciloleucel (Axi-cel) CD19 chimeric antigen receptor (CAR) T-cell therapy for relapsed/refractory large B-cell lymphoma: real world experience. *Blood*. 2018;132:91.
21. Locke FL, Neelapu SS, Bartlett NL, et al. Phase 1 results of ZUMA-1: a multicenter study of KTE-C19 anti-CD19 CAR T cell therapy in refractory aggressive lymphoma. *Mol Ther*. 2017;25(1):285-295.
22. Bishop MR, Dickinson M, Purtill D, et al. Second-line tisagenlecleucel or standard care in aggressive B-cell lymphoma. *N Engl J Med*. 2022;386(7):629-639.
23. Kamdar M, Solomon SR, Arnason J, et al. Lisocabtagene maraleucel versus standard of care with salvage chemotherapy followed by autologous stem cell transplantation as second-line treatment in patients with relapsed or refractory large B-cell lymphoma (TRANSFORM): results from an interim analysis of an open-label, randomised, phase 3 trial. *Lancet*. 2022;399(10343):2294-2308.
24. Locke FL, Miklos DB, Jacobson CA, et al. Axicabtagene ciloleucel as second-line therapy for large B-cell lymphoma. *N Engl J Med*. 2022;386(7):640-654.
25. Abramson JS, Palomba ML, Gordon LI, et al. Two-year follow-up of lisocabtagene maraleucel in relapsed or refractory large B-cell lymphoma in TRANSCEND NHL 001. *Blood*. 2024;143(5):404-416. doi:10.1182/blood.2023020854
26. Neelapu SS, Jacobson CA, Ghobadi A, et al. 5-Year follow-up supports curative potential of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1). *Blood*. 2023;141(19):2307-2315. doi:10.1182/blood.2022018893
27. Bishop MR, Dickinson M, Purtill D, et al. Second-line tisagenlecleucel or standard care in aggressive B-cell lymphoma. *N Engl J Med*. 2022;386(7):629-639. doi:10.1056/NEJMoa2116596
28. Kwon M, Iacoboni G, Reguera JL, et al. Axicabtagene ciloleucel compared to tisagenlecleucel for the treatment of aggressive B-cell lymphoma. *Haematologica*. 2023;108(1):110-121. doi:10.3324/haematol.2022.280805
29. Herrera AF, Mei M, Low L, et al. Relapsed or refractory double-expressor and double-hit lymphomas have inferior progression-free survival after autologous stem-cell transplantation. *J Clin Oncol*. 2017;35(1):24-31. doi:10.1200/jco.2016.68.2740
30. Joseph J, Ma J, Hennawy F, et al. Impact of cell of origin classification on survival outcomes after autologous transplantation in relapsed/refractory diffuse large B cell lymphoma. *Transplant Cell Ther*. 2021;27(5):404.e1-404.e5. doi:10.1016/j.jtct.2021.02.009
31. Moskowitz CH, Zelenetz AD, Kewalramani T, et al. Cell of origin, germinal center versus nongerminal center, determined by immunohistochemistry on tissue microarray, does not correlate with outcome in patients with relapsed and refractory DLBCL. *Blood*. 2005;106(10):3383-3385. doi:10.1182/blood-2005-04-1603
32. Locke FL, Miklos DB, Jacobson CA, et al. Axicabtagene ciloleucel as second-line therapy for large B-cell lymphoma. *N Engl J Med*. 2022;386(7):640-654. doi:10.1056/NEJMoa2116133
33. Jacobson CA, Hunter BD, Redd R, et al. Axicabtagene ciloleucel in the non-trial setting: outcomes and correlates of response, resistance, and toxicity. *J Clin Oncol*. 2020;38(27):3095-3106. doi:10.1200/jco.19.02103
34. Zurko J, Shouse G, Torka P, et al. Double hit/double expressor lymphomas: a multicenter analysis of survival outcomes with CD19-directed CAR T-cell therapy. *Blood*. 2022;140(suppl 1):375-377. doi:10.1182/blood-2022-163438
