Received: 9 June 2024 | Accepted: 26 August 2024

DOI: 10.1111/bjh.19751

BJHaem
BRITISH JOURNAL OF HAEMATOLOGY

REVIEW

Chimeric antigen receptor T-cell therapy for haematological malignancies: Insights from fundamental and translational research to bedside practice

Céline Grégoire[1,2] | **Beatriz Coutinho de Oliveira**[1] | **Paolo F. Caimi**[3] | **Jo Caers**[2] | **Jan Joseph Melenhorst**[1]

[1]Center for ImmunoTherapy and Precision Immuno-Oncology (CITI), Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio, USA

[2]Department of Clinical Hematology and Laboratory of Hematology (GIGA I3), University Hospital Center of Liège and University of Liège, Liège, Belgium

[3]Department of Hematology and Oncology, Cleveland Clinic Taussig Cancer Institute, Cleveland, Ohio, USA

Correspondence
Jan Joseph Melenhorst, Center for ImmunoTherapy and Precision Immuno-Oncology (CITI), Lerner Research Institute, Cleveland Clinic, Cleveland, OH, USA.
Email: melenhj@ccf.org

Funding information
Cleveland Clinic Centre of Excellence; Velosano

Summary
Autologous chimeric antigen receptor (CAR) T-cell therapy has revolutionized the treatment of lymphoid malignancies, leading to the approval of CD19-CAR T cells for B-cell lymphomas and acute leukaemia, and more recently, B-cell maturation antigen-CAR T cells for multiple myeloma. The long-term follow-up of patients treated in the early clinical trials demonstrates the possibility for long-term remission, suggesting a cure. This is associated with a low incidence of significant long-term side effects and a rapid improvement in the quality of life for responders. In contrast, other types of immunotherapies require prolonged treatments or carry the risk of long-term side effects impairing the quality of life. Despite impressive results, some patients still experience treatment failure or ultimately relapse, underscoring the imperative to improve CAR T-cell therapies and gain a better understanding of their determinants of efficacy to maximize positive outcomes. While the next-generation of CAR T cells will undoubtingly be more potent, there are already opportunities for optimization when utilizing the currently available CAR T cells. This review article aims to summarize the current evidence from clinical, translational and fundamental research, providing clinicians with insights to enhance their understanding and use of CAR T cells.

KEYWORDS
biomarkers, CAR T-cell, immunotherapy, leukaemia therapy, multiple myeloma, non-Hodgkin's lymphoma

INTRODUCTION

T cells are potent components of adaptive immunity, relying on their T-cell receptor (TCR) to selectively recognize any peptide presented by the major histocompatibility complex (MHC) proteins. In addition to the TCR-peptide–MHC interaction (signal 1), their activation depends on costimulation (signal 2) and cytokines (signal 3). As a mechanism of escape, tumour cells can downregulate their expression of MHC and costimulatory molecules or express co-inhibitory molecules. B cells are activated by directly recognizing an antigen through their B-cell receptor (BCR) without the need for MHC presentation. Chimeric antigen receptors (CARs) are receptors engineered using components of the BCR and TCR to leverage the strengths of both receptors: the variable fragment of the BCR enables direct recognition of a surface peptide without the need for MHC presentation, while the costimulatory domain (4-1BB and/or CD28) and activation domain (CD3ζ) from the TCR complex transmit a potent activation signal following antigen recognition.

To produce CAR T cells, autologous T cells are first collected via apheresis, then activated and transduced to

express the CAR. Following this, the cells are expanded in vitro before being re-infused into the patient. Their encounter with the antigen in vivo prompts activation, proliferation and tumour killing. Notably, CAR T cells not only induce responses, including complete remissions, but also demonstrate the potential for sustaining long-term remission in a subset of patients, suggesting the potential for achieving disease cure.[1–3] The success of CAR T cells relies on (a) the targeting of an antigen selectively expressed on tumour cells and (b) the presence of functional T cells, capable of both cytotoxic and memory functions. Numerous aspects of the CAR design can influence CAR T-cell function and efficacy. The significance of incorporating a costimulatory domain is evidenced by the limited efficacy of first-generation CARs lacking this feature, and the remarkable enhancement observed in second-generation CARs upon inclusion of a 4-1BB or CD28 costimulatory domain.[4] CAR T cells engineered with different costimulatory domains have never been directly compared in clinical trials, but preclinical studies have demonstrated functional difference between CAR T cells manufactured with a 4-1BB costimulatory domain (BBζ-CAR) or the CD28 costimulatory domain (28ζ-CAR) (reviewed in Ref [5]). Results have varied depending on the experimental design (for instance regarding the propensity for activation-induced cell death),[5] but overall findings indicate a higher cytokine secretion by 28ζ-CAR T cells and a prolonged persistence of BBζ-CAR T cells,[4] in relation to a lower level of tonic signalling (defined as ligand-independent constitutive signalling).[6] Indeed, sustained CAR signalling induced by elevated tonic signalling can lead to exhaustion (a state of altered effector function and proliferation in T cells exposed to chronic antigen stimulation) and subsequent lack of CAR T-cell persistence.[6]

Several other factors affect the efficacy of CAR T cells including T-cell fitness, the manufacturing process, tumour antigen expression and its interaction with the tumour microenvironment (TME). Many of these aspects still require comprehensive examination through both preclinical and translational studies to design next-generation CAR T cells capable of unlocking the full potential of this therapeutic modality.

OUTCOMES OF CURRENT CAR T-CELL THERAPIES: KEY RESULTS FROM CLINICAL TRIALS (SUMMARIZED IN TABLE 1; FIGURE 1)

The first approved CAR T-cell products were CD19-CAR T cells, initially the BBz-CAR tisagenlecleucel (Tisa-cel) for relapsed/refractory (R/R) B-cell acute lymphoblastic leukaemia (B-ALL) in children and young adults, and the 28z-CAR axicabtagene ciloleucel (Axi-cel) for R/R large B-cell lymphoma (LBCL) after ≥2 lines of previous therapy. These approvals were based on the impressive results of the ELIANA trial in paediatric B-ALL (overall response rate [ORR] of 81% with complete response [CR] rate of 60%)[7] and the ZUMA-1 trial in LBCL (ORR 82%, CR 54%)[10] respectively. Subsequently, Tisa-cel was approved for R/R LBCL[8] and brexucabtagene autoleucel (Brexu-Cel) (similar to Axi-cel, but with a T-cell enrichment phase in the manufacturing process to remove the malignant cells present in the apheresis product) for R/R mantle cell lymphoma (MCL),[15] as well as for adult B-ALL.[14,22] Another CAR T-cell product, the BBz-CAR lisocabtagene maraleucel (Liso-cel), was later developed and approved in R/R LBCL.[16] This product comprises separate formulations of $CD8^+$ (enriched in central memory T cells [T_{CM}]) and $CD4^+$ T cells at a 1:1 ratio, infused sequentially,[23] which was shown to be superior to unselected CAR T cells in preclinical analyses.[24] CAR T cells have also demonstrated their efficacy in indolent B-cell malignancies: Axi-cel and Tisa-cel are now approved for the treatment of R/R follicular lymphoma (FL),[3,9,25] as well as Liso-cel for R/R chronic lymphoid leukaemia (CLL).[19] Importantly, although results are lower in CLL than in other B-cell malignancies (ORR/CR rates 43%/18% in the TRANSCEND CLL 004 trial),[19] CD19-CAR T cells can induce decade-long remissions in a subset of CLL patients.[1]

The four commercial CD19-CAR T-cell products have never been directly compared in a clinical trial. Most indirect comparisons have shown a higher toxicity of Axi-cel, while no definitive conclusion on efficacy can be drawn (possible superior efficacy of Tisa-cel in ALL, and similar or lower efficacy in LBCL[26–29]). Recent and ongoing trials in B-cell malignancies aim to assess their efficacy in earlier lines of therapy (Table 2). The three products used in LBCL have been evaluated against the current standard of care (SOC) second-line therapy (platinum-based immunochemotherapy followed by autologous haematopoietic stem cell transplantation [autoHSCT]). While Tisa-cel failed to meet the primary outcome of event-free survival (EFS) (BELINDA trial[30]), treatment with Axi-cel (ZUMA-7 trial[11]) and Liso-cel (TRANSFORM trial[17]) significantly improved EFS compared to the SOC arm. Notably, no bridging therapy was allowed in the Axi-cel trial, except for corticosteroids, which likely resulted in enrolling only patients with less aggressive disease. Regarding the Tisa-cel trial, several factors might have contributed to the negative results, including an imbalance between the two groups, the study design (i.e. two lines of immunochemotherapy allowed as bridging therapy) and the definition of the outcomes (EFS evaluated at week 12 after randomization, which might have been too soon to assess the response, given the long duration from leukapheresis to infusion in this study) (reviewed in Ref [31]). Despite the overall positive results, a debate has persisted regarding whether CAR T cells should be used in second line or reserved for further relapse after autoHSCT.[32] However, the long-term follow-up with Axi-cel is now available, demonstrating a significant advantage in overall survival (OS).[12]

Regarding multiple myeloma, two BBzBCMA-CAR T-cell products have been approved following impressive results (far superior to historical SOC) in phase 2 trials in R/R multiple myeloma: ORR/CR rates of 73%/33% with

TABLE 1 Currently FDA-approved indications for CAR T-cell therapies, and the trials leading to their approval.

Product	Structure	Disease	Phase	Indication	Study	Results (ORR/CR)	Ref
Tisa-cel (Kymriah)	CD19-BBζ	B-ALL	2	R/R, ≤25 years	ELIANA	81%/60% (CRi 21%)	7
		LBCL	2	R/R after ≥2 lines of therapy	JULIET	53%/39%	8
		FL	2	R/R after ≥2 lines of therapy	ELARA	86%/69%	9
Axi-cel (Yescarta)	CD19-28ζ	LBCL	2	R/R after ≥2 lines of therapy	ZUMA-1	82%/54%	10
			3	Refractory to or relapsing at <12 months of first-line therapy	ZUMA-7	83%/65% (vs. 50%/32% in SOC arm[a])	11, 12
			2	Refractory to or relapsing at <12 months of first-line therapy, ineligible for autoHSCT	ALYCANTE	90%/79%	13
		FL	2	R/R after ≥2 lines of therapy	ZUMA-5	92%/74%	3
Brexu-Cel (Tecartus)	CD19-28ζ	B-ALL	2	R/R, ≥18 years	ZUMA-3	71%/56% (CRi 15%)	14
		MCL	2	R/R	ZUMA-2	91%/68%	15
Liso-cel (Breyanzi)	CD19-BBζ, with a 1:1 CD4:CD8 ratio	LBCL	2	R/R after ≥2 lines of therapy	TRANSCEND NHL 001	73%/53%	16
			3	Refractory to or relapsing at <12 months of first-line therapy	TRANSFORM	80%/74% (vs 45%/43% in SOC arm[a])	17
			2	R/R and not eligible for autoHSCT	PILOT	80%/54%	18
		CLL		R/R after ≥2 lines of therapy[b]	TRANSCEND CLL 004	43%/18%	19
Ide-cel (Abecma)	BCMA-BBζ	Multiple myeloma	2	R/R after ≥4 lines of therapy[c]	KarMMa	73%/33%	20
Cilta-cel (Carvykti)	Dual BCMA-BBζ	Multiple myeloma	2	R/R after ≥2 lines of therapy[c]	CARTITUDE-1	97%/67%	21

Abbreviations: autoHSCT, autologous haematopoietic stem cell transplantation; B-ALL, B-cell acute lymphoblastic leukaemia; BCMA, B-cell maturation antigen; CAR, chimeric antigen receptor; CLL, chronic lymphocytic leukaemia; CR, complete remission; CRi, complete remission with incomplete haematological recovery; FDA, Food and Drug Administration; FL, follicular lymphoma; LBCL, large B-cell lymphoma; MCL, mantle cell lymphoma; ORR, overall response rate; R/R, relapsed/refractory; SOC, standard of care.

[a]SOC arm: immunochemotherapy + autoHSCT.

[b]Including a Bruton's tyrosine kinase (BTK) inhibitor and a B-cell lymphoma 2 (BCL-2) inhibitor.

[c]Including an immunomodulatory agent, a proteasome inhibitor and an anti-CD38 monoclonal antibody.

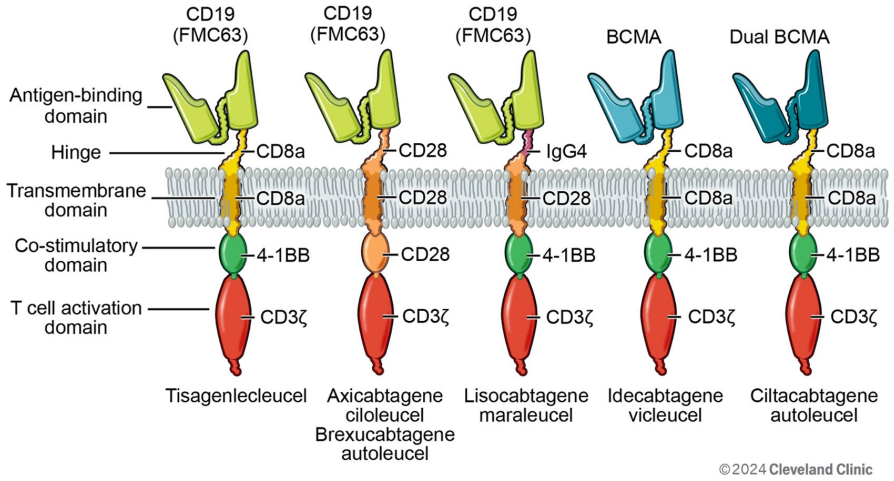

FIGURE 1 Schematic structure of the currently Food and Drug Administration-approved indications for chimeric antigen receptor T-cell therapies. Illustration by David Schumick, BS, CMI. Reprinted with the permission of the Cleveland Clinic Enterprise Creative Services © 2024. All Rights Reserved. BCMA, B-cell maturation antigen.

idecabtagene vicleucel (Ide-cel) (≥4 lines of therapy) and 97%/67% with ciltacabtagene autoleucel (Cilta-cel) (≥2 lines of therapy).[20,21] Intriguingly, while both products are undetectable 3–6 months after infusion, some patients experience durable remission.[33,34] Whether CAR T cells can cure multiple myeloma, despite this apparent absence of persistence, is still to be determined by longer follow-up studies. Further phase 3 clinical trials with both B-cell maturation antigen (BCMA)-CAR T cells confirmed their superiority over SOC.[35,36] Similar to the trend in B-cell malignancies, the benefits of CAR T cells when used as an earlier line of therapy in multiple myeloma is currently being explored, and Cilta-cel has been recently approved by the Food and Drug Administration (FDA) in patients with R/R multiple myeloma who have received at least one prior line of therapy, including a proteasome inhibitor and an immunomodulatory agent, and are refractory to lenalidomide.

FACTORS INFLUENCING CAR T-CELL THERAPY OUTCOMES: INSIGHTS FROM CORRELATIVE AND RETROSPECTIVE STUDIES (SUMMARIZED IN TABLE 3; FIGURE 2)

T-cell subsets and CAR T-cell proliferation

A consistent observation across most clinical trials, regardless of the CAR T-cell product and disease, is the correlation between the in vivo expansion of CAR T cells following infusion, as assessed by flow cytometry and/or quantitative polymerase chain reaction, and the efficacy and duration of response.[10,11,14,15,25,30,34,37–39] In most of these trials, a higher CAR T-cell expansion was also associated with a higher risk of severe acute toxicity, but prevention and management of these side effects improved with time. In some cohorts, expansion of the BBz-CAR T cells also correlated with tumour burden.[16,76]

Although effector cells are needed for the immediate cytotoxic function of CAR T cells, several studies have now demonstrated that the proliferation, efficacy and persistence of CAR T cells depend on the proportion of early memory or stem cell memory (SCM) T cells.[12,40–44] In CLL patients treated with BBz-CAR T cells, a higher frequency of early memory $CD8^+$ T cells ($CD27^+CD45RO^-$, expressing the memory-related genes *TCF7* and *LEF1*) was observed in the apheresis product of responders, and these patients had a significantly higher CAR T-cell expansion after infusion. On the contrary, non-responder patients had more effector cells, with features related to glycolysis, exhaustion and apoptosis, co-expressing more inhibitory receptors such as PD-1 associated with LAG3 or TIM3, and expanding significantly less in vivo. This was proved to be mechanistically relevant as this population of $CD27^+PD\text{-}1^-$ early memory $CD8^+$ CAR T cells derived from patient cells was essential to obtain a tumour control in mice.[41] Similar observations on the importance of early memory T cells were made in LBCL[45,46] and ALL.[43,47] In LBCL, poor responders have a higher proportion of exhausted $CD8^+$ CAR T cells upregulating the inhibitory receptor TIGIT, and TIGIT blockade improves CAR T-cell function in vivo.[48] A higher frequency of $LAG3^+TNFa^{low}$ CD8 T cells in the starting material has also been associated with negative outcomes in young ALL patients.[49] Similarly, a lower level of $LAG3^+$ cells in the TME was associated with longer PFS and duration of remission (DOR) in FL.[25] When examining the clonal diversity of $CD8^+$ CAR T cells, a progressive decrease is observed, indicating that CAR T-cell expansion relies on the expansion of a few clones.[77] The CAR T_{SCM}, although present in low proportion in the manufactured product, undergoes a rapid expansion after infusion

TABLE 2 Selected phase 2/3 clinical trials with FDA-approved CD19-CAR T-cell therapies in B-cell malignancies.

Product	Structure	Disease	Phase	Indication	Intervention	Study	NCT
Tisa-cel (Kymriah)	BBζ	FL	3	R/R ≥2 lines	Tisa-cel vs. SOC (R2 or R-CHOP)	LEDA	05888493
		B-ALL	2	≤25 years, CR MRD^{-}, previous first infusion	Early second infusion (30–60 days after the first dose)	REFUEL	05460533
			2	≤25 years, HR, EOC MRD^{+}	Tisa-cel	CASSIOPEIA	03876769
			1/2	≤25 years, early loss of BCA after first infusion	Second infusion + nivolumab	CAPTiRALL	05310591
Axi-cel (Yescarta)	28ζ	LBCL	3	First line, HR (IPI 4–5)	After two cycles of R-chemo: Axi-cel vs. SOC (four additional cycles)	ZUMA-23	05605899
			2	R/R ≥1 line	Axi-cel + prophylactic corticosteroids	ZUMA-24	05459571
			2	R/R ≥1 line, ineligible to autoHSCT	Axi-cel	ALYCANTE	04531046
		FL	3	R/R, ≥1 line and HR (POD24) or ≥2 lines	Axi-cel vs. SOC (R2 or R-CHOP)	ZUMA-22	05371093
Brexu-Cel (Tecartus)	28ζ	B-ALL	2	Adult, CR but MRD^{+} after induction	Brexu-Cel	-	06144606
		Rare B-cell malignancies[a]	2	R/R ≥1 line	Brexu-Cel	ZUMA-25	05537766
Liso-cel (Breyanzi)	BBζ, with a 1:1 CD4:CD8 ratio	LBCL	2	R/R after ≥2 lines	Acalabrutinib + Liso-cel	-	05583149
		Richter's Transformation	2	R/R ≥1 line	Zanibrutinib + Liso-cel	-	05873712
			2	R/R ≥2 lines, or ≥1 line if refractory, relapse <1 year or HSCT-ineligible	Liso-cel + nivolumab + ibrutinib	-	05672173

Abbreviations: autoHSCT, autologous haematopoietic stem cell transplantation; B-ALL, B-cell acute lymphoblastic leukaemia; BCA, B-cell aplasia; CAR, chimeric antigen receptor; CR, complete remission; EOC, end of consolidation; FDA, Food and Drug Administration; FL, follicular lymphoma; HR, high-risk; IPI, International Prognostic Index; LBCL, large B-cell lymphoma; MRD, minimal residual disease; POD24, progression of disease within 24 months; R/R, relapsed/refractory; R2, rituximab, lenalidomide (Revlimid); R-CHOP, rituximab, cyclophosphamide, doxorubicin hydrochloride (hydroxydaunomycin), vincristine sulphate (oncovin), prednisone; SOC, standard of care.

[a]Waldenstrom macroglobulinaemia, Richter transformation, Burkitt lymphoma, hairy cell leukaemia.

TABLE 3 Factors associated with resistance to CAR T-cell therapy.