35. Chong EA, Chong ER, Landsburg DJ, et al. Anti-CD19 CAR-T for treatment of double expressor and double hit large B-cell lymphomas: a single institution real-world analysis. *Blood*. 2020;136(suppl 1):19-20. doi:10.1182/blood-2020-143348
36. Ali A, Goy A, Dunleavy K. CAR T-cell therapy in highly aggressive B-cell lymphoma: emerging biological and clinical insights. *Blood*. 2022;140(13):1461-1469. doi:10.1182/blood.2022016226
37. Vercellino L, Di Blasi R, Kanoun S, et al. Predictive factors of early progression after CAR T-cell therapy in relapsed/refractory diffuse large B-cell lymphoma. *Blood Adv*. 2020;4(22):5607-5615. doi:10.1182/bloodadvances.2020003001
38. Kittai AS, Huang Y, Gordon M, et al. Comorbidities predict inferior survival in patients receiving chimeric antigen receptor T cell therapy for diffuse large B cell lymphoma: a multicenter analysis. *Transplant Cell Ther*. 2021;27(1):46-52. doi:10.1016/j.bbmt.2020.09.028
39. Berning P, Shumilov E, Maulhardt M, et al. Chimeric antigen receptor-T cell therapy shows similar efficacy and toxicity in patients with diffuse large B-cell lymphoma aged 70 and older compared to younger patients: a multicenter cohort study. *HemaSphere*. 2024;8(3):e54. doi:10.1002/hem3.54
40. Shouse G, Kaempf A, Gordon MJ, et al. A validated composite comorbidity index predicts outcomes of CAR T-cell therapy in patients with diffuse large B-cell lymphoma. *Blood Adv*. 2023;7(14):3516-3529. doi:10.1182/bloodadvances.2022009309
41. Sermer D, Batlevi C, Palomba ML, et al. Outcomes in patients with DLBCL treated with commercial CAR T cells compared with alternate therapies. *Blood Adv*. 2020;4(19):4669-4678. doi:10.1182/bloodadvances.2020002118
42. Iovino L, Wu QV, Voutsinas J, et al. Predictors of response to axicabtagene-ciloleucel CAR T cells in aggressive B cell lymphomas: a real-world study. *J Cell Mol Med*. 2022;26(24):5976-5983. doi:10.1111/jcmm.17550

43. Hirayama AV, Gauthier J, Hay KA, et al. The response to lymphodepletion impacts PFS in patients with aggressive non-Hodgkin lymphoma treated with CD19 CAR T cells. *Blood*. 2019;133(17):1876-1887. doi:10.1182/blood-2018-11-887067
44. Galtier J, Vercellino L, Chartier L, et al. Positron emission tomography-imaging assessment for guiding strategy in patients with relapsed/refractory large B-cell lymphoma receiving CAR T cells. *Haematologica*. 2023;108(1):171-180. doi:10.3324/haematol.2021.280550
45. Voltin C-A, Gödel P, Beckmann L, et al. Outcome prediction in patients with large B-cell lymphoma undergoing chimeric antigen receptor T-cell therapy. *HemaSphere*. 2023;7(1):e817. doi:10.1097/hs9.0000000000000817
46. Iacoboni G, Simó M, Villacampa G, et al. Prognostic impact of total metabolic tumor volume in large B-cell lymphoma patients receiving CAR T-cell therapy. *Ann Hematol*. 2021;100(9):2303-2310. doi:10.1007/s00277-021-04560-6
47. Bethge WA, Martus P, Schmitt M, et al. GLA/DRST real-world outcome analysis of CAR T-cell therapies for large B-cell lymphoma in Germany. *Blood*. 2022;140(4):349-358. doi:10.1182/blood.2021015209
48. Roddie C, Neill L, Osborne W, et al. Effective bridging therapy can improve CD19 CAR-T outcomes while maintaining safety in patients with large B-cell lymphoma. *Blood Adv*. 2023;7(12):2872-2883. doi:10.1182/bloodadvances.2022009019
49. Dean EA, Kimmel GJ, Frank MJ, et al. Circulating tumor DNA adds specificity to PET after axicabtagene ciloleucel in large B-cell lymphoma. *Blood Adv*. 2023;7(16):4608-4618. doi:10.1182/bloodadvances.2022009426