T cells	- Poor in vivo expansion following infusion	10, 11, 14, 15, 25, 30, 34, 37–39
	- Low proportion of early memory or SCM T-cells	12, 34, 40–47
	- High proportion of senescent $CD8^+$ T-cells	34
	- High proportion of exhausted $CD8^+$ T-cells	25, 48, 49
	- High proportion of Treg	33, 45, 50
	- Low proportion of type 2 cells	47
	- High expression of genes involved in IFN response and type 1 differentiation	43
Tumour-related factors	Tumour burden	
	- Lymphoma	
	○ High tumour volume	13, 25, 42, 51–53
	○ High circulating tumour DNA	54
	○ Number of extranodal sites	53
	- ALL: high percentage of BM blasts	26, 55, 56
	- Multiple myeloma: high BM infiltration, high serum BCMA	33, 57
	Tumour genetic alterations	
	- *TP53* genomic alterations	54, 58
	- DNA copy number alterations	59
	- Loss of the FAS death receptor[59]	59
	- Mutation of *MYC*, *BCL2* or *CDKN2A*	60
	Tumour inflammation/immunosuppressive microenvironment	
	- Tumour IFN signature	51
	- Tumour expression of T-cell inhibitory ligands (including PD-L1 and PD-L2)	51
	- Intra-tumoural regulatory T cells	54
	- Higher stromal and immunosuppressive signature (including hypoxia)	61
Systemic factors	- Systemic inflammation (IL6, ferritin, CRP)	42, 51, 62
	- Circulating M-MDSCs	51
Therapy-related factors	- Higher number of previous lines of therapy	62
	- Recent exposure to bendamustine	63–66
	- Previous exposure to blinatumomab in ALL?	26, 67, 68
	- Previous exposure to other BCMA-directed therapies in multiple myeloma?	69, 70
	- Absence of response to bridging therapy?	71
	- Suboptimal lymphodepletion	33, 72–75

Abbreviations: ALL, acute lymphoblastic leukaemia; BCMA, B-cell maturation antigen; BM, bone marrow; CRP, C-reactive protein; IFN, interferon; IL6, interleukin 6; M-MDSCs, monocytic myeloid-derived suppressor cells.; SCM, stem cell memory; Treg, regulatory T cells.

and contribute the most to the clonal pool in patients with CAR T-cell persistence.[78] A similar importance of naïve and early memory T cells has been observed with Ide-cel in multiple myeloma.[34] Overall, these findings underscore the crucial role of a functional $CD8^+$ cell pool capable of sustaining both self-renewal and differentiation into effector cells, ensuring not only optimal functionality but also persistence, thereby providing a long-term anti-tumour immune response.

The anti-tumour efficacy of CAR T cells relies not only on $CD8^+$ but also on $CD4^+$ CAR T cells, and $CD4^+$ CAR T cells support the proliferation and persistence of $CD8^+$ CAR T cells.[24] In the longest phenotypic follow-up available to date, BBz-CAR T cells persisting for 10 years in two CLL patients, CAR T-cell kinetics could be divided into two phases: (a) an initial expansion of $CD8^+$ CAR T cells and also $CD4^-CD8^-$ γδ CAR T cells expressing cytotoxic markers, and (b) a long-term remission associated with a dominating $CD4^+$ CAR T-cell population. Those long-lasting $CD4^+$ CAR T cells managed to remain functional despite chronic antigen stimulation, showing signs of ongoing activation (high expression levels of activation markers as well as co-inhibitory proteins such as PD1, TIGIT and CTLA4; secretion of cytokines and production of cytotoxic enzymes) and proliferation (associated with oxidative phosphorylation and aerobic glycolysis).[1] A population of $CD4^-CD8^-$ with a similar signature (by unbiased cell-to-cell matching) was also identified as long-lasting functional CAR T cells in paediatric B-ALL treated with a low-affinity CD19-CAR T-cell product.[79] Different subsets of T helper cells also have

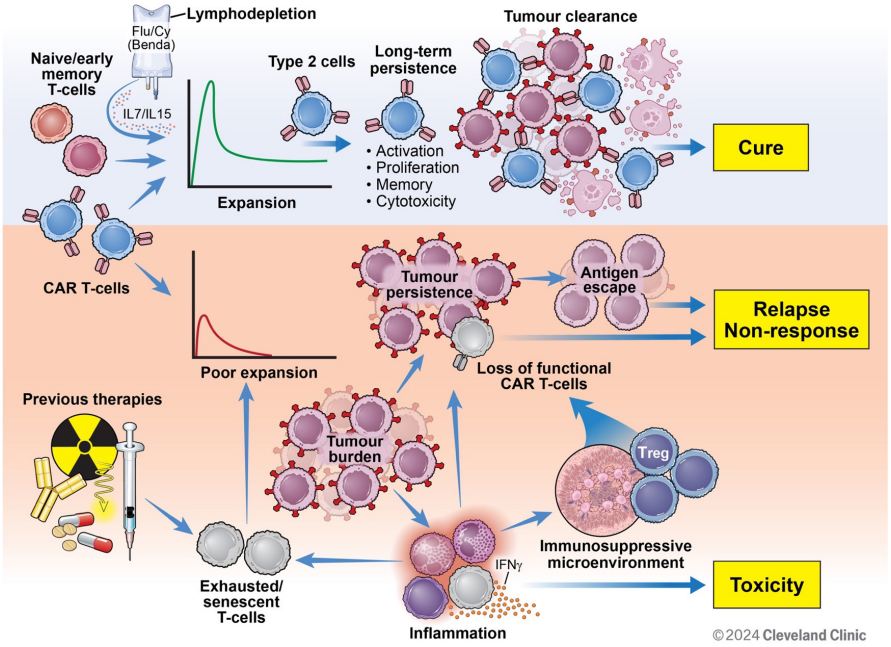

FIGURE 2 Major determinants of response and failure after CAR T-cell therapy. Illustration by David Schumick, BS, CMI. Reprinted with the permission of the Cleveland Clinic Enterprise Creative Services © 2024. All Rights Reserved. CAR, chimeric antigen receptor; Flu/Cy, fludarabine/cyclophosphamide; IFN, interferon; IL, interleukin.

different impact on CAR T-cell efficacy. Regulatory $CD4^+$ T cells (T_{Reg}) were shown to be associated with lower response rate (but also lower neurotoxicity) in LBCL patients treated with the 28ζ-CAR T-cell product Axi-cel,[45,50] as well as in multiple myeloma patients treated with Ide-cel.[33] Moreover, in addition to early memory cells, T helper 2 (Th2) cells (producing interleukin 4 [IL4], IL5 and IL13) are associated with long-term response in paediatric ALL.[47] Similarly, loss of CAR T cells in ALL patients is associated with high expression of genes involved in interferon (IFN) response and Th1 differentiation in the apheresis product.[43] Furthermore, preclinical data show that blockage of IFNg signalling does not affect CAR T-cell killing of tumour cells and mitigates toxicity, confirming that production of IFNg by CAR T cells is not responsible for their efficacy in haematological malignancies.[80,81] Moreover, accumulating evidence underscores the role of IFNg in the toxicity observed following CAR T-cell infusion, including cytokine release syndrome (CRS) and neurological toxicities,[81] and early rise of IFNg can even be used as a predictive biomarker of severe CRS in ALL.[82] Additionally, prolonged cytopenia has been associated with BM infiltration by clonal $CD8^+$ T cells expressing IFNg (and increased expression of IFNg-induced genes in haematopoietic stem cells)[83] and higher peak serum of IFNg.[84] Finally, instead of a specific T-cell subtype, the polyfunctionality (ability to secrete several cytokines and/or chemokines) of 28z-CAR T cells from lymphoma patients upon in vitro stimulation has been associated with better outcomes (but also higher incidence of severe CRS).[85]

Lentiviral integration sites have also been linked to outcomes after CAR T-cell infusion. Because CAR T cells are manufactured using viruses to integrate the CAR into the genome, the site of the CAR integration is not controlled, yielding a heterogeneous CAR T-cell product with varied integration sites. The location of CAR insertion within the genome can disrupt certain genes, thereby impacting the expansion, phenotype and function of CAR T cells in either positive or negative ways. This was discovered in a CLL patient who experienced long-term remission associated with the expansion of a predominant clone displaying a T_{CM} phenotype. Dissecting the mechanism of this clonal expansion, it appeared that this patient presented a hypomorphic mutation in one allele of the TET2 gene, and that the CAR transgene was inserted in the other allele of this single clone, resulting in a low TET2 activity in this CAR T-cell clone.[86] A recent study correlating the efficacy of BCMA CAR-T cells with the CAR density suggested that a higher CAR density on the surface of T cells, resulting from a greater number of viral integrations, could be predictive of poor outcomes in patients due to high tonic signalling and exhaustion-like signatures.[87]

Correlations between the infused CAR T-cell dose and anti-tumour responses were not consistently found in previous studies in CLL[38] and ALL,[55,88] but a correlation was observed with associated toxicity.[23,89,90] However, response of multiple myeloma to Ide-cel was dose-dependent in a dose escalation phase.[34] Overall, the total dose of CAR T cells infused is probably not the most critical parameter, but rather the composition of the CAR T-cell product, and low dose of highly functional CAR T cells with high proliferative capacity is sufficient to induce response,[91] while high dose of pro-inflammatory effector CAR T cells might result in higher toxicity.

Tumour characteristics, tumour burden and inflammation

Several tumour-related factors have been identified as predictors of poor response to CAR T-cell therapy, and some of them differ from the prognostic factors following conventional treatments. In the randomized trials, CAR T-cell therapy improved outcomes compared to SOC even in categories associated with lower response to conventional treatments, such as high International Prognostic Index or activated B-cell-like LBCL.[11,92] On the contrary, resistance to CAR T-cell therapy in LBCL was found to be associated with TP53 genomic alterations (which correlates with reduced tumour infiltration by $CD8^+$ T cells and dysregulation of cytotoxic pathways[54,58]), higher DNA copy number alterations and loss of the FAS death receptor.[59] However, the impact of TP53 mutations on CAR T-cell responses is still unclear, as it was not predictive of poor outcomes in other CAR T-cell studies (contrary to other mutations such as *MYC*, *BCL2* or *CDKN2A*).[60,93] Fas expression by tumour cells appears to contribute to CAR T-cell-mediated cytotoxicity, and high pretreatment *FAS* expression in diffuse large B cell lymphoma (DLBCL) cells was associated with longer responses after CAR T-cell therapy, while it had the opposite effect after conventional therapy.[94] A higher tumour IFN signature also strongly correlates with lower CAR T-cell efficacy, higher tumour expression of T-cell inhibitory ligands (including PD-L1 and PD-L2) and distinct myeloid cell signatures.[51] In multiple myeloma, response rates are high across all subgroups, but patients with unfavourable cytogenetic profiles have a shorter duration of response.[57]

The efficacy of CAR T-cell therapy is impacted by the environment encountered by the T cells after infusion. In LBCL, lack of durable responses has been linked to higher baseline (pre- and/or post-lymphodepletion) tumour burden (total tumour volume, LDH or circulating tumour DNA), higher systemic and tumoural inflammation (IL6, ferritin, C-reactive protein, tumour IFN signalling) and higher level of immune-suppressor cells, such as circulating monocytic myeloid-derived suppressor cells (M-MDSCs), as well as intra-tumoural regulatory T cells or myeloid cells.[13,42,51–54,62] A higher baseline tumour volume has also been correlated with shorter PFS and DOR in B-ALL,[26,55,56] FL (especially if associated with a poor CAR T-cell expansion)[25] and multiple myeloma.[33,57] While the effector-to-tumour ratio has been highlighted in some studies,[42] it is important to remind that the effector parameter is not the dose of infused CAR T cells but the peak CAR T cells after infusion, which is determined by several factors including the phenotype of the manufactured CAR T product and the post-infusion environment. These parameters seem to matter not only at the time of CAR T-cell infusion but also at the time of T-cell collection, as higher tumour burden, inflammation and circulating myeloid cells have been associated with a lower CAR T-cell expansion during the manufacturing[95] and a more differentiated phenotype of the CAR T product.[42] In CLL, a disease characterized by low response rates to CAR T-cell therapy, the immune dysfunction induced by the tumour is well documented, with higher level of suppressive myeloid cells, Treg and exhausted T cells (reviewed in Ref [96]). In an analysis of the gene expression signatures in lymph node biopsies from LBCL patients treated with Axi-Cell in second line (ZUMA-7[11,12]), a higher B-cell lineage signature and CD19 expression were associated with better outcomes, while a higher stromal and immunosuppressive signature (including hypoxia) was associated with lower EFS and DOR.[61] T-cell signatures associated with functionality and trafficking to the TME were inversely correlated with tumour burden and number of lines of therapy. Importantly, tumour burden was not associated with outcomes, unlike in the standard-of-care arm and third-line LBCL (ZUMA-1 trial).[42] These observations suggest that earlier administration of CAR T-cell therapy may mitigate the negative impact of high tumour burden due to improved T-cell trafficking and a less immunosuppressive TME.[61] However, the exact mechanisms underlying these observations are still incompletely understood.

Higher systemic inflammation before CAR T-cell infusion is also associated with higher risk of toxicity, including acute CRS and neurotoxicity[97] as well as prolonged cytopenia.[84] This observation has led to the development of a risk score for haematotoxicity, incorporating markers of hemin, haematopoietic reserve and baseline inflammation, and validated in LBCL, MCL and multiple myeloma[98–100]. The pattern of neutrophil recovery after CAR T-cell therapy is also associated with both different baseline characteristics and different outcomes. In a retrospective cohort of LBCL patients, those experiencing severe neutropenia lasting for more than 14 days after CAR T-cell therapy (aplastic phenotype; 18% of patients) tend to have higher baseline inflammation and tumour burden, impaired haematopoietic function and higher levels of inhibitory checkpoint ligands and inflammation markers post-infusion, leading to poorer outcomes. Conversely, patients with a biphasic neutrophil recovery pattern, characterized by an initial rapid recovery followed by a second nadir after day 21 (intermittent phenotype; 42% of patients), exhibit better outcomes. Compared to patients with either a quick and sustained neutrophil recovery (quick phenotype; 40% of patients) or those with an aplastic phenotype, patients in the intermittent group demonstrate longer PFS and OS, associated with a higher CAR T-cell expansion and longer CAR T-cell persistence. Interestingly, non-CAR T-cell recovery was also faster in this group of patients, suggesting a better T-cell fitness and/or a more favourable environment for T-cell expansion.[84]

Overall, these observations suggest that tumour burden both at the time of apheresis and infusion impacts the CAR T-cell product. Higher tumour burden seems to induce the higher level of inflammation and immunosuppression (including higher levels of MDSCs), which can simultaneously impair the phenotype of the apheresed T cells and the expansion and function of the infused CAR T cells. Reducing tumour burden before CAR T-cell infusion, and potentially even before apheresis, may enhance the efficacy of CAR T cells, although further correlative and mechanistic studies are needed to fully elucidate these complex interactions.

OPTIMIZING CURRENT CAR T-CELL THERAPY: TRANSLATING LESSONS FROM PAST CLINICAL STUDIES INTO PRACTICE

Impact of treatments before T-cell collection

The phenotype and proliferative capacity of T cells are frequently altered in patients diagnosed with haematological malignancies, secondary to the disease itself, advanced age and/or previous treatments. Patients with B-cell malignancies have lower CD4/CD8 ratio and naïve T cells,[24] and this skewing towards a more differentiated phenotype worsens with successive lines of chemotherapy.[101,102] Accordingly, a higher number of previous lines of therapy has been associated with lower response to CAR T cells in LBCL.[62] Based on these observations, early T-cell collection might leverage a higher CAR T-cell efficacy. In a small prospective trial, T-cell collection at first relapse, with CAR T cells manufactured and administered only upon failure of second-line therapy, resulted in higher percentages of naïve T cells, lower expression of exhaustion markers and a higher ORR, but no significant difference in PFS and OS.[103] This could be due to limited statistical power, but also other factors affecting CAR T-cell function post-infusion, such as TME and inflammation. In multiple myeloma, comparison of post-induction versus R/R patients reveals a higher proportion of early memory $CD8^+$ T cells, a higher CD4/CD8 ratio, and superior proliferative capacity of T cells.[104]

When choosing a treatment for potential CAR T-cell therapy candidates, it is essential to consider its impact on T-cell phenotype and function. Bendamustine induces long-lasting lymphopenia and immunomodulatory effects (increased T_{Reg} and enhanced function of MDSCs),[105,106] and recent administration is associated with manufacturing failure (particularly with shorter washout periods and/or prolonged treatment durations), lower peak CAR T cells and shorter response in aggressive lymphomas.[63–66] In multiple myeloma, recent exposure to alkylating agents or autoHSCT favours more senescent $CD8^+$ T cells, while immunomodulatory drugs (IMiDs) have the opposite effect.[34] The Bruton's tyrosine kinase inhibitor (BTKi) ibrutinib also impacts T cells, both indirectly by reducing the immunosuppressive effect of malignant cells, and directly through its irreversible inhibition of the IL2-inducible T-cell kinase (ITK). CLL patients receiving ibrutinib at the time of apheresis have a higher proportion of T_{CM} and a higher CAR T-cell ex vivo expansion.[107] In preclinical models, ibrutinib decreases PD-1 expression on CAR T cells and improves their expansion and efficacy.[108] In CLL patients not achieving CR after ≥6 months of ibrutinib, BBz-CAR T cells in adjunction to ibrutinib resulted in CR and minimal residual disease negativity (MRD^-) rates of 50% and 72.2% at 12 months, which compares favourably to previous trials.[109] In contrast, ibrutinib administration ≥2 weeks before leukapheresis and for ≥3 months after infusion of T_{CM}-enriched CAR T cells resulted in equivalent CAR T-cell expansion, phenotype and efficacy compared to historical controls.[110] However, $CD4^+$ and $CD8^+$ T cells were manufactured separately, which has since been shown to profoundly reduce $CD8^+$ T-cell function.[111] These findings underscore the need to interrogate more extensively the impact of ibrutinib on T cells, including optimal timing of administration. In the TARMAC trial (20 R/R MCL patients), combination of BBz-CAR T cells with time-limited ibrutinib (≥7 days before leukapheresis, and continued until MRD^-) resulted in an encouraging CR of 80% (70% MRD^-).[112] Importantly, these observations might not be generalized to all BTK inhibitors: In the ZUMA-2 trial, a higher peak CAR T-cell level was observed only in patients having received ibrutinib, compared to those who had received acalabrutinib, a more selective BTKi with no effect on ITK.[63]

With the increasing number of immunotherapeutic options in lymphoid malignancies, the question is their optimal sequence of use, especially when targeting the same antigen, remains unanswered. Clinical data reporting outcomes of patients having received CD19 immunotherapies (monoclonal antibody tafasitamab and antibody-drug conjugate loncastuximab) before CAR T cells are scarce, but these therapies do not seem to exert positive pressure on $CD19^-$ clones at relapse.[113,114] Given their relatively long half-life and the potential competition with CAR T cells for CD19 binding, a washout period may be recommended. Multiple myeloma patients whose disease progressed after BCMA-directed therapies can subsequently respond to BCMA-CAR T cells, but response rates appear to be lower.[69,70] Prolonged exposure to bispecific antibodies, which activate T cells through the TCR without providing a costimulatory signal, could potentially lead to T-cell exhaustion or anergy, thereby reducing the efficacy of subsequent CAR T-cell therapy. Continuous infusion of blinatumomab results in decreased T-cell cytotoxic function and IFNg production within 2 weeks of treatment, although preclinical data suggest that treatment-free intervals could rescue T-cell phenotype and function.[115] While blinatumomab exposure was not associated with poorer outcomes in infants treated with Tisa-cel,[116] larger cohort studies of paediatric B-ALL show a correlation with higher risk of early failure (especially in patients not responding to blinatumomab) and reduced CD19 expression.[26,67,68] In a large retrospective cohort of 420 children and young adults with relapsed or refractory ALL treated with CD19-CAR T cells, CR rates were comparable for blinatumomab-naïve patients and responders but were lower for non-responders.[26] Given the shorter remission in blinatumomab-exposed patients, post-CAR consolidation with HSCT may be a viable strategy to prevent relapse. Importantly, even in patients with robust CD19 expression, outcomes were inferior in blinatumomab-exposed patients,[26] suggesting a probable induction of T-cell dysfunction. Nevertheless, CAR T cells still outperformed chemotherapy in heavily pretreated multiple myeloma patients progressing after therapy with a bispecific antibody.[117]

Impact of bridging therapy and lymphodepletion

Because high tumour burden at the time of infusion is associated with poorer outcomes and higher CAR T-related toxicity, bridging therapy could both control the disease during the CAR T-cell manufacturing and potentially improve outcomes. However, we lack data regarding the optimal strategy for bridging. Analysis of retrospective data is complex since LBCL patients receiving bridging therapy also tend to have a higher risk disease.[118] However, response to bridging therapy is associated with lower incidence of severe neurological toxicity and higher ORR and survival.[71] Similarly, in ALL, the achievement of a morphologic response to bridging therapy is associated with improved OS, whereas increasing the intensity or the number of cycles of the bridging therapy increases the risk of severe infection without improving the response rates.[119,120] Overall, these studies underscore the importance of implementing bridging therapy in patients with high disease burden, with the challenge of frequent lack of efficacy in chemoresistant diseases. Aiming to reduce tumour load while prioritizing low-intensity treatment regimens and minimizing the number of cycles seem to be associated with reduced toxicities after CAR T-cell infusion, while the effect on CAR T-cell efficacy remains unclear.

The type and intensity of lymphodepletion also significantly impact outcomes. Addition of fludarabine (Flu) to cyclophosphamide (Cy) improves CAR T-cell expansion,[23] and optimal fludarabine exposure is associated with a lower risk of relapse and higher progression-free survival, even in patients with high tumour burden.[72,73] This beneficial effect of optimal lymphodepletion has been linked to a favourable cytokine profile (higher monocyte chemoattractant protein-1, IL7 or IL15) and improved CAR T-cell expansion after infusion.[73–75] In paediatric B-ALL treated with 28z-CAR T cells, increasing the dose of Cy was also shown to be associated with higher response rates and CAR T-cell expansion, without increased toxicity.[74,121] Similarly, in multiple myeloma patients treated with Ide-cel, non-responders exhibited higher T-cell counts on day 0, reflecting less effective lymphodepletion.[33] Finally, although an initial report raised concerns about the use of bendamustine as a conditioning regimen for anti-CD30 CAR T cells in Hodgkin lymphoma,[122] further retrospective studies in LBCL and multiple myeloma suggest that bendamustine is a valid alternative to Flu/Cy, resulting in similar CAR T efficacy with reduced haematological toxicity.[123,124] Overall, the lymphodepletion plays an important role in creating optimal conditions for CAR T-cell expansion and efficacy.