50. Shi H, Zheng P, Liu R, et al. Genetic landscapes and curative effect of CAR T-cell immunotherapy in patients with relapsed or refractory DLBCL. *Blood Adv*. 2023;7(6):1070-1075. doi:10.1182/bloodadvances.2021006845
51. Shouval R, Alarcon Tomas A, Fein JA, et al. Impact of TP53 genomic alterations in large B-cell lymphoma treated with CD19-chimeric antigen receptor T-cell therapy. *J Clin Oncol*. 2022;40(4):369-381. doi:10.1200/jco.21.02143
52. Jain MD, Zicchedda B, Coughlin CA, et al. Whole-genome sequencing reveals complex genomic features underlying anti-CD19 CAR T-cell treatment failures in lymphoma. *Blood*. 2022;140(5):491-503. doi:10.1182/blood.2021015008
53. Locke FL, Oluwole OO, Kuruvilla J, et al. Axicabtagene ciloleucel vs standard of care in second-line large B-cell lymphoma: outcomes by metabolic tumor volume. *Blood*. 2024;143:2464-2473. doi:10.1182/blood.2023021620
54. Frank MJ, Hossain NM, Bukhari A, et al. Monitoring of circulating tumor DNA improves early relapse detection after axicabtagene ciloleucel infusion in large B-cell lymphoma: results of a prospective multi-institutional trial. *J Clin Oncol*. 2021;39(27):3034-3043. doi:10.1200/jco.21.00377
55. Iacoboni G, Navarro V, Martín-López AÁ, et al. Recent bendamustine treatment before Apheresis has a negative impact on outcomes in patients with large B-cell lymphoma receiving chimeric antigen receptor T-cell therapy. *J Clin Oncol*. 2024;42:205-217. doi:10.1200/jco.23.01097
56. Jain MD, Zhao H, Wang X, et al. Tumor interferon signaling and suppressive myeloid cells are associated with CAR T-cell failure in large B-cell lymphoma. *Blood*. 2021;137(19):2621-2633. doi:10.1182/blood.2020007445
57. Wright GW, Huang DW, Phelan JD, et al. A Probabilistic Classification Tool for genetic subtypes of diffuse large B cell lymphoma with therapeutic implications. *Cancer Cell*. 2020;37(4):551-568.e14. doi:10.1016/j.ccell.2020.03.015
58. Ram R, Grisariu S, Shargian-Alon L, et al. Toxicity and efficacy of chimeric antigen receptor T-cell therapy in patients with diffuse large B-cell lymphoma above the age of 70 years compared to younger patients—a matched control multicenter cohort study. *Haematologica*. 2022;107(5):1111-1118. doi:10.3324/haematol.2021.278288
59. Neelapu SS, Jacobson CA, Oluwole OO, et al. Outcomes of older patients in ZUMA-1, a pivotal study of axicabtagene ciloleucel in refractory large B-cell lymphoma. *Blood*. 2020;135(23):2106-2109. doi:10.1182/blood.2019004162
60. Sehgal A, Hoda D, Riedell PA, et al. Lisocabtagene maraleucel as second-line therapy in adults with relapsed or refractory large B-cell lymphoma who were not intended for haematopoietic stem cell transplantation (PILOT): an open-label, phase 2 study. *Lancet Oncol*. 2022;23(8):1066-1077. doi:10.1016/s1470-2045(22)00339-4
61. Dreger P, Holtick U, Subklewe M, et al. Impact of age on outcome of CAR-T cell therapies for large B-cell lymphoma: the GLA/DRST experience. *Bone Marrow Transplant*. 2023;58(2):229-232. doi:10.1038/s41409-022-01867-4
62. Mamlouk O, Nair R, Iyer SP, et al. Safety of CAR T-cell therapy in kidney transplant recipients. *Blood*. 2021;137(18):2558-2562. doi:10.1182/blood.2020008759
63. Locke FL, Rossi JM, Neelapu SS, et al. Tumor burden, inflammation, and product attributes determine outcomes of axicabtagene ciloleucel in large B-cell lymphoma. *Blood Adv*. 2020;4(19):4898-4911. doi:10.1182/bloodadvances.2020002394