Treatments after CAR T-cell infusion

Some therapies also hold the potential to boost CAR T-cell efficacy after infusion. In LBCL relapsing after CAR T cells, lenalidomide-based therapy was associated with better OS compared to chemotherapy.[28] Because IMiDs can both exert anti-tumoural effects and boost T-cell function, their use after CAR T-cell therapy could be a promising approach. Post-CAR T-cell maintenance with lenalidomide or pomalidomide in multiple myeloma is safe and leads to a second CAR T-cell expansion and late-onset response in some patients.[125] Positive selection of malignant clones overexpressing PD-L1 and PD-L2 has been associated with relapse,[54] but association of CAR T cells with anti-PDL1 or anti-PD1 therapy has not convincingly demonstrated increased efficacy.[126,127] Administration of a second infusion of CAR T cells has also been attempted in patients with persistent/relapsed B-cell malignancy. Although safe and leading to 20% of complete response, most patients ultimately relapsed or received consolidative therapies.[128] Finally, the role of allogenic haematopoietic stem cell transplantation (alloHSCT) in maintaining remission for B-ALL patients is still debated (reviewed in Ref [129]). While the follow-up of children and young adults treated with Tisa-Cel in the ELIANA study showed a median EFS of 24 months,[130] long-term outcomes were considerably poorer in adults, both with Tisa-Cel and Brexu-Cel.[90,131] Importantly, long-term remissions have been reported in B-ALL without consolidative alloHSCT, suggesting that CAR T cells can induce a cure in a subset of B-ALL patients. However, consolidative alloHSCT is still recommended in adult patients, as it improves EFS.[90]

Loss of CAR T-cell (functional) persistence often precedes relapse and its detection can allow early intervention, before clinical relapse. However, in LBCL and multiple myeloma, long-term remissions have been observed in patients with undetectable circulating CAR T cells. Because CD19-CAR T cells also target normal B cells, B-cell aplasia (BCA) is a marker of CAR T-cell functionality and persistence. In B-ALL, early loss of BCA is strongly associated with loss of CAR T cells and higher risk of $CD19^+$ relapse,[67,88,132] and can be used to identify the patients who could benefit from a consolidative alloHSCT. However, $CD19^-$ relapse can occur despite persisting BCA and detectable CAR T cells. Patients with high tumour burden have a higher risk of early $CD19^-$ relapse, while relapses in patients with low tumour burden are mostly $CD19^+$ and are preceded by loss of BCA.[26,67,132] Therefore, BCA, as a surrogate marker for threatening relapse, should be used cautiously in patients with high tumour burden at the time of infusion. In LBCL, a higher level of circulating tumour DNA (ctDNA) before lymphodepletion is a poor prognostic factor, while undetectable ctDNA at early time points (day 7 and 28 after Axi-Cel infusion) is predictive of durable response, and detection of ctDNA occurs earlier than radiologic relapse.[133] Although these measures are not yet used in clinical practice, combining measure of early CAR T-cell expansion and early tumour response can provide a strong prognostic score.[54] Similarly, in multiple myeloma patients treated with BCMA-CAR T cells, the depth of initial response (clinical response, serum BCMA) correlates with a longer duration of response.[33,34]

Overall, combining measures of CAR T-cell kinetics and functional persistence with follow-up of the residual disease can help predict disease recurrence and allow intervention

before morphological relapse occurs. However, with the possible exception of lenalidomide,[125] we currently lack innovative strategies to boost CAR T-cell function after infusion when identifying a patient at risk of relapse.

CONCLUSION

Much like alloHSCT revolutionized the prognosis of many haematological diseases 50 years ago, CAR T cells are currently reshaping the landscape of haematology. Harnessing the full potential of this therapeutic modality will require a better understanding of the complex immunological interactions taking place in cancer and after CAR T-cell infusion. CAR T cells can cure cancer in some patients; we need to make them so efficient that they can cure cancer in all patients. Moreover, we will need to make them easier to use and more affordable, so that they can be used in patients around the world. Manufacturing processes favouring T_{SCM} and early memory T cells (shorter manufacturing, selection of T-cell subsets),[91,134] as well as academic and/or point-of-care production of CAR T cells are increasingly being developed[135–138] and might be a way to achieve this goal. Varnimcabtagene autoleucel, an academic BBzCD19-CAR T-cell product (based on a different scFv), has a cost estimated to be more than three times inferior the commercial CAR T cells. This is approved in Spain as Hospital Exemption in adult patients with R/R B-ALL and as compassionate use in R/R B-cell lymphoma, and is supported by the European Medicines Agency to accelerate its development and approval.[135,139]

A major question that is still not answered is the impact of other types of T-cell-based immunotherapy on T-cell phenotype and function, which might be important to determine the best therapeutic sequence. Bispecific T-cell engagers and bispecific antibodies, now widely employed in B-ALL, B-cell lymphoma and multiple myeloma, may potentially lead to T-cell exhaustion due to recurrent T-cell stimulation. A high tumour burden is associated with lower response rates, and these targeted therapies might be a good option for debulking before CAR T-cell therapy, as their short-term use might preserve the T-cell compartment compared to chemotherapy. CAR T cells could also be combined with other immunotherapeutic modalities, such as monoclonal antibodies (Rituximab in the ZUMA-14 trial, NCT04002401) or checkpoint inhibitors.[127,140] Finally, numerous preclinical studies and clinical trials are underway to develop and refine off-the-shelf allogeneic CAR T cells. This approach has the potential to enhance both the availability and quality of CAR T-cell therapy, using T cells with a more favourable subset distribution, and therefore potentially a better functionality. However, it requires further engineering to prevent rejection and graft-versus-host disease. Current clinical data indicate promising remission rates, but further efforts may be necessary to enhance long-term persistence.[141]

Immunotherapies for cancer are developing exponentially, and many fundamental, translational and clinical studies are still needed to better understand and ultimately improve the outcomes with these therapies. While many compare and oppose these treatments, another approach might be to study how to better use them sequentially or in combination. Current treatments for promyelocytic AML or chronic myeloid leukaemia have demonstrated that the dream of curing cancer without chemotherapy is not a utopia; CAR T cells have the potential to become a cornerstone in realizing this vision for other haematological malignancies, and possibly solid tumours.

AUTHOR CONTRIBUTIONS

CG and JJM initiated and conceptualized the manuscript. CG performed the literature review and wrote the manuscript under the supervision of JJM, PFC and JC. BCdO contributed to the literature review and writing. All authors actively reviewed the manuscript and approved its final version.

FUNDING INFORMATION

The writing of this review article was supported by financing from Cleveland Clinic Centre of Excellence and Velosano grants.

CONFLICT OF INTEREST STATEMENT

JJM has patents related to biomarkers and manufacturing of chimeric antigen receptor-engineered T cells; the remaining authors have no competing interests.

ORCID

Jan Joseph Melenhorst https://orcid.org/0000-0001-7677-537X

REFERENCES

1. Melenhorst JJ, Chen GM, Wang M, Porter DL, Chen C, Collins MA, et al. Decade-long leukaemia remissions with persistence of CD4(+) CAR T cells. Nature. 2022;602(7897):503–9.
2. Neelapu SS, Jacobson CA, Ghobadi A, Miklos DB, Lekakis LJ, Oluwole OO, et al. 5-Year follow-up supports curative potential of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1). Blood. 2023;141(19):2307–15.
3. Jacobson CA, Chavez JC, Sehgal AR, William BM, Munoz J, Salles G, et al. Axicabtagene ciloleucel in relapsed or refractory indolent non-Hodgkin lymphoma (ZUMA-5): a single-arm, multicentre, phase 2 trial. Lancet Oncol. 2022;23(1):91–103.
4. Milone MC, Fish JD, Carpenito C, Carroll RG, Binder GK, Teachey D, et al. Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo. Mol Ther. 2009;17(8):1453–64.
5. Cappell KM, Kochenderfer JN. A comparison of chimeric antigen receptors containing CD28 versus 4-1BB costimulatory domains. Nat Rev Clin Oncol. 2021;18(11):715–27.
6. Long AH, Haso WM, Shern JF, Wanhainen KM, Murgai M, Ingaramo M, et al. 4-1BB costimulation ameliorates T cell exhaustion induced by tonic signaling of chimeric antigen receptors. Nat Med. 2015;21(6):581–90.
7. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med. 2018;378(5):439–48.
8. Schuster SJ, Bishop MR, Tam CS, Waller EK, Borchmann P, McGuirk JP, et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. N Engl J Med. 2019;380(1):45–56.
9. Fowler NH, Dickinson M, Dreyling M, Martinez-Lopez J, Kolstad A, Butler J, et al. Tisagenlecleucel in adult relapsed or