64. Sworder BJ, Kurtz DM, Alig SK, et al. Determinants of resistance to engineered T cell therapies targeting CD19 in large B cell lymphomas. *Cancer Cell*. 2023;41(1):210-225.e5. doi:10.1016/j.ccell.2022.12.005
65. Keane C, Tobin J, Talaulikar D, et al. A high LDH to absolute lymphocyte count ratio in patients with DLBCL predicts for a poor intratumoral immune response and inferior survival. *Oncotarget*. 2018;9(34):23620-23627. doi:10.18632/oncotarget.25306
66. Faramand RG, Lee SB, Jain MD, et al. Baseline serum inflammatory proteins predict poor CAR T outcomes in diffuse large B-cell lymphoma. *Blood Cancer Discov*. 2024;5(2):106-113. doi:10.1158/2643-3230.Bcd-23-0056
67. The International Non-Hodgkin's Lymphoma Prognostic Factor Project. A predictive model for aggressive non-Hodgkin's lymphoma. *N Engl J Med*. 1993;329(14):987-994. doi:10.1056/nejm199309303291402
68. Hamlin PA, Zelenetz AD, Kewalramani T, et al. Age-adjusted International Prognostic Index predicts autologous stem cell transplantation outcome for patients with relapsed or primary refractory diffuse large B-cell lymphoma. *Blood*. 2003;102(6):1989-1996. doi:10.1182/blood-2002-12-3837
69. Garcia-Recio M, Wudhikarn K, Pennisi M, et al. The International Prognostic Index is Associated with outcomes in diffuse large B cell lymphoma after chimeric antigen receptor T cell therapy. *Transplant Cell Ther*. 2021;27(3):233-240. doi:10.1016/j.jtct.2020.10.022
70. Pennisi M, Sanchez-Escamilla M, Flynn JR, et al. Modified EASIX predicts severe cytokine release syndrome and neurotoxicity after chimeric antigen receptor T cells. *Blood Adv*. 2021;5(17):3397-3406. doi:10.1182/bloodadvances.2020003885
71. Rejeski K, Perez A, Sesques P, et al. CAR-HEMATOTOX: a model for CAR T-cell-related hematologic toxicity in relapsed/refractory large B-cell lymphoma. *Blood*. 2021;138(24):2499-2513. doi:10.1182/blood.2020010543
72. Rejeski K, Perez A, Iacoboni G, et al. The CAR-HEMATOTOX risk-stratifies patients for severe infections and disease progression after CD19 CAR-T in R/R LBCL. *J Immunother Cancer*. 2022;10(5):e004475. doi:10.1136/jitc-2021-004475
73. Bhaskar ST, Patel VG, Porter DL, et al. Chimeric antigen receptor T-cell therapy yields similar outcomes in patients with and without cytokine release syndrome. *Blood Adv*. 2023;7(17):4765-4772. doi:10.1182/bloodadvances.2022008937

74. Vercellino L, Cottereau AS, Casasnovas O, et al. High total metabolic tumor volume at baseline predicts survival independent of response to therapy. *Blood*. 2020;135(16):1396-1405. doi:10.1182/blood.2019003526
75. Dean EA, Mhaskar RS, Lu H, et al. High metabolic tumor volume is associated with decreased efficacy of axicabtagene ciloleucel in large B-cell lymphoma. *Blood Adv*. 2020;4(14):3268-3276. doi:10.1182/bloodadvances.2020001900
76. Derlin T, Schultze-Florey C, Werner RA, et al. ^{18}F-FDG PET/CT of off-target lymphoid organs in CD19-targeting chimeric antigen receptor T-cell therapy for relapsed or refractory diffuse large B-cell lymphoma. *Ann Nucl Med*. 2021;35(1):132-138. doi:10.1007/s12149-020-01544-w
77. Breen WG, Hathcock MA, Young JR, et al. Metabolic characteristics and prognostic differentiation of aggressive lymphoma using one-month post-CAR-T FDG PET/CT. *J Hematol Oncol*. 2022;15(1):36. doi:10.1186/s13045-022-01256-w
78. Hong R, Tan S, Yin E, Wang L, et al. Tumor burden measured by ^{18}F-FDG PET/CT in predicting efficacy and adverse effects of chimeric antigen receptorT-cell therapy in non-Hodgkin lymphoma. *Front Oncol*. 2021;11:713577. doi:10.3389/fonc.2021.713577
79. Lutfi F, Goloubeva O, Kowatli A, et al. Imaging biomarkers to predict outcomes in patients with large B-cell lymphoma with a day 28 partial response by ^{18}F-FDG PET/CT imaging following CAR-T therapy. *Clin Lymphoma Myeloma Leuk*. 2023;23(10):757-763. doi:10.1016/j.clml.2023.06.005
80. Sesques P, Tordo J, Ferrant E, et al. Prognostic impact of ^{18}F-FDG PET/CT in patients with aggressive B-cell lymphoma treated with anti-CD19 chimeric antigen receptor T cells. *Clin Nucl Med*. 2021;46(8):627-634. doi:10.1097/rlu.0000000000003756
81. Cohen D, Luttwak E, Beyar-Katz O, et al. [^{18}F]FDG PET-CT in patients with DLBCL treated with CAR-T cell therapy: a practical approach of reporting pre- and post-treatment studies. *Eur J Nucl Med Mol Imaging*. 2022;49(3):953-962. doi:10.1007/s00259-021-05551-5
82. Kuhnl A, Roddie C, Kirkwood AA, et al. Early FDG-PET response predicts CAR-T failure in large B-cell lymphoma. *Blood Adv*. 2022;6(1):321-326. doi:10.1182/bloodadvances.2021005807
83. Al Zaki A, Feng L, Watson G, et al. Day 30 SUVmax predicts progression in patients with lymphoma achieving PR/SD after CAR T-cell therapy. *Blood Adv*. 2022;6(9):2867-2871. doi:10.1182/bloodadvances.2021006715
84. Guidetti A, Dodero A, Lorenzoni A, et al. Combination of Deauville score and quantitative positron emission tomography parameters as a predictive tool of anti-CD19 chimeric antigen receptor T-cell efficacy. *Cancer*. 2023;129(2):255-263. doi:10.1002/cncr.34532
85. Saifi O, Breen WG, Lester SC, et al. Consolidative radiotherapy for residual fluorodeoxyglucose activity on day +30 post CAR T-cell therapy in non-Hodgkin lymphoma. *Haematologica*. 2023;108(11):2982-2992. doi:10.3324/haematol.2023.283311
86. Mulgaonkar A, Udayakumar D, Yang Y, et al. Current and potential roles of immuno-PET/-SPECT in CAR T-cell therapy. *Front Med*. 2023;10:1199146. doi:10.3389/fmed.2023.1199146
87. Hossain NM, Dahiya S, Le R, et al. Circulating tumor DNA assessment in patients with diffuse large B-cell lymphoma following CAR T-cell therapy. *Leuk Lymphoma*. 2019;60(2):503-506. doi:10.1080/10428194.2018.1474463
88. Zhou L, Zhao H, Shao Y, et al. Serial surveillance by circulating tumor DNA profiling after chimeric antigen receptor T therapy for the guidance of r/r diffuse large B cell lymphoma precise treatment. *J Cancer*. 2021;12(18):5423-5431. doi:10.7150/jca.60390
89. Yakoub-Agha I, Chabannon C, Bader P, et al. Management of adults and children undergoing chimeric antigen receptor T-cell therapy: best practice recommendations of the European Society for Blood and Marrow Transplantation (EBMT) and the Joint Accreditation Committee of ISCT and EBMT (JACIE). *Haematologica*. 2020;105(2):297-316. doi:10.3324/haematol.2019.229781
90. Pinnix CC, Gunther JR, Dabaja BS, et al. Bridging therapy prior to axicabtagene ciloleucel for relapsed/refractory large B-cell lymphoma. *Blood Adv*. 2020;4(13):2871-2883. doi:10.1182/bloodadvances.2020001837
91. Lyu C, Cui R, Wang J, et al. Intensive Debulking chemotherapy improves the short-term and long-term efficacy of anti-CD19-CAR-T in refractory/relapsed DLBCL with high tumor bulk. *Front Oncol*. 2021;11:706087. doi:10.3389/fonc.2021.706087