refractory follicular lymphoma: the phase 2 ELARA trial. Nat Med. 2022;28(2):325–32.
10. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. N Engl J Med. 2017;377(26):2531–44.
11. Locke FL, Miklos DB, Jacobson CA, Perales MA, Kersten MJ, Oluwole OO, et al. Axicabtagene ciloleucel as second-line therapy for large B-cell lymphoma. N Engl J Med. 2022;386(7):640–54.
12. Westin JR, Oluwole OO, Kersten MJ, Miklos DB, Perales MA, Ghobadi A, et al. Survival with axicabtagene ciloleucel in large B-cell lymphoma. N Engl J Med. 2023;389(2):148–57.
13. Houot R, Bachy E, Cartron G, Gros FX, Morschhauser F, Oberic L, et al. Axicabtagene ciloleucel as second-line therapy in large B cell lymphoma ineligible for autologous stem cell transplantation: a phase 2 trial. Nat Med. 2023;29(10):2593–601.
14. Shah BD, Ghobadi A, Oluwole OO, Logan AC, Boissel N, Cassaday RD, et al. KTE-X19 for relapsed or refractory adult B-cell acute lymphoblastic leukaemia: phase 2 results of the single-arm, open-label, multicentre ZUMA-3 study. Lancet. 2021;398(10299):491–502.
15. Wang M, Munoz J, Goy A, Locke FL, Jacobson CA, Hill BT, et al. KTE-X19 CAR T-cell therapy in relapsed or refractory mantle-cell lymphoma. N Engl J Med. 2020;382(14):1331–42.
16. Abramson JS, Palomba ML, Gordon LI, Lunning MA, Wang M, Arnason J, et al. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. Lancet. 2020;396(10254):839–52.
17. Abramson JS, Solomon SR, Arnason J, Johnston PB, Glass B, Bachanova V, et al. Lisocabtagene maraleucel as second-line therapy for large B-cell lymphoma: primary analysis of the phase 3 TRANSFORM study. Blood. 2023;141(14):1675–84.
18. Sehgal A, Hoda D, Riedell PA, Ghosh N, Hamadani M, Hildebrandt GC, 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):1066–77.
19. Siddiqi T, Maloney DG, Kenderian SS, Brander DM, Dorritie K, Soumerai J, et al. Lisocabtagene maraleucel in chronic lymphocytic leukaemia and small lymphocytic lymphoma (TRANSCEND CLL 004): a multicentre, open-label, single-arm, phase 1-2 study. Lancet. 2023;402(10402):641–54.
20. Munshi NC, Anderson LD Jr, Shah N, Madduri D, Berdeja J, Lonial S, et al. Idecabtagene vicleucel in relapsed and refractory multiple myeloma. N Engl J Med. 2021;384(8):705–16.
21. Berdeja JG, Madduri D, Usmani SZ, Jakubowiak A, Agha M, Cohen AD, et al. Ciltacabtagene autoleucel, a B-cell maturation antigen-directed chimeric antigen receptor T-cell therapy in patients with relapsed or refractory multiple myeloma (CARTITUDE-1): a phase 1b/2 open-label study. Lancet. 2021;398(10297):314–24.
22. Wayne AS, Huynh V, Hijiya N, Rouce RH, Brown PA, Krueger J, et al. Three-year results from phase I of ZUMA-4: KTE-X19 in pediatric relapsed/refractory acute lymphoblastic leukemia. Haematologica. 2023;108(3):747–60.
23. Turtle CJ, Hanafi LA, Berger C, Gooley TA, Cherian S, Hudecek M, et al. CD19 CAR-T cells of defined CD4+:CD8+ composition in adult B cell ALL patients. J Clin Invest. 2016;126(6):2123–38.
24. Sommermeyer D, Hudecek M, Kosasih PL, Gogishvili T, Maloney DG, Turtle CJ, et al. Chimeric antigen receptor-modified T cells derived from defined CD8+ and CD4+ subsets confer superior antitumor reactivity in vivo. Leukemia. 2016;30(2):492–500.
25. Dreyling M, Fowler NH, Dickinson M, Martinez-Lopez J, Kolstad A, Butler J, et al. Durable response after tisagenlecleucel in adults with relapsed/refractory follicular lymphoma: ELARA trial update. Blood. 2024;143(17):1713–25.
26. Myers RM, Taraseviciute A, Steinberg SM, Lamble AJ, Sheppard J, Yates B, et al. Blinatumomab nonresponse and high-disease burden are associated with inferior outcomes after CD19-CAR for B-ALL. J Clin Oncol. 2022;40(9):932–44.
27. Maloney DG, Kuruvilla J, Liu FF, Kostic A, Kim Y, Bonner A, et al. Matching-adjusted indirect treatment comparison of liso-cel versus axi-cel in relapsed or refractory large B cell lymphoma. J Hematol Oncol. 2021;14(1):140.
28. Alarcon Tomas A, Fein JA, Fried S, Flynn JR, Devlin SM, Fingrut WB, et al. Outcomes of first therapy after CD19-CAR-T treatment failure in large B-cell lymphoma. Leukemia. 2023;37(1):154–63.
29. Bachy E, Le Gouill S, Di Blasi R, Sesques P, Manson G, Cartron G, 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):2145–54.
30. Bishop MR, Dickinson M, Purtill D, Barba P, Santoro A, Hamad N, et al. Second-line tisagenlecleucel or standard care in aggressive B-cell lymphoma. N Engl J Med. 2022;386(7):629–39.
31. Bommier C, Lambert J, Thieblemont C. Comparing apples and oranges: the ZUMA-7, TRANSFORM and BELINDA trials. Hematol Oncol. 2022;40(5):1090–3.
32. Shadman M, Pasquini M, Ahn KW, Chen Y, Turtle CJ, Hematti P, et al. Autologous transplant vs chimeric antigen receptor T-cell therapy for relapsed DLBCL in partial remission. Blood. 2022;139(9):1330–9.
33. Fischer L, Grieb N, Born P, Weiss R, Seiffert S, Boldt A, et al. Cellular dynamics following CAR T cell therapy are associated with response and toxicity in relapsed/refractory myeloma. Leukemia. 2024;38(2):372–82.
34. Lin Y, Raje NS, Berdeja JG, Siegel DS, Jagannath S, Madduri D, et al. Idecabtagene vicleucel for relapsed and refractory multiple myeloma: post hoc 18-month follow-up of a phase 1 trial. Nat Med. 2023;29(9):2286–94.
35. Rodriguez-Otero P, Ailawadhi S, Arnulf B, Patel K, Cavo M, Nooka AK, et al. Ide-cel or standard regimens in relapsed and refractory multiple myeloma. N Engl J Med. 2023;388(11):1002–14.
36. San-Miguel J, Dhakal B, Yong K, Spencer A, Anguille S, Mateos MV, et al. Cilta-cel or standard care in lenalidomide-refractory multiple myeloma. N Engl J Med. 2023;389(4):335–47.
37. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med. 2014;371(16):1507–17.
38. Frey NV, Gill S, Hexner EO, Schuster S, Nasta S, Loren A, et al. Long-term outcomes from a randomized dose optimization study of chimeric antigen receptor modified T cells in relapsed chronic lymphocytic leukemia. J Clin Oncol. 2020;38(25):2862–71.
39. Cappell KM, Sherry RM, Yang JC, Goff SL, Vanasse DA, McIntyre L, et al. Long-term follow-up of anti-CD19 chimeric antigen receptor T-cell therapy. J Clin Oncol. 2020;38(32):3805–15.
40. Xu Y, Zhang M, Ramos CA, Durett A, Liu E, Dakhova O, 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):3750–9.
41. Fraietta JA, Lacey SF, Orlando EJ, Pruteanu-Malinici I, Gohil M, Lundh S, et al. Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. Nat Med. 2018;24(5):563–71.
42. Locke FL, Rossi JM, Neelapu SS, Jacobson CA, Miklos DB, Ghobadi A, et al. Tumor burden, inflammation, and product attributes determine outcomes of axicabtagene ciloleucel in large B-cell lymphoma. Blood Adv. 2020;4(19):4898–911.
43. Chen GM, Chen C, Das RK, Gao P, Chen CH, Bandyopadhyay S, et al. Integrative bulk and single-cell profiling of premanufacture T-cell populations reveals factors mediating long-term persistence of CAR T-cell therapy. Cancer Discov. 2021;11(9):2186–99.
44. Deng Q, Han G, Puebla-Osorio N, Ma MCJ, Strati P, Chasen B, 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):1878–87.
45. Haradhvala NJ, Leick MB, Maurer K, Gohil SH, Larson RC, Yao N, et al. Distinct cellular dynamics associated with response to CAR-T therapy for refractory B cell lymphoma. Nat Med. 2022;28(9):1848–59.

46. Cuffel A, Allain V, Faivre L, Di Blasi R, Morin F, Vercellino L, et al. Real-world characteristics of T-cell apheresis and clinical response to tisagenlecleucel in B-cell lymphoma. Blood Adv. 2022;6(15):4657–60.
47. Bai Z, Woodhouse S, Zhao Z, Arya R, Govek K, Kim D, et al. Single-cell antigen-specific landscape of CAR T infusion product identifies determinants of CD19-positive relapse in patients with ALL. Sci Adv. 2022;8(23):eabj2820.
48. Jackson Z, Hong C, Schauner R, Dropulic B, Caimi PF, de Lima M, et al. Sequential single cell transcriptional and protein marker profiling reveals TIGIT as a marker of CD19 CAR-T cell dysfunction in patients with non-Hodgkin's lymphoma. Cancer Discov. 2022;12(8):1886–903.
49. Finney OC, Brakke HM, Rawlings-Rhea S, Hicks R, Doolittle D, Lopez M, et al. CD19 CAR T cell product and disease attributes predict leukemia remission durability. J Clin Invest. 2019;129(5):2123–32.
50. Good Z, Spiegel JY, Sahaf B, Malipatlolla MB, Ehlinger ZJ, Kurra S, et al. Post-infusion CAR TReg cells identify patients resistant to CD19-CAR therapy. Nat Med. 2022;28(9):1860–71.
51. Jain MD, Zhao H, Wang X, Atkins R, Menges M, Reid K, 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):2621–33.
52. Iovino L, Wu QV, Voutsinas J, Panaite L, Mullane E, Lynch RC, 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):5976–83.
53. Vercellino L, Di Blasi R, Kanoun S, Tessoulin B, Rossi C, D'Aveni-Piney M, 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):5607–15.
54. Sworder BJ, Kurtz DM, Alig SK, Frank MJ, Shukla N, Garofalo A, 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.
55. Park JH, Riviere I, Gonen M, Wang X, Senechal B, Curran KJ, et al. Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. N Engl J Med. 2018;378(5):449–59.
56. Schultz LM, Baggott C, Prabhu S, Pacenta HL, Phillips CL, Rossoff J, et al. Disease burden affects outcomes in pediatric and young adult B-cell lymphoblastic leukemia after commercial tisagenlecleucel: a pediatric real-world chimeric antigen receptor consortium report. J Clin Oncol. 2022;40(9):945–55.
57. Martin T, Usmani SZ, Berdeja JG, Agha M, Cohen AD, Hari P, et al. Ciltacabtagene autoleucel, an anti-B-cell maturation antigen chimeric antigen receptor T-cell therapy, for relapsed/refractory multiple myeloma: CARTITUDE-1 2-year follow-up. J Clin Oncol. 2023;41(6):1265–74.
58. Shouval R, Alarcon Tomas A, Fein JA, Flynn JR, Markovits E, Mayer S, 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):369–81.
59. Cherng HJ, Sun R, Sugg B, Irwin R, Yang H, Le CC, et al. Risk assessment with low-pass whole-genome sequencing of cell-free DNA before CD19 CAR T-cell therapy for large B-cell lymphoma. Blood. 2022;140(5):504–15.
60. Hill BT, Roth CJ, Kositsky R, Dave T, Love C, McKinney M, et al. Impact of molecular features of diffuse large B-cell lymphoma on treatment outcomes with anti-CD19 chimeric antigen receptor (CAR) T-cell therapy. Blood. 2021;138(Suppl 1):165.
61. Locke FL, Filosto S, Chou J, Vardhanabhuti S, Perbost R, Dreger P, 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(2):507–18.
62. Sesques P, Ferrant E, Safar V, Wallet F, Tordo J, Dhomps A, 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):1324–33.
63. Wang M, Munoz J, Goy A, Locke FL, Jacobson CA, Hill BT, et al. Three-year follow-up of KTE-X19 in patients with relapsed/refractory mantle cell lymphoma, including high-risk subgroups, in the ZUMA-2 study. J Clin Oncol. 2023;41(3):555–67.
64. Wang Y, Jain P, Locke FL, Maurer MJ, Frank MJ, Munoz JL, et al. Brexucabtagene autoleucel for relapsed or refractory mantle cell lymphoma in standard-of-care practice: results from the US lymphoma CAR T consortium. J Clin Oncol. 2023;41(14):2594–606.
65. Iacoboni G, Navarro V, Martin-Lopez AA, Rejeski K, Kwon M, Jalowiec KA, 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(2):205–17.
66. Jo T, Yoshihara S, Okuyama Y, Fujii K, Henzan T, Kahata K, et al. Risk factors for CAR-T cell manufacturing failure among DLBCL patients: a nationwide survey in Japan. Br J Haematol. 2023;202(2):256–66.
67. Dourthe ME, Rabian F, Yakouben K, Chevillon F, Cabannes-Hamy A, Mechinaud F, et al. Determinants of CD19-positive vs CD19-negative relapse after tisagenlecleucel for B-cell acute lymphoblastic leukemia. Leukemia. 2021;35(12):3383–93.
68. Pillai V, Muralidharan K, Meng W, Bagashev A, Oldridge DA, Rosenthal J, et al. CAR T-cell therapy is effective for CD19-dim B-lymphoblastic leukemia but is impacted by prior blinatumomab therapy. Blood Adv. 2019;3(22):3539–49.
69. Ferreri CJ, Hildebrandt MAT, Hashmi H, Shune LO, McGuirk JP, Sborov DW, et al. Real-world experience of patients with multiple myeloma receiving ide-cel after a prior BCMA-targeted therapy. Blood Cancer J. 2023;13(1):117.
70. Cohen AD, Mateos MV, Cohen YC, Rodriguez-Otero P, Paiva B, van de Donk N, et al. Efficacy and safety of cilta-cel in patients with progressive multiple myeloma after exposure to other BCMA-targeting agents. Blood. 2023;141(3):219–30.
71. Roddie C, Neill L, Osborne W, Iyengar S, Tholouli E, Irvine D, 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):2872–83.
72. Fabrizio VA, Boelens JJ, Mauguen A, Baggott C, Prabhu S, Egeler E, et al. Optimal fludarabine lymphodepletion is associated with improved outcomes after CAR T-cell therapy. Blood Adv. 2022;6(7):1961–8.
73. Dekker L, Calkoen FG, Jiang Y, Blok H, Veldkamp SR, De Koning C, et al. Fludarabine exposure predicts outcome after CD19 CAR T-cell therapy in children and young adults with acute leukemia. Blood Adv. 2022;6(7):1969–76.
74. Hirayama AV, Gauthier J, Hay KA, Voutsinas JM, Wu Q, Gooley T, 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):1876–87.
75. Kochenderfer JN, Somerville RPT, Lu T, Shi V, Bot A, Rossi J, et al. Lymphoma remissions caused by anti-CD19 chimeric antigen receptor T cells are associated with high serum interleukin-15 levels. J Clin Oncol. 2017;35(16):1803–13.
76. Mueller KT, Maude SL, Porter DL, Frey N, Wood P, Han X, et al. Cellular kinetics of CTL019 in relapsed/refractory B-cell acute lymphoblastic leukemia and chronic lymphocytic leukemia. Blood. 2017;130(21):2317–25.
77. Sheih A, Voillet V, Hanafi LA, DeBerg HA, Yajima M, Hawkins R, et al. Clonal kinetics and single-cell transcriptional profiling of CAR-T cells in patients undergoing CD19 CAR-T immunotherapy. Nat Commun. 2020;11(1):219.
78. Biasco L, Izotova N, Rivat C, Ghorashian S, Richardson R, Guvenel A, et al. Clonal expansion of T memory stem cells determines early anti-leukemic responses and long-term CAR T cell persistence in patients. Nat Cancer. 2021;2(6):629–42.