92. Jain MD, Jacobs MT, Gao F, et al. Bridging therapy with axicabtagene ciloleucel for large B-cell lymphoma: Results from the US lymphoma CAR-T consortium. *Blood Adv*. 2024;8:1042-1050. doi:10.1182/bloodadvances.2023011489
93. Bishop MR, Maziarz RT, Waller EK, et al. Tisagenlecleucel in relapsed/refractory diffuse large B-cell lymphoma patients without measurable disease at infusion. *Blood Adv*. 2019;3(14):2230-2236. doi:10.1182/bloodadvances.2019000151
94. Le Gouill S, Bachy E, Di Blasi R, et al. First results of DLBCL patients treated with CAR-T cells and enrolled in DESCAR-T Registry, a French real-life database for CAR-T cells in hematologic malignancies. *Hematol Oncol*. 2021;39(S2). doi:10.1002/hon.84_2879
95. Roddie C, Lekakis LJ, Marzolini MAV, et al. Dual targeting of CD19 and CD22 with bicistronic CAR-T cells in patients with relapsed/refractory large B-cell lymphoma. *Blood*. 2023;141(20):2470-2482. doi:10.1182/blood.2022018598
96. Saifi O, Breen WG, Rule WG, et al. Comprehensive bridging radiotherapy for limited pre-CART non-Hodgkin lymphoma. *JAMA Oncol*. 2024:e241113. In press. doi:10.1001/jamaoncol.2024.1113
97. Saifi O, Lester SC, Breen WG, et al. Incorporating radiation with anti-CD19 chimeric antigen receptor T-cell therapy for relapsed/refractory non-Hodgkin lymphoma: a multicenter consensus approach. *Am J Hematol*. 2024;99(1):124-134. doi:10.1002/ajh.27155
98. DeSelm C, Palomba ML, Yahalom J, et al. Low-dose radiation conditioning enables CAR T cells to mitigate antigen escape. *Mol Ther*. 2018;26(11):2542-2552. doi:10.1016/j.ymthe.2018.09.008
99. Liebers N, Duell J, Fitzgerald D, et al. Polatuzumab vedotin as a salvage and bridging treatment in relapsed or refractory large B-cell lymphomas. *Blood Adv*. 2021;5(13):2707-2716. doi:10.1182/bloodadvances.2020004155
100. Kuhnl A, Roddie C, Kirkwood AA, et al. Outcome and feasibility of radiotherapy bridging in large B-cell lymphoma patients receiving CD19 CAR T in the UK. *Br J Haematol*. 2024. In press. doi:10.1111/bjh.19453
101. Song EZ, Milone MC. Pharmacology of chimeric antigen receptor-modified T cells. *Annu Rev Pharmacol Toxicol*. 2021;61:805-829. doi:10.1146/annurev-pharmtox-031720-102211
102. Henning AN, Roychoudhuri R, Restifo NP. Epigenetic control of CD8$^+$ T cell differentiation. *Nat Rev Immunol*. 2018;18(5):340-356. doi:10.1038/nri.2017.146
103. van den Broek T, Borghans JAM, van Wijk F. The full spectrum of human naive T cells. *Nat Rev Immunol*. 2018;18(6):363-373. doi:10.1038/s41577-018-0001-y
104. Ando M, Ito M, Srirat T, Kondo T, Yoshimura A. Memory T cell, exhaustion, and tumor immunity. *Immunol Med*. 2020;43(1):1-9. doi:10.1080/25785826.2019.1698261
105. Chan JD, Lai J, Slaney CY, Kallies A, Beavis PA, Darcy PK. Cellular networks controlling T cell persistence in adoptive cell therapy. *Nat Rev Immunol*. 2021;21(12):769-784. doi:10.1038/s41577-021-00539-6
106. Štach M, Pytlík R, Šmilauerová K, et al. Characterization of the input material quality for the production of tisagenlecleucel by multiparameter flow cytometry and its relation to the clinical outcome. *Pathol Oncol Res*. 2023;29:1610914. doi:10.3389/pore.2023.1610914

107. Wang Y, Tong C, Lu Y, et al. Characteristics of premanufacture CD8+ T cells determine CAR-T efficacy in patients with diffuse large B-cell lymphoma. *Signal Transduct Target Ther*. 2023;8(1):409. doi:10.1038/s41392-023-01659-2