79. Anderson ND, Birch J, Accogli T, Criado I, Khabirova E, Parks C, et al. Transcriptional signatures associated with persisting CD19 CAR-T cells in children with leukemia. Nat Med. 2023;29(7):1700–9.
80. Larson RC, Kann MC, Bailey SR, Haradhvala NJ, Llopis PM, Bouffard AA, et al. CAR T cell killing requires the IFNgammaR pathway in solid but not liquid tumours. Nature. 2022;604(7906):563–70.
81. Manni S, Del Bufalo F, Merli P, Silvestris DA, Guercio M, Caruso S, et al. Neutralizing IFNgamma improves safety without compromising efficacy of CAR-T cell therapy in B-cell malignancies. Nat Commun. 2023;14(1):3423.
82. Teachey DT, Lacey SF, Shaw PA, Melenhorst JJ, Maude SL, Frey N, et al. Identification of predictive biomarkers for cytokine release syndrome after chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. Cancer Discov. 2016;6(6):664–79.
83. Strati P, Li X, Deng Q, Marques-Piubelli ML, Henderson J, Watson G, et al. Prolonged cytopenia following CD19 CAR T cell therapy is linked with bone marrow infiltration of clonally expanded IFNgamma-expressing CD8 T cells. Cell Rep Med. 2023;4(8):101158.
84. Rejeski K, Perez A, Iacoboni G, Blumenberg V, Bucklein VL, Volkl S, et al. Severe hematotoxicity after CD19 CAR-T therapy is associated with suppressive immune dysregulation and limited CAR-T expansion. Sci Adv. 2023;9(38):eadg3919.
85. Rossi J, Paczkowski P, Shen YW, Morse K, Flynn B, Kaiser A, et al. Preinfusion polyfunctional anti-CD19 chimeric antigen receptor T cells are associated with clinical outcomes in NHL. Blood. 2018;132(8):804–14.
86. Fraietta JA, Nobles CL, Sammons MA, Lundh S, Carty SA, Reich TJ, et al. Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells. Nature. 2018;558(7709):307–12.
87. Rodriguez-Marquez P, Calleja-Cervantes ME, Serrano G, Oliver-Caldes A, Palacios-Berraquero ML, Martin-Mallo A, et al. CAR density influences antitumoral efficacy of BCMA CAR T cells and correlates with clinical outcome. Sci Adv. 2022;8(39):eabo0514.
88. Mueller KT, Waldron E, Grupp SA, Levine JE, Laetsch TW, Pulsipher MA, et al. Clinical pharmacology of tisagenlecleucel in B-cell acute lymphoblastic leukemia. Clin Cancer Res. 2018;24(24):6175–84.
89. Shah BD, Bishop MR, Oluwole OO, Logan AC, Baer MR, Donnellan WB, et al. KTE-X19 anti-CD19 CAR T-cell therapy in adult relapsed/refractory acute lymphoblastic leukemia: ZUMA-3 phase 1 results. Blood. 2021;138(1):11–22.
90. Frey NV, Shaw PA, Hexner EO, Pequignot E, Gill S, Luger SM, et al. Optimizing chimeric antigen receptor T-cell therapy for adults with acute lymphoblastic leukemia. J Clin Oncol. 2020;38(5):415–22.
91. Dickinson MJ, Barba P, Jager U, Shah NN, Blaise D, Briones J, et al. A novel autologous CAR-T therapy, YTB323, with preserved T-cell stemness shows enhanced CAR T-cell efficacy in preclinical and early clinical development. Cancer Discov. 2023;13(9):1982–97.
92. Kamdar M, Solomon SR, Arnason J, Johnston PB, Glass B, Bachanova V, 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):2294–308.
93. Porpaczy E, Wohlfarth P, Konigsbrugge O, Rabitsch W, Skrabs C, Staber P, et al. Influence of TP53 mutation on survival of diffuse large B-cell lymphoma in the CAR T-cell era. Cancers (Basel). 2021;13(22):5592. https://doi.org/10.3390/cancers13225592
94. Upadhyay R, Boiarsky JA, Pantsulaia G, Svensson-Arvelund J, Lin MJ, Wroblewska A, et al. A critical role for Fas-mediated off-target tumor killing in T-cell immunotherapy. Cancer Discov. 2021;11(3):599–613.
95. Stroncek DF, Ren J, Lee DW, Tran M, Frodigh SE, Sabatino M, et al. Myeloid cells in peripheral blood mononuclear cell concentrates inhibit the expansion of chimeric antigen receptor T cells. Cytotherapy. 2016;18(7):893–901.
96. Arruga F, Gyau BB, Iannello A, Vitale N, Vaisitti T, Deaglio S. Immune response dysfunction in chronic lymphocytic leukemia: dissecting molecular mechanisms and microenvironmental conditions. Int J Mol Sci. 2020;21(5):1825.
97. Faramand R, Jain M, Staedtke V, Kotani H, Bai R, Reid K, et al. Tumor microenvironment composition and severe cytokine release syndrome (CRS) influence toxicity in patients with large B-cell lymphoma treated with axicabtagene ciloleucel. Clin Cancer Res. 2020;26(18):4823–31.
98. Rejeski K, Perez A, Sesques P, Hoster E, Berger C, Jentzsch L, et al. CAR-HEMATOTOX: a model for CAR T-cell-related hematologic toxicity in relapsed/refractory large B-cell lymphoma. Blood. 2021;138(24):2499–513.
99. Rejeski K, Wang Y, Albanyan O, Munoz J, Sesques P, Iacoboni G, et al. The CAR-HEMATOTOX score identifies patients at high risk for hematological toxicity, infectious complications, and poor treatment outcomes following brexucabtagene autoleucel for relapsed or refractory MCL. Am J Hematol. 2023;98(11):1699–710.
100. Rejeski K, Hansen DK, Bansal R, Sesques P, Ailawadhi S, Logue JM, et al. The CAR-HEMATOTOX score as a prognostic model of toxicity and response in patients receiving BCMA-directed CAR-T for relapsed/refractory multiple myeloma. J Hematol Oncol. 2023;16(1):88.
101. Singh N, Perazzelli J, Grupp SA, Barrett DM. Early memory phenotypes drive T cell proliferation in patients with pediatric malignancies. Sci Transl Med. 2016;8(320):320ra3.
102. Das RK, Vernau L, Grupp SA, Barrett DM. Naive T-cell deficits at diagnosis and after chemotherapy impair cell therapy potential in pediatric cancers. Cancer Discov. 2019;9(4):492–9.
103. Dubnikov Sharon T, Assayag M, Avni B, Kfir-Erenfeld S, Lebel E, Gatt ME, et al. Early lymphocyte collection for anti-CD19 CART production improves T-cell fitness in patients with relapsed/refractory diffuse large B-cell lymphoma. Br J Haematol. 2023;202(1):74–85.
104. Garfall AL, Dancy EK, Cohen AD, Hwang WT, Fraietta JA, Davis MM, et al. T-cell phenotypes associated with effective CAR T-cell therapy in postinduction vs relapsed multiple myeloma. Blood Adv. 2019;3(19):2812–5.
105. Stokes J, Molina MS, Hoffman EA, Simpson RJ, Katsanis E. Immunomodulatory effects of bendamustine in hematopoietic cell transplantation. Cancers (Basel). 2021;13(7):1702.
106. Martinez-Calle N, Hartley S, Ahearne M, Kasenda B, Beech A, Knight H, et al. Kinetics of T-cell subset reconstitution following treatment with bendamustine and rituximab for low-grade lymphoproliferative disease: a population-based analysis. Br J Haematol. 2019;184(6):957–68.
107. Geyer MB, Riviere I, Senechal B, Wang X, Wang Y, Purdon TJ, et al. Safety and tolerability of conditioning chemotherapy followed by CD19-targeted CAR T cells for relapsed/refractory CLL. JCI Insight. 2019;5(9):e122627.
108. Fraietta JA, Beckwith KA, Patel PR, Ruella M, Zheng Z, Barrett DM, et al. Ibrutinib enhances chimeric antigen receptor T-cell engraftment and efficacy in leukemia. Blood. 2016;127(9):1117–27.
109. Gill S, Vides V, Frey NV, Hexner EO, Metzger S, O'Brien M, et al. Anti-CD19 CAR T cells in combination with ibrutinib for the treatment of chronic lymphocytic leukemia. Blood Adv. 2022;6(21):5774–85.
110. Gauthier J, Hirayama AV, Purushe J, Hay KA, Lymp J, Li DH, et al. Feasibility and efficacy of CD19-targeted CAR T cells with concurrent ibrutinib for CLL after ibrutinib failure. Blood. 2020;135(19):1650–60.
111. Lee SY, Lee DH, Sun W, Cervantes-Contreras F, Basom RS, Wu F, et al. $CD8^+$ chimeric antigen receptor T cells manufactured in absence of $CD4^+$ cells exhibit hypofunctional phenotype. J Immunother Cancer. 2023;11(11):e007803.
112. Minson A, Hamad N, Cheah CY, Tam C, Blombery P, Westerman D, et al. CAR T cells and time-limited ibrutinib as treatment for relapsed/refractory mantle cell lymphoma: the phase 2 TARMAC study. Blood. 2024;143(8):673–84.
113. Duell J, Obr A, Augustin M, Endell J, Liu H, Geiger S, et al. CD19 expression is maintained in DLBCL patients after treatment