108. Filosto S, Vardhanabhuti S, Canales MA, et al. Product attributes of CAR T-cell therapy differentially associate with efficacy and toxicity in second-line large B-cell lymphoma (ZUMA-7). *Blood Cancer Discov*. 2023;5:21-33. doi:10.1158/2643-3230.Bcd-23-0112
109. Worel N, Grabmeier-Pfistershammer K, Kratzer B, et al. The frequency of differentiated $CD3^+CD27^-CD28^-$ T cells predicts response to CART cell therapy in diffuse large B-cell lymphoma. *Front Immunol*. 2023;13:1004703. doi:10.3389/fimmu.2022.1004703
110. Locke FL, Filosto S, Chou J, et al. Impact of tumor microenvironment on efficacy of anti-CD19 CAR T cell therapy or chemotherapy and transplant in large B cell lymphoma. *Nat Med*. 2024;30:507-518. doi:10.1038/s41591-023-02754-1
111. Deng Q, Han G, Puebla-Osorio N, et al. Characteristics of anti-CD19 CAR T cell infusion products associated with efficacy and toxicity in patients with large B cell lymphomas. *Nat Med*. 2020;26(12):1878-1887. doi:10.1038/s41591-020-1061-7
112. Wang X, Borquez-Ojeda O, Stefanski J, et al. Depletion of high-content $CD14^+$ cells from apheresis products is critical for successful transduction and expansion of CAR T cells during large-scale cGMP manufacturing. *Mol Ther Methods Clin Dev*. 2021;22:377-387. doi:10.1016/j.omtm.2021.06.014
113. Locke FL, Ghobadi A, Jacobson CA, et al. Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1–2 trial. *Lancet Oncol*. 2019;20(1):31-42. doi:10.1016/s1470-2045(18)30864-7
114. Monfrini C, Stella F, Aragona V, et al. Phenotypic composition of commercial anti-CD19 CAR T cells affects in vivo expansion and disease response in patients with large B-cell lymphoma. *Clin Cancer Res*. 2022;28(15):3378-3386. doi:10.1158/1078-0432.Ccr-22-0164
115. Awasthi R, Pacaud L, Waldron E, et al. Tisagenlecleucel cellular kinetics, dose, and immunogenicity in relation to clinical factors in relapsed/refractory DLBCL. *Blood Adv*. 2020;4(3):560-572. doi:10.1182/bloodadvances.2019000525
116. Wada F, Jo T, Arai Y, et al. T-cell counts in peripheral blood at leukapheresis predict responses to subsequent CAR-T cell therapy. *Sci Rep*. 2022;12(1):18696. doi:10.1038/s41598-022-23589-9
117. Jo T, Yoshihara S, Okuyama Y, et al. Risk factors for CAR-T cell manufacturing failure among DLBCL patients: a nationwide survey in Japan. *Br J Haematol*. 2023;202(2):256-266. doi:10.1111/bjh.18831
118. Turtle CJ, Hanafi LA, Berger C, et al. Immunotherapy of non-Hodgkin's lymphoma with a defined ratio of $CD8^+$ and $CD4^+$ CD19-specific chimeric antigen receptor-modified T cells. *Sci Transl Med*. 2016;8(355):355ra116. doi:10.1126/scitranslmed.aaf8621
119. Chong EA, Ruella M, Schuster SJ. Five-year outcomes for refractory B-cell lymphomas with CAR T-cell therapy. *N Engl J Med*. 2021;384(7):673-674. doi:10.1056/NEJMc2030164
120. Labanieh L, Majzner RG, Mackall CL. Programming CAR-T cells to kill cancer. *Nat Biomed Eng*. 2018;2(6):377-391. doi:10.1038/s41551-018-0235-9
121. Mangal JL, Handlos JL, Esrafili A, et al. Engineering metabolism of chimeric antigen receptor (CAR) cells for developing efficient immunotherapies. *Cancers*. 2021;13(5):1123. doi:10.3390/cancers13051123
122. Weinkove R, George P, Dasyam N, McLellan AD. Selecting costimulatory domains for chimeric antigen receptors: functional and clinical considerations. *Clin Transl Immunol*. 2019;8(5):e1049. doi:10.1002/cti2.1049
123. Kawalekar OU, O'Connor RS, Fraietta JA, et al. Distinct signaling of coreceptors regulates specific metabolism pathways and impacts memory development in CAR T cells. *Immunity*. 2016;44(2):380-390. doi:10.1016/j.immuni.2016.01.021
124. Turtle CJ, Hanafi LA, Berger C, et al. CD19 CAR-T cells of defined $CD4^+$:$CD8^+$ composition in adult B cell ALL patients. *J Clin Invest*. 2016;126(6):2123-2138. doi:10.1172/jci85309
125. Salter AI, Ivey RG, Kennedy JJ, et al. Phosphoproteomic analysis of chimeric antigen receptor signaling reveals kinetic and quantitative differences that affect cell function. *Sci Signal*. 2018;11(544):eaat6753. doi:10.1126/scisignal.aat6753
126. Good Z, Spiegel JY, Sahaf B, et al. Post-infusion CAR TReg cells identify patients resistant to CD19-CAR therapy. *Nat Med*. 2022;28(9):1860-1871. doi:10.1038/s41591-022-01960-7
127. Chan JD, Scheffler CM, Munoz I, et al. FOXO1 enhances CAR T cell stemness, metabolic fitness and efficacy. *Nature*. 2024;629:201-210. doi:10.1038/s41586-024-07242-1
128. Doan AE, Mueller KP, Chen AY, et al. FOXO1 is a master regulator of memory programming in CAR T cells. *Nature*. 2024;629:211-218. doi:10.1038/s41586-024-07300-8
129. Gauthier J, Gazeau N, Hirayama AV, et al. Impact of CD19 CAR T-cell product type on outcomes in relapsed or refractory aggressive B-NHL. *Blood*. 2022;139(26):3722-3731. doi:10.1182/blood.2021014497
130. Alvarez-Fernández C, Escribà-Garcia L, Caballero A, et al. Memory stem T cells modified with a redesigned CD30-chimeric antigen receptor show an enhanced antitumor effect in Hodgkin lymphoma. *Clin Transl Immunol*. 2021;10(4):e1268. doi:10.1002/cti2.1268
131. Xu Y, Zhang M, Ramos CA, et al. Closely related T-memory stem cells correlate with in vivo expansion of CAR.CD19-T cells and are preserved by IL-7 and IL-15. *Blood*. 2014;123(24):3750-3759. doi:10.1182/blood-2014-01-552174
132. Sabatino M, Hu J, Sommariva M, et al. Generation of clinical-grade CD19-specific CAR-modified $CD8^+$ memory stem cells for the treatment of human B-cell malignancies. *Blood*. 2016;128(4):519-528. doi:10.1182/blood-2015-11-683847
133. Jiang W, He Y, He W, et al. Exhausted $CD8^+$ T cells in the tumor immune microenvironment: new pathways to therapy. *Front Immunol*. 2021;11:622509. doi:10.3389/fimmu.2020.622509
134. Gumber D, Wang LD. Improving CAR-T immunotherapy: overcoming the challenges of T cell exhaustion. *EBioMedicine*. 2022;77:103941. doi:10.1016/j.ebiom.2022.103941
135. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. *Nat Rev Immunol*. 2015;15(8):486-499. doi:10.1038/nri3862
136. Scholler N, Perbost R, Locke FL, et al. Tumor immune contexture is a determinant of anti-CD19 CAR T cell efficacy in large B cell lymphoma. *Nat Med*. 2022;28(9):1872-1882. doi:10.1038/s41591-022-01916-x
137. Pearce EL, Poffenberger MC, Chang CH, Jones RG. Fueling immunity: insights into metabolism and lymphocyte function. *Science*. 2013;342(6155):1242454. doi:10.1126/science.1242454.
138. Fahrmann JF, Saini NY, Chia-Chi C, et al. A polyamine-centric, blood-based metabolite panel predictive of poor response to CAR-T cell therapy in large B cell lymphoma. *Cell Rep Med*. 2022;3(11):100720. doi:10.1016/j.xcrm.2022.100720
139. Schwabe RF, Jobin C. The microbiome and cancer. *Nat Rev Cancer*. 2013;13(11):800-812. doi:10.1038/nrc3610
140. Smith M, Dai A, Ghilardi G, et al. Gut microbiome correlates of response and toxicity following anti-CD19 CAR T cell therapy. *Nat Med*. 2022;28(4):713-723. doi:10.1038/s41591-022-01702-9