with tafasitamab plus lenalidomide in the L-MIND study. Leuk Lymphoma. 2022;63(2):468–72.
114. Thapa B, Caimi PF, Ardeshna KM, Solh M, Carlo-Stella C, Kahl BS, et al. CD19 antibody-drug conjugate therapy in DLBCL does not preclude subsequent responses to CD19-directed CAR T-cell therapy. Blood Adv. 2020;4(16):3850–2.
115. Philipp N, Kazerani M, Nicholls A, Vick B, Wulf J, Straub T, et al. T-cell exhaustion induced by continuous bispecific molecule exposure is ameliorated by treatment-free intervals. Blood. 2022;140(10):1104–18.
116. Ghorashian S, Jacoby E, De Moerloose B, Rives S, Bonney D, Shenton G, et al. Tisagenlecleucel therapy for relapsed or refractory B-cell acute lymphoblastic leukaemia in infants and children younger than 3 years of age at screening: an international, multicentre, retrospective cohort study. Lancet Haematol. 2022;9(10):e766–e775.
117. Mouhieddine TH, Van Oekelen O, Melnekoff DT, Li J, Ghodke-Puranik Y, Lancman G, et al. Sequencing T-cell redirection therapies leads to deep and durable responses in patients with relapsed/refractory myeloma. Blood Adv. 2023;7(6):1056–64.
118. Jain MD, Jacobs MT, Gao F, Nastoupil LJ, Spiegel JY, Lin Y, et al. Bridging therapy with axicabtagene ciloleucel for large B-cell lymphoma: results from the US lymphoma CAR-T consortium. Blood Adv. 2024;8(4):1042–50.
119. Perica K, Flynn J, Curran KJ, Rivere I, Wang X, Senechal B, et al. Impact of bridging chemotherapy on clinical outcome of CD19 CAR T therapy in adult acute lymphoblastic leukemia. Leukemia. 2021;35(11):3268–71.
120. Shahid S, Ramaswamy K, Flynn J, Mauguen A, Perica K, Park JH, et al. Impact of bridging chemotherapy on clinical outcomes of CD19-specific CAR T cell therapy in children/young adults with relapsed/refractory B cell acute lymphoblastic leukemia. Transplant Cell Ther. 2022;28(2):72.e1–72.e8.
121. Curran KJ, Margossian SP, Kernan NA, Silverman LB, Williams DA, Shukla N, et al. Toxicity and response after CD19-specific CAR T-cell therapy in pediatric/young adult relapsed/refractory B-ALL. Blood. 2019;134(26):2361–8.
122. Ramos CA, Grover NS, Beaven AW, Lulla PD, Wu MF, Ivanova A, et al. Anti-CD30 CAR-T cell therapy in relapsed and refractory Hodgkin lymphoma. J Clin Oncol. 2020;38(32):3794–804.
123. Ghilardi G, Chong EA, Svoboda J, Wohlfarth P, Nasta SD, Williamson S, et al. Bendamustine is safe and effective for lymphodepletion before tisagenlecleucel in patients with refractory or relapsed large B-cell lymphomas. Ann Oncol. 2022;33(9):916–28.
124. Sidana S, Hosoya H, Jensen A, Liu L, Goyal A, Hovanky V, et al. Bendamustine vs. fludarabine/cyclophosphamide lymphodepletion prior to BCMA CAR-T cell therapy in multiple myeloma. Blood Cancer J. 2023;13(1):158.
125. Garfall AL, Cohen AD, Susanibar-Adaniya SP, Hwang WT, Vogl DT, Waxman AJ, et al. Anti-BCMA/CD19 CAR T cells with early immunomodulatory maintenance for multiple myeloma responding to initial or later-line therapy. Blood Cancer Discov. 2023;4(2):118–33.
126. Jacobson CA, Westin JR, Miklos DB, Herrera AF, Lee J, Seng J, et al. Abstract CT055: phase 1/2 primary analysis of ZUMA-6: axicabtagene ciloleucel (Axi-Cel) in combination with atezolizumab (atezo) for the treatment of patients (pts) with refractory diffuse large B cell lymphoma (DLBCL). Cancer Res. 2020;80(16_Suppl):CT055.
127. Jaeger U, Worel N, McGuirk JP, Riedell PA, Fleury I, Du Y, et al. Safety and efficacy of tisagenlecleucel plus pembrolizumab in patients with r/r DLBCL: phase 1b PORTIA study results. Blood Adv. 2023;7(11):2283–6.
128. Gauthier J, Bezerra ED, Hirayama AV, Fiorenza S, Sheih A, Chou CK, et al. Factors associated with outcomes after a second CD19-targeted CAR T-cell infusion for refractory B-cell malignancies. Blood. 2021;137(3):323–35.
129. Cappell KM, Kochenderfer JN. Long-term outcomes following CAR T cell therapy: what we know so far. Nat Rev Clin Oncol. 2023;20(6):359–71.
130. Laetsch TW, Maude SL, Rives S, Hiramatsu H, Bittencourt H, Bader P, et al. Three-year update of tisagenlecleucel in pediatric and young adult patients with relapsed/refractory acute lymphoblastic leukemia in the ELIANA trial. J Clin Oncol. 2023;41(9):1664–9.
131. Shah BD, Ghobadi A, Oluwole OO, Logan AC, Boissel N, Cassaday RD, et al. Two-year follow-up of KTE-X19 in patients with relapsed or refractory adult B-cell acute lymphoblastic leukemia in ZUMA-3 and its contextualization with SCHOLAR-3, an external historical control study. J Hematol Oncol. 2022;15(1):170.
132. Molinos-Quintana A, Alonso-Saladrigues A, Herrero B, Caballero-Velazquez T, Galan-Gomez V, Panesso M, et al. Impact of disease burden and late loss of B cell aplasia on the risk of relapse after CD19 chimeric antigen receptor T cell (tisagenlecleucel) infusion in pediatric and young adult patients with relapse/refractory acute lymphoblastic leukemia: role of B-cell monitoring. Front Immunol. 2023;14:1280580.
133. Frank MJ, Hossain NM, Bukhari A, Dean E, Spiegel JY, Claire GK, 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):3034–43.
134. Meyran D, Zhu JJ, Butler J, Tantalo D, MacDonald S, Nguyen TN, et al. T(STEM)-like CAR-T cells exhibit improved persistence and tumor control compared with conventional CAR-T cells in preclinical models. Sci Transl Med. 2023;15(690):eabk1900.
135. Ortiz-Maldonado V, Rives S, Castella M, Alonso-Saladrigues A, Benitez-Ribas D, Caballero-Banos M, et al. CART19-BE-01: a multicenter trial of ARI-0001 cell therapy in patients with CD19(+) relapsed/refractory malignancies. Mol Ther. 2021;29(2):636–44.
136. Oliver-Caldes A, Gonzalez-Calle V, Cabanas V, Espanol-Rego M, Rodriguez-Otero P, Reguera JL, et al. Fractionated initial infusion and booster dose of ARI0002h, a humanised, BCMA-directed CAR T-cell therapy, for patients with relapsed or refractory multiple myeloma (CARTBCMA-HCB-01): a single-arm, multicentre, academic pilot study. Lancet Oncol. 2023;24(8):913–24.
137. Shah NN, Johnson BD, Schneider D, Zhu F, Szabo A, Keever-Taylor CA, et al. Bispecific anti-CD20, anti-CD19 CAR T cells for relapsed B cell malignancies: a phase 1 dose escalation and expansion trial. Nat Med. 2020;26(10):1569–75.
138. Palani HK, Arunachalam AK, Yasar M, Venkatraman A, Kulkarni U, Lionel SA, et al. Decentralized manufacturing of anti CD19 CAR-T cells using CliniMACS prodigy(R): real-world experience and cost analysis in India. Bone Marrow Transplant. 2023;58(2):160–7.
139. Martinez-Cibrian N, Ortiz-Maldonado V, Espanol-Rego M, Blazquez A, Cid J, Lozano M, et al. The academic point-of-care anti-CD19 chimeric antigen receptor T-cell product varnimcabtagene autoleucel (ARI-0001 cells) shows efficacy and safety in the treatment of relapsed/refractory B-cell non-Hodgkin lymphoma. Br J Haematol. 2024;204(2):525–33.
140. Chong EA, Alanio C, Svoboda J, Nasta SD, Landsburg DJ, Lacey SF, et al. Pembrolizumab for B-cell lymphomas relapsing after or refractory to CD19-directed CAR T-cell therapy. Blood. 2022;139(7):1026–38.
141. Mailankody S, Matous JV, Chhabra S, Liedtke M, Sidana S, Oluwole OO, et al. Allogeneic BCMA-targeting CAR T cells in relapsed/refractory multiple myeloma: phase 1 UNIVERSAL trial interim results. Nat Med. 2023;29(2):422–9.

How to cite this article: Grégoire C, Coutinho de Oliveira B, Caimi PF, Caers J, Melenhorst JJ. Chimeric antigen receptor T-cell therapy for haematological malignancies: Insights from fundamental and translational research to bedside practice. Br J Haematol. 2024;00:1–15. https://doi.org/10.1111/bjh.19